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EDITORIAL

Prediction of gestational diabetes mellitus: are we ready for a biomarker lead screening strategy for GDM?

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Gestational diabetes mellitus (GDM) is known to be associated with adverse pregnancy outcomes and more importantly with a substantial future metabolic risk for both the mother and the offspring [1]. It is also well established that recognition and timely management of GDM is associated with significant reductions in these risks. Strategies such as universal screening of all pregnant women for GDM have now been adopted in several countries and have also been recommended in government-approved guidelines for GDM in our country. By adopting such strategy, it has been reported that over 70% of GDM women can be identified at their first antenatal visit [2]. Appropriate management of these women during pregnancy followed by suitable lifestyle interventions in the mother in the postpartum period can go a long way in halting the spiraling burden of type 2 diabetes mellitus in our country.

While efforts at the detection and management of GDM during and after pregnancy through universal screening strategies are laudable, opportunities for intervention in early first trimester pregnancy may still be lost. Recognition of risk factors for GDM early in pregnancy that could reliably predict GDM in the second trimester in those who are not already detected with GDM could form the basis of a useful cost-effective biomarker lead GDM screening strategy. Early detection of women at risk for GDM would also help put in place a focused antenatal care model that allows targeted nutrition and lifestyle interventions in the first trimester to prevent GDM besides providing for greater surveillance and prompt management of GDM. However, this would only be possible if a suitable biomarker could be discovered, which is easy to measure and predicts GDM with a high degree of accuracy and consistency. The quest for such a biomarker such biomarker has led to extensive research in this area over the last couple of decades.

In gestational diabetes mellitus (GDM), risk prediction has traditionally been based mostly on maternal history and clinical risk factors. These clinical risk prediction tools for GDM are useful and have been validated in large populations. However, sensitivity and specificity have been inadequate and they may not optimally identify high-risk pregnancies [2]. Biomarkers can help improve risk prediction and facilitate early intervention that could prevent and effectively manage GDM.

Biomarkers for prediction of GDM have included inflammatory markers and adipocytokines [3–5] such as adiponectin, leptin, and hsCRP; nutritional biomarkers; markers of endothelial dysfunction like tissue plasminogen activator (TPA), FGF 21 and glycosylated fibronectin [6] and lipids; proteomics-based biomarkers [7]; multianalyte models of biomarkers [8]; metabolomic biomarkers [9] in GDM based on NMR; and genetic markers such as microRNAs [1].

In a recent systematic review [10], it was reported that circulating adiponectin had a pooled diagnostic odds ratio (DOR) of 6.4, a sensitivity of 64.7%, and a specificity of 77.8% for predicting future GDM. A recent study from North India [11] observed that first-trimester adiponectin was the strongest predictor of GDM. Receiver operating characteristic curves revealed that a cut-off value of adiponectin of 9.1 µg/mL in the first trimester was associated with a sensitivity of 100% and specificity of 95.6% in predicting GDM in Indian patients. The study concluded that this might be a very useful biomarker of GDM among Asian Indians. The detection rate of GDM improved from 58% in screening by maternal factors alone to 68% if these were combined with serum visfatin and adiponectin measurements [12]. Leptin appears to have a role in the inflammation and pathophysiology of GDM. Hyperleptinemia in early pregnancy appears to be predictive of an increased risk to development of GDM later in pregnancy independent of maternal obesity [2]. A recent meta-analysis evaluating eight prospective studies

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found that leptin levels in the first or early second trimester were significantly higher (7.25 ng/mL) in women who later developed GDM compared to those in women who did not [5]. However, the confounding influence of BMI/adiposity and gestational weight gain on leptin levels limits its role as a reliable biomarker. GDM risk prediction based on other adipocytokines has not been very encouraging. The combination of low SHBG and high hsCRP had a good predictive value for detection of GDM with sensitivity 74% and specificity 76% [5]. The role of altered lipid metabolism in early pregnancy for the prediction of GDM independent of BMI and insulin-resistant phenotypes has not been adequately explored. A multianalyte maternal serum biomarker [8] which was evaluated as a universal screening test for GDM had a high (87%) detection rate and a low false positivity (< 1%). Proteomics-based [7] and metabolomics-based tests [9] have also been developed but results were inconsistent. Proteomic methods are too complex and expensive for routine clinical use [2].

The current issue features several manuscripts which have evaluated different biomarkers in GDM. Guleroglu et al. [13] reported significant differences in oxidative stress markers as well as markers of oxidative DNA damage and pro-apoptosis in 69 GDM women with 21–42 weeks gestation when compared with those with non-GDM pregnant women. Similarly, Argun et al. [14] have shown significantly higher levels of endotrophin, a newly discovered adipocytokine, in second-trimester GDM subjects compared to those in normal pregnant women which correlated well with fasting and 1-h post-OGTT glucose values and HOMA-IR. Yang et al. [15] from China demonstrated a low-grade inflammatory state in a small set of diagnosed GDM women who were shown to have significantly higher levels of IL2, IL6, and hsCRP than non-GDM pregnant women. While the findings of these studies point to possible pathogenetic links between inflammation, oxidative stress, and adipocytokines to GDM, they need to be confirmed in larger studies. In terms of prediction, these studies are of limited value as all 3 studies have been done in diagnosed second-trimester GDM patients. To serve as biomarkers for prediction of GDM, significant associations will need to be demonstrated between first trimester measurements of one or more of these markers and GDM diagnosed later in the 2nd trimester in well-designed longitudinal studies of women followed up through different trimesters of pregnancy. The evaluation of first-trimester total cholesterol and postprandial triglyceride levels as biomarkers for prediction of future GDM which were also included in the current issue [16] is one such prospective longitudinal study which has reported that simple lipid parameters such as total cholesterol and postprandial triglyceride levels estimated at 11–13 weeks gestation could be useful in identifying women at risk for future GDM. The study concludes that a combination of elevated fasting cholesterol and postprandial triglyceride (PPTg) levels at an early stage of gestation can significantly predict future GDM in Indian women. If replicated, appropriate lifestyle strategies could be instituted early in pregnancy to prevent GDM. The incremental benefit of lipid measurements over existing clinical parameters and glucose measurements should also be evaluated to understand their additional value in GDM risk prediction.

Recent studies have also indicated that miRNA profiling in the first trimester or early second trimester can predict GDM. Most promising of these are mir29a, mir 132, and mir 222 [1]. However, as is the case for other biomarkers, these positive findings from small studies need to be confirmed in larger longitudinal studies. Also, low-cost assays for the same need to be developed which could be put to routine use [2]. Recently, machine learning or artificial intelligence methods using demographic variables and previous laboratory results have been applied to improve predictive power [17]. Deep learning and fog computing methods have also shown promise [18].

There are a number of candidate biomarkers that are being evaluated for GDM risk prediction. Models of prediction have also been developed with multiple markers [19, 20] and so have risk scoring systems with variable success [21].

Overall, adding inflammatory and other biomarkers to clinical GDM risk prediction markers has not resulted in clinically important improvements in prediction so far despite a demonstrable increase in sensitivity and specificity [1]. It has also been said that prediction was better for obese GDM than for non-obese GDM and different biomarkers may be needed for both [2]. A recent study concluded that simple clinical models [22] for prediction of GDM when combined with early pregnancy glucose measurements performed best in predicting GDM. Unless we are able to find a biomarker which improves accuracy in a clinically meaningful way and is affordable and convenient, the strategy of future risk prediction of GDM will have a limited scope and universal screening will continue to be the best strategy for the detection and management of GDM. Biomarkers will need to perform substantially better than clinical markers in predicting GDM and pregnancy outcomes to warrant a change in strategy.

References

1. Lorenzo-Almorós A, Hang T, Peiró C, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. Cardiovasc Diabetol. 2019;18:140. https://doi.org/10.1186/s12933-019-0935-9.
2. McIntyre HD, Kapur A, Divakar H, Hod M. Gestational diabetes mellitus-innovative approach to prediction, diagnosis, management, and prevention of future NCD-mother and offspring. Front

Endocrinol (Lausanne). 2020;3(11): 614533. https://doi.org/10.3389/fendo.2020.614533.
3. Williams MA, Qiu C, Muy-Rivera M, Vadachkoria S, Song T, Luthy DA. Plasma adiponectin concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. J Clin Endocrinol Metab. 2004;89(5):2306–11.
4. Winzer C, Wagner O, Festa A, Schneider B, Roden M, Bancher-Todesca D, et al. Plasma adiponectin, insulin sensitivity, and subclinical inflammation in women with prior gestational diabetes mellitus. Diabetes Care. 2004;27(7):1721–7.
5. Abell SK, De Courten B, Boyle JA, Teede HJ. Inflammatory and other biomarkers: role in pathophysiology and prediction of gestational diabetes mellitus. Int J Mol Sci. 2015;16(6):13442–73. https://doi.org/10.3390/ijms160613442.
6. Rasanen JP, Snyder CK, Rao PV, Mihalache R, Heinonen S, Gravett MG, Roberts CT Jr, Nagalla SR. Glycosylated fibronectin as a first-trimester biomarker for prediction of gestational diabetes. Obstet Gynecol. 2013;122(3):586–94. https://doi.org/10.1097/AOG.0b013e3182a0c88b.
7. Singh A, Subramani E, Datta Ray C, Rapole S, Chaudhury K. Proteomic driven biomarker discovery in gestational diabetes mellitus: a review. J Proteomics. 2015;127:44–9.
8. Srinivasa R. Nagalla, Caryn K. Snyder, John E. Michaels, Mary J. Laughlin, Charles T. Roberts Jr1,Madhuri Balaji2, V. Balaji2, V. Seshiah2, Paturi V. Rao. Maternal serum biomarkers for risk assessment in GDM: a potential universal screening test to predict GDM status. IJEM 2015;19:155–9.
9. Pinto J, Almeida LM, Martins AS, Duarte D, Barros AS, Galhano E, Pita C, Almeida Mdo C, Carreira IM, Gil AM. Prediction of gestational diabetes through NMR metabolomics of maternal blood. J Proteome Res. 2015;14(6):2696–706. https://doi.org/10.1021/acs.jproteome.5b00260.
10. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson SM. Accuracy of circulating adiponectin for predicting gestational diabetes: a systematic review and meta-analysis. Diabetologia. 2016;59(4):692–9. https://doi.org/10.1007/s00125-015-3855-6.
11. Madhu SV, Bhardwaj S, Jhamb R, Srivastava H, Sharma S, Raizada N. Prediction of gestational diabetes from first trimester serum adiponectin levels in Indian women. Indian J Endocrinol Metab. 2019 Sep-Oct;23(5):536–539. https://doi.org/10.4103/ijem.IJEM_319_19
12. Ferreira AF, Rezende JC, Vaikousi E, Akolekar R, Nicolaides KH. Maternal serum visfatin at 11–13 weeks of gestation in gestational diabetes mellitus. Clin Chem. 2011;57(4):609–13. https://doi.org/10.1373/clinchem.2010.159806.
13. Guleroglu FY, Bafali IO, Topaktas M et al. Comparison of biomarkers of oxidative stress, 8-isoprostane, advanced oxidation protein products, and 8-hydroxy-2′-deoxyguanosine and pro-apoptosis, cytokeratin 18 M30, in women with normal glucose tolerance and gestational diabetes mellitus. International J Diab Dev countries 2021. https://doi.org/10.1007/s13410-022-01072-5
14. Argun D, Eren EC. Evaluation of the circulating serum endotrophin in women with and without gestational diabetes mellitus during second trimester. International J Diab Dev countries 2021. https://doi.org/10.1007/s13410-021-01005-8
15. Yang Y, Zeng H, Yin P, Wang Z. Comparison of serum cytokines between gestational diabetes mellitus and normal pregnancy women: a pilot study. Int J Diabetes Dev Ctries. https://doi.org/10.1007/s13410-022-01060-9.
16. Madhu SV, Bhardwaj S, Mishra BK and Aslam M. Total cholesterol and postprandial triglyceride levels as early markers of GDM in Asian Indian women. International Journal of Diabetes in Developing Countries 2022. https://doi.org/10.1007/s13410-022-01046-7
17. Wu YT, Zhang CJ, Mol BW, Kawai A, Li C, Chen L, Wang Y, Sheng JZ, Fan JX, Shi Y, Huang HF. Early prediction of gestational diabetes mellitus in the Chinese population via advanced machine learning. J Clin Endocrinol Metab. 2021;106(3):e1191–205. https://doi.org/10.1210/clinem/dgaa899.
18. El-Rashidy N, ElSayed NE, El-Ghamry A, et al. Prediction of gestational diabetes based on explainable deep learning and fog computing. Soft Comput. 2022;26:11435–50. https://doi.org/10.1007/s00500-022-07420.
19. Palanlappan Y, Samal S, Lakshmi M, Prabhu K. First trimester prediction of gestational diabetes using a predictive model of biochemical parameters - a longitudinal study. J Clin Diagnostic Res. 2022. https://doi.org/10.7860/JCDR/2022/55641.16831
20. Kinneret Tenenbaum-Gavish, Adi Sharabi-Nov, Dana Binyamin, Holger Jon Møller, David Danon, Lihi Rothman, Eran Hadar, Ana Idelson, Ida Vogel, Omry Koren, Kypros H. Nicolaides, Henning Gronbaek, Hamutal Meiri, First trimester biomarkers for prediction of gestational diabetes mellitus, Placenta,2020; 101:80-89.
21. Wang Y, Ge Z, Chen L, et al. Risk prediction model of gestational diabetes mellitus in a Chinese population based on a risk scoring system. Diabetes Ther. 2021;12:1721–34. https://doi.org/10.1007/s13300-021-01066-2.
22. van Hoorn F, Koster M, Naaktgeboren CA, Groenendaal F, Kwee A, Lamain- de Ruiter M, et al. Prognostic models versus single risk factor approach in first-trimester selective screening for gestational diabetes mellitus: a prospective population-based multicentre cohort study. Bjog (2020). https://doi.org/10.1111/1471-0528.16446

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GUIDELINES

RSSDI Guidelines for the management of hypertension in patients with diabetes mellitus

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Abstract

Hypertension and diabetes mellitus (DM) are two of the leading lifestyle diseases in the Indian and South Asian populations that often co-exist due to overlapping pathophysiological factors. Obesity, insulin resistance, inflammation, and oxidative stress are thought to be some common pathways. Up to 50% of hypertensive cases in India are diagnosed with type 2 diabetes mellitus (T2DM), which defines the need for a comprehensive guideline for managing hypertension in diabetic patients. These RSSDI guidelines have been formulated based on consultation with expert endocrinologists in India and Southeast Asia, acknowledging the needs of the Indian population. Ambulatory blood pressure monitoring and office and home-based blood pressure (BP) monitoring are recommended for the early analysis of risks. Cardiovascular risks, end-organ damage, and renal disorders are the primary complications associated with diabetic hypertension that needs to be managed with the help of non-pharmacological and pharmacological interventions. The non-pharmacological interventions include the nutrition education of the patient to reduce the intake of salt, sodium, and trans fats and increase the consumption of nuts, fresh fruits, vegetables, and potassium-rich foods. It is also recommended to initiate 50 to 60 min of exercise three to four times a week since physical activity has shown to be more beneficial for hypertension control in Indian patients than dietary modulation. For the pharmacological management of hypertension in patients with T2DM, angiotensin II receptor blockers (ARBs) are recommended as the first line of therapy, demonstrating their superiority over other antihypertensive agents such as ACEi. However, most of the global hypertension guidelines recommend initiation with combination therapy to achieve better BP control in most patients and to reduce the risk of adverse events. For combination therapy, calcium channel blockers (CCBs) are recommended to be administered along with ARBs instead of beta-blockers or diuretics to avoid the risk of cardiovascular events and hyperglycaemia. Among the CCBs, novel molecules (e.g. cilnidipine) are recommended in combination with ARBs for better cardiovascular and reno-protection in diabetic hypertensive patients.

Keywords Diabetes mellitus · Hypertension · Macrovascular complication · Treatment

Abbreviations

ABPM	Ambulatory blood pressure monitoring
ACC	American College of Cardiology
ACEi	Angiotensin-converting enzyme inhibitors
AD	Alzheimer's disease
ADA	American Diabetes Association
AHA	American Heart Association
ALDO ANT	Aldosterone antagonist
ARBs	Angiotensin II receptor blockers
ASCVD	Atherosclerotic cardiovascular disease
BB	Beta-blockers
BP	Blood pressure
CAD	Coronary artery disease
CCB	Calcium channel blockers
CKD	Chronic kidney disease
CVD	Cardiovascular disorders
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic blood pressure
DM	Diabetes mellitus
ED	Erectile dysfunction

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eGFR	Estimated glomerular filtration rate
eNOS	Endothelial nitric oxide synthase
HBPM	Home blood pressure monitoring
HFrEF	Heart failure with reduced ejection fraction
HMOD	Hypertension-mediated organ damage
HTN	Hypertension
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
LMIC	Low- and middle-income countries
LV	Left ventricular
MACCE	Major adverse cardiac and cerebrovascular events
MHSBP	Morning home systolic blood pressure
MI	Myocardial infarction
OSA	Obstructive sleep apnoea
PAD	Peripheral arterial disease
PE	Preeclampsia
RAAS	Renin–angiotensin–aldosterone system
RAS	Renin–angiotensin system
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RSSDI	Research Society for the Study of Diabetes in India
SBP	Systolic blood pressure
SGLT2 inhibitors	Sodium–glucose cotransporter 2 inhibitors
SNA	Sympathetic nerve activity
SPC	Single-pill combination
SUA	Serum uric acid
T2DM	Type 2 diabetes mellitus
TIA	Transient ischaemic attack
UACR	Urine albumin–creatinine ratio
UPCR	Urine protein–creatinine ratio
VSM	Vascular smooth muscle
WHO	World Health Organization

Introduction

Currently, hypertension is a major public health issue in India, causing over 1.6 million annual deaths accounting for 10.8% of the total mortalities and 4.6% of the disability-adjusted life years [1]. Both conditions' co-existence is common in the middle and older age groups across all geographic and sociodemographic groups in India [2]. A crucial consideration for the management of hypertension in Indian subjects is the management of risk factors, which can be achieved through a combination of treatment approaches [3]. To manage the co-existing diseases, there is a need for detailed guidelines that consider the safety and efficacies of various treatment agents in patients with DM. This guideline by the Research Society for the Study of Diabetes in India (RSSDI) provides a detailed account of the standard, approved, and novel treatment agents to be used in India for controlling hypertension in patients with DM and for managing and reducing the risks of associated complications and organ damage. It also will describe lifestyle modification strategies and dietary approaches recommended for patient education, which is a pressing need for the Indian population [4]. This guideline focusses only on the management of hypertension in diabetic patients. Discussion on the non-pharmacological or pharmacological management of diabetes and special populations like pregnancy may not be in the purview of this guideline.

Definition of hypertension

Hypertension or systemic arterial hypertension refers to persistently high blood pressure in the systemic arteries beyond 140 mmHg [5]. ACC/AHA guidelines have changed the range to 130/80; however, Indian Guideline of Hypertension IV (IGH IV) guideline defines hypertension as systolic blood pressure (SBP) of $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) of $\geq$ 90 mmHg [6].

Classification of blood pressure

Blood pressure is defined as the pressure exerted on the blood vessels due to blood flow. Measurement of systolic pressure refers the pressure in arteries when the heart beats while the diastolic pressure refers the pressure in arteries when the heart rests between beats. A pressure below 120/80 mmHg is normal for all age groups. Table 1 shows a classification of normal and elevated blood pressure. When the BP levels of the patient are far beyond the normal and range higher than 180/120 mmHg, they are in a state of hypertensive crisis (as referred by AHA) requiring immediate medical attention [6].

Other criteria based on office, ambulatory (ABPM), and home blood pressure (HBPM) measurements are shown in Table 2.

Types of hypertension

Based on causative factors

Primary or essential hypertension

- Primary hypertension is mostly asymptomatic and is diagnosed based on repeated BP measurements or community screening [5].

Table 1 Various blood pressure categories and definitions of hypertension grade [6]

Blood pressure category	Systolic mmHg (upper number)		Diastolic mmHg (lower number)
Normal	Less than 120	and	Less than 80
Elevated	120–129	and	Less than 80
High blood pressure (hypertension) stage 1	130–139	or	80–89
High blood pressure (hypertension) stage 2	140 or higher	or	90 or higher
High blood pressure (hypertension) stage 3 OR hypertensive crisis	Higher than 180	and/or	Higher than 120

Table 2 Office, ambulatory (ABPM), and home blood pressure (HBPM) measurements [6]

	SBP/DBP, mmHg
Office BP	≥ 140 and/or ≥ 90
ABPM	
24-h average	≥ 130 and/or ≥ 80
Daytime (or awake) average	≥ 135 and/or ≥ 85
Nighttime (or asleep) average	≥ 120 and/or ≥ 70
HBPM	≥ 135 and/or ≥ 85

- Positive family history because of involvement of multiple genes and their allelic variants [5]
- Up to 60% of the population above 60 years of age is more susceptible to primary hypertension [7].
- Indian patients with primary hypertension are mostly unaware of their status and remain undetected. Hence, the Ministry of Health and Family Welfare Guidelines have advised that patients with positive risk factors such as obesity, diabetes mellitus, previous history of cardiovascular disease, patients above 60 years, and current smokers must be screened regularly [8].

Salt-sensitive hypertension

- Patients' response to salt due to the genetic build-up is one of the described factors for the development of essential hypertension. Not all individuals demonstrate a rise in BP due to the intake of a salt-rich diet.
- Salt sensitivity is a crucial element in the pathophysiology of hypertension. It is involved in both mechanisms of hypertension: (a) increased pulse volume and inability to excrete sodium in the urine and (b) endothelial dysfunction and increased peripheral resistance.
- Salt-sensitive hypertension is presented by a significant increase or decrease in the BP levels of the patient depending on the salt content of the diet [9].
- In Indian patients, salt intake has been a significant barrier to managing hypertension, as the average intake is as high as 13.8 g per day [4].
- Approximately 17–30% of cases of hypertension and associated cardiovascular conditions have been attributed to high salt consumption in India [4]. It also increases the risk of endothelial dysfunction and renal function decline [9]

The possible mechanism by which excess salt intake contributes to hypertension involves its effects on cardiac output. Excessive salt intake leads to an expansion of extracellular volume in the presence of sodium, which causes an increase in the cardiac output, increasing the cardiac workload. Reduction of dietary salt intake, thus, has a positive effect and is thus recommended in Indian patients [10].

Secondary hypertension

- Secondary hypertension is often due to an identifiable reason showing sudden worsening of BP. It is secondary to other diagnoses such as aldosteronism, reno-vascular hypertension, renal disease, and obstructive sleep apnea (OSA) [5].
- The prevalence of secondary hypertension is about 5–10% of hypertensive cases, wherein 2–3% cases are reno-parenchymal hypertension, and 1–2% are reno-vascular [11].
- As per the International Society of Hypertension, the following signs and symptoms should suggest the possibility of secondary hypertension [12]:

  Muscle weakness/tetany
  Cramps, arrhythmias
  Hypokalemia
  Pulmonary edema
  Sweating
  Palpitations
  Frequent headaches (pheochromocytoma)
  Snoring, daytime sleepiness (obstructive sleep apnea)

- Across all adult ages, renal disease, reno-vascular hypertension, aldosteronism, and OSA represent the most common causes of secondary hypertension.
- In patients with secondary hypertension, besides blood pressure assessment, further investigations should include blood investigations for the sodium, potassium, serum creatinine, estimated glomerular filtration rate

(eGFR), lipid profile, and fasting glucose levels, along with urinalysis.
- Clinical recommendations in practice for the evaluation of hypertension are for early detection of secondary hypertension, for prevention of hypertension-mediated organ damage (HMOD) and associated cardiovascular complications [13].
- Young adults prone to secondary hypertension should be assessed for renal parenchymal disease [14].

Based on disease severity

Resistant hypertension

- Patients in whom hypertension remains unmanaged despite being treated with 3 or more antihypertensive medications, including diuretics, are classified to have treatment-resistant hypertension after ruling out non-adherence to treatment and sub-optimal choices in antihypertensive therapy [5].
- It affects about 10% of the population and is associated with a high risk of cardiovascular disorders, end-organ damage, and all-cause mortality [12].
- Patients with resistant hypertension must be screened for secondary causes with the help of lab investigations that have been outlined above as per guidelines by the International Society of Hypertension.

Hypertension in special populations

Isolated systolic hypertension

- In elderly patients, isolated systolic hypertension is the predominant type that carries a significant cardiovascular or cerebrovascular risks, leading to significant morbidity and mortality [15].
- Approximately 60% of individuals above the age of 60 years have isolated systolic hypertension, and its prevalence is expected to rise substantially in the future [15].
- The incidence of isolated systolic hypertension is lower among generalized adult age groups in India, with 5.1% of men and 3.6% of women being diagnosed in North India as per the findings of a community cross-sectional survey conducted in the year 2010 [16]. However, isolated diastolic hypertension has also been identified in parts of rural India, which has a much higher prevalence of 70%, according to another cross-sectional survey of 3148 adults [17].
- Overall, Asian populations have been identified to be at a greater risk of systolic hypertension when compared with Western counterparts putting, which increases the risk of cardiovascular disorders, renal functional decline, and mortality [18].

Gestational hypertension: In pregnant women, hypertension with or without the diagnosis of preeclampsia is termed gestational hypertension, which increases the risk of maternal mortality and fetal abnormalities [19].

Refer to Table 3 for the diagnosis and blood pressure range for various types of hypertension [20].

Other sub-types of hypertension

White coat hypertension

- White coat hypertension, also called isolated clinic hypertension, is characterized by elevated office BP readings but normal out-of-the-office values [1].
- Ambulatory blood pressure monitoring is recommended for the diagnosis of white coat hypertension. Patients with office BP values at least 20/10 mmHg higher when compared with their ambulatory values are positive for white coat hypertension [21].
- In Indian patients, the risk of white coat hypertension is higher among younger populations compared to the elderly [21].

Table 3 Types of hypertension with a blood pressure range [20]

Type of hypertension	Description	Blood pressure range
Essential hypertension (most common type)	Chronic elevation in blood pressure with no underlying disease	Both systolic and diastolic blood pressures are elevated at more than 140/90 mmHg
Secondary hypertension (second most common type)	Chronic elevation in blood pressure due to underlying pathology (mostly due to renal problems)	Both systolic and diastolic blood pressure are elevated at more than 140/90 mmHg
Isolated systolic hypertension	Common in the elderly due to the loss of elasticity of major arteries	The systolic blood pressure is higher than 140 mmHg, while the diastolic blood pressure is close to the normal range
Resistant hypertension	When more than three different antihypertensive agents are prescribed, including a diuretic, and blood pressure remains elevated	Both systolic and diastolic blood pressures are elevated at more than 140/90 mmHg

Masked hypertension

- Masked hypertension, or isolated ambulatory hypertension, refers to a state where the patient has normal office readings but elevated out-of-the-office BP levels [5, 22].
- It is diagnosed with the help of office blood pressure monitoring and ambulatory monitoring.
- The risk of masked hypertension is not related to the age group of patients in the Indian population.
- Overall, patients who are receiving appropriate treatment for the management of hypertension are at a lower risk of masked hypertension [23].

Refer to Table 4 for the diagnosis of masked and white coat hypertension.

Table 4 Criteria for the diagnosis of white coat hypertension and masked hypertension in clinical practice [21]

- White-coat hypertension (isolated clinical hypertension)
  - Untreated patients with elevated office BP ≥ 140/90 mmHg and 24-h ambulatory BP < 130/80 mmHg and
    Awake ambulatory BP < 135/85 mmHg and
    Sleep ambulatory BP < 120/70 mmHg
- Masked hypertension
  - Untreated patients with office BP < 140/90 mmHg and
    24-h ambulatory BP ≥ 130/80 mmHg and/or
    Awake ambulatory BP ≥ 135/85 mmHg and/or
    Sleep ambulatory BP ≥ 120/70 mmHg
- Pseudo- or false-resistant hypertension because of the white-coat effect
  - Treated patients with elevated office BP ≥ 140/90 mmHg and
    24-h ambulatory BP < 130/80 mmHg and
    Awake ambulatory BP < 135/85 mmHg and
    Sleep ambulatory BP < 120/70 mmHg
- Masked uncontrolled hypertension
  - Treated patients with office BP < 140/90 mmHg and
    24-h ambulatory BP ≥ 130/80 mmHg and/or
    Awake ambulatory BP ≥ 135/85 mmHg and/or
    Sleep ambulatory BP ≥ 120/70 mmHg

Global and Indian scenarios of hypertension

- As per WHO, worldwide, about 1.13 billion people have hypertension. Among them, two thirds are from low- and middle-income countries (LMICs). The percentage of adults having hypertension is higher in LMICs (31.5%) than in high-income countries (28.5%) [24].
- A systemic analysis of population-based studies from 90 countries showed the age-standardized prevalence of hypertension was slightly higher in men (31.9%) than in women (30.1%) [25].
- As the Global Burden of Diseases 2016, 1.63 million deaths in India were attributed to hypertension. This was 108% higher than in 1990 [26].
- The fourth National Family Health Survey reported hypertension in 13.8% of men vs. 8.8% of women aged 15–49 and 15–54, respectively [26].
- In India, about 33% and 25% of urban and rural residents, respectively, are hypertensive. Among them, over 50–75% are unaware of their hypertensive state [27].

Prevalence of hypertension in patients with diabetes

- The prevalence of hypertension is higher in patients with diabetes mellitus (DM), with 50% of cases of hypertension also being diagnosed with type 2 diabetes mellitus (T2DM) [28].
- This risk is marked in the elderly population, who are at a greater risk of complications, including macro- and microvascular diseases due to the co-existence of DM and hypertension [29].
- In India, an increase in the coexistence of diabetes and hypertension is being reported. Patients with diabetes showed 1.5–2.0 times higher prevalence of hypertension than those without diabetes [30].

Good blood pressure measurement and its significance

Office blood pressure measurement

Blood pressure measurement in the office or clinic is a standard diagnostic test for hypertension and follow-up. A minimum of 2–3 office visits at 1–4-week intervals (depending on the BP level) is required to confirm the diagnosis of hypertension. Diagnosis must be performed in the first visit if BP is ≥ 180/110 mmHg with an evidence of cardiovascular disease (CVD). Figure 1 shows recommendations for Office BP measurement.

An automated oscillometric upper-arm cuff device (validated) is recommended for BP measurement. To measure BP, the cuff bladder must be wrapped so that it covers 80% of the arm circumference of the patient and 40% of the width. Accurate positioning of the patient is most crucial for precisely monitoring his/her BP levels. AHA recommends measuring blood pressure while the patient sits upright with legs uncrossed and arms supported. The centre of the cuff on the upper arm must be at the level of the right atrium of the patient, which lies at the midpoint of the

sternum. Chair positioning and pillows as support must be made to achieve the desired level.

Ambulatory blood pressure and home BP measurement

Ambulatory BP monitoring (ABPM) provides a 24-h account of the patient's state and is useful for diagnosing primary and secondary hypertension and differentiating it from the white coat and masked subtypes [31]. ABPM is accurate in predicting the risk of cardiovascular disease and mortality [31]. BP level measurement using home or ambulatory BP monitoring is recommended in patients with office BP classified as high-normal BP or grade 1 hypertension (systolic 130–159 mmHg and/or diastolic 85–99 mmHg) (Table 5).

Blood pressure monitoring in patients with diabetes

- ABPM is recommended for patients with DM and hypertension to reduce the risk of post-treatment complications and improve BP control [36]. In elderly patients, it is recommended to opt for a standing position during blood pressure monitoring [37].
- In a systematic review of 95 randomized control trials (RCTs), it was stated that for patients with hypertension and diabetes mellitus, the blood pressure target must be kept close to 120 to 130 mmHg to reduce the risk of stroke [38].
- In a clinical trial of 244 subjects with uncontrolled SBP and DM, it was found that home blood pressure monitoring alleviated the risk of cardiovascular disorders due to the reduction of BP by 9 mmHg and better control of BP fluctuations during the day [39]. More than half of the patients achieved better adjustment of antihypertensives with the help of home blood pressure monitoring [39].
- As per the American Diabetes Association (ADA) recommendations, blood pressure must be monitored at every office visit in patients with DM [40]. In patients with elevated BP, multiple readings must be taken to diagnose hypertension [36].
- If automated devices are used during office measurement of BP, the machine must be calibrated and validated regularly. Generally, a 5- to 10-mmHg variation from a traditional sphygmomanometer is expected [36].

Digital mediums being used for blood pressure measurement in India

Digital semiautomatic and fully automatic devices have been introduced in India for ABPM [40]. While it has the advantage of reducing the risk of human errors, especially in busy clinics, there is a mistrust related to the use of digital monitoring devices among the clinicians, primarily because they are not formally validated and, thus, have a range of error [40]. Population-based studies in India

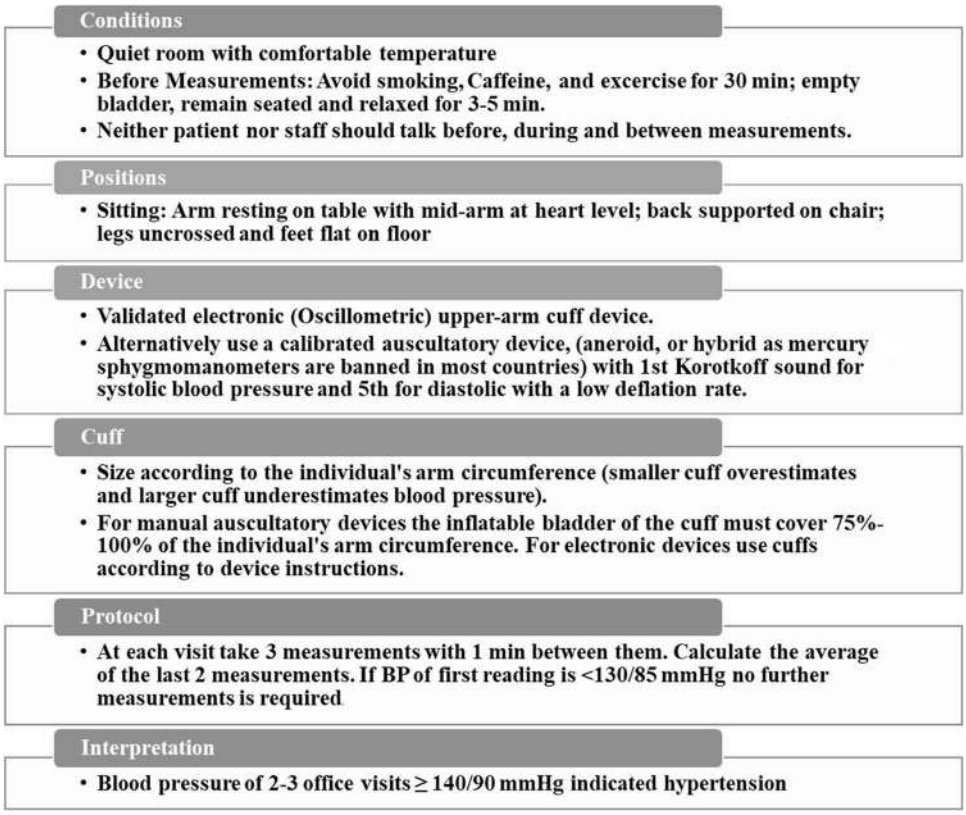

Fig. 1 Recommendations for office blood pressure measurement [12]

Table 5 Home and ambulatory blood pressure (BP) measurement [32–35]

	Condition	Position	Device	Cuff	Measurement protocol	Interpretation
Home blood pressure monitoring	As for office blood pressure	As for office blood pressure	Validated electronic (oscillometric) upper-arm cuff device	Size according to the individual's arm circumference	Before each visit to the health professional: • 3–7-day monitoring in the morning (before drug intake if treated and the evening) • Two measurements on each occasion after 5 min sitting rest and 1 min between measurements Long term follow-up of treated hypertension: • 1–2 measurements per week or month	• 24-h monitoring at 15–30 min intervals during daytime and night-time • At least 20 valid daytime and 7 nighttime BP readings are required. If less, the test should be repeated
24-h ambulatory blood pressure monitoring	Routine working day	Avoid strenuous activity. Arm still and relaxed during each measurement			• Average home blood pressure after excluding readings of the first day ≥ 135 or 85 mmHg indicates hypertension	• 24-h ambulatory blood pressure after ≥ 130/80 mmHg indicates hypertension. (Primary criterion) • Daytime (awake) ambulatory blood pressure ≥ 135/85 mmHg and night-time (asleep) ≥ 120/70 mmHg indicated hypertension

indicate that an aneroid sphygmomanometer must be preferred over digital devices since their readings are more like a traditional mercury-based sphygmomanometer, the most reliable device. However, digital mediums can be preferred for home-based measurement for higher patient comfort, which has a specificity of 80% and a sensitivity of 67.7% in the Indian population [41].

Hypertension and diabetes: the relationship

A complex cause–effect relationship between hypertension and diabetes involves obesity, visceral adiposity, and insulin resistance as the probable main pathogenic factors [42] (Fig. 1). Hypertension and diabetes are the consequence of metabolic syndrome. Inflammatory markers such as C-reactive protein are elevated in patients with DM or hypertension, indicating that both the conditions are associated with low-grade inflammation and genetic factors such as single nucleotide polymorphisms [43]. Insulin being a pleiotropic hormone has a role in developing hypertension, diabetes, and metabolic syndrome [44].

In patients with DM and hypertension, the risk of isolated systolic hypertension is the highest due to autonomic neuropathy (Fig. 2) [38]. They have a higher baseline heart rate and are at an increased risk of cardiovascular disorders (CVDs) [37]. Furthermore, nocturnal BP elevation is not significant in these patients, but they face greater day fluctuations [37].

The incidence of resistant hypertension (RH) is higher among people with diabetes than in the general or hypertensive population.

The peculiar presentation of hypertensive patients with DM reflects the need for specific guidelines. Indian guidelines need to be more comprehensive in terms of patient education and consideration of dietary factors so that the overall risk and burden of hypertensive disorders is reduced in patients with DM. This objective is addressed in present guideline, which provides recommendations related to the use of various antihypertensive combinations and treatments in patients with DM along with dietary and lifestyle changes as well as the need for monitoring.

Hypertension-mediated/related conditions

Hypertension, diabetes, and cardiovascular disorders

Hypertension is common among patients with diabetes mellitus and so is the risk of cardiovascular complications (Fig. 3). Hypertension alleviates the risk of atherosclerotic cardiovascular disease (ASCVD), heart failure, and microvascular complications. In subjects with diabetes, ACSVD significantly increases the morbidity and mortality [36].

It has been observed that the management of hypertension in patients with DM lowers the risk of ASCVD events. With every 10 mmHg decrease in systolic BP, the risk of complications of DM is reduced by 12%, and the associated mortality is reduced by 15%. The risk of MI in these patients is reduced by 11%, and microvascular complications are reduced by 13% [46]. This states the significance of good blood pressure management in patients with DM, especially those at a high risk of cardiovascular complications.

Hypertension-mediated organ damage

Undesired changes in the structure and function of arteries or organs lead to hypertension-mediated organ damage, a critical marker of cardiovascular (CV) disease. Higher incidences of all-cause death and CV events including ischemic heart disease, ischemic stroke, hemorrhagic stroke, cardiac death, and major adverse cardiac and cerebrovascular events (MACCE) may be observed. Nephropathy is the

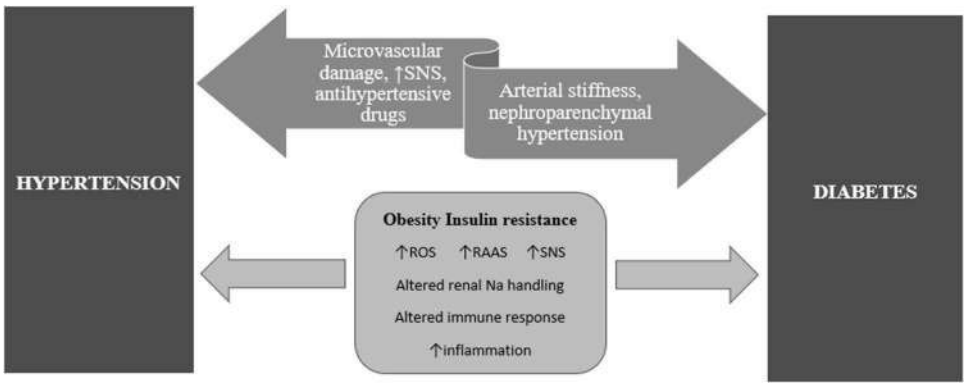

Fig. 2 Relationship between hypertension and diabetes

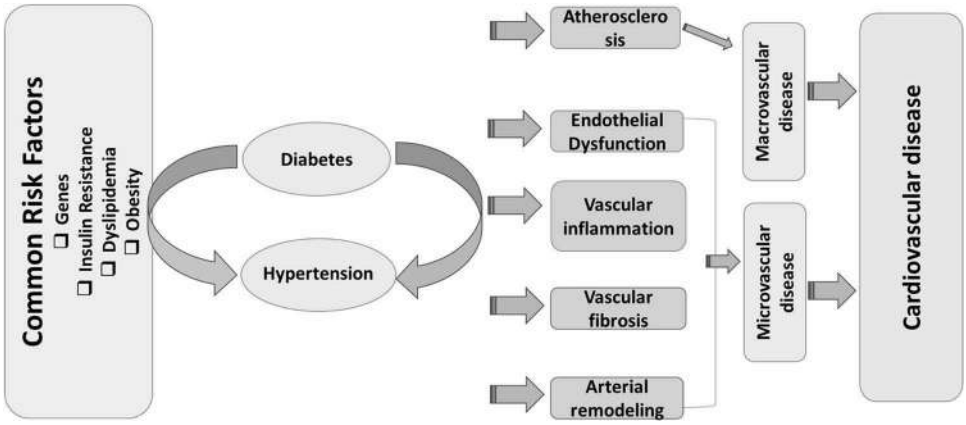

Fig. 3 Diabetes and hypertension predispose to cardiovascular disease [45]

most common form of organ damage seen in hypertensive patients. European Society of Cardiology guidelines for hypertension recommend basic screening of HMOD in all hypertensive patients [47].

Hypertension and chronic kidney disease

Hypertension is closely linked with chronic kidney disease because a sustained state of elevated BP worsens kidney function (Fig. 4) [48]. The pathophysiology of CKD and hypertension is complex. A loss of kidney function worsens blood pressure or vice versa.

Tight control of BP reduces the risk of chronic kidney disease by attenuating the pathophysiological pathways that contribute to eGFR progression and loss of kidney function [49]. Along with antihypertensive medications, dietary management, including salt restriction, forms the mainstay in the management of BP in CKD patients [50], which has been discussed in the dietary recommendations made by RSSDI ahead.

Proteinuria/microalbuminuria and end-organ damage

- Microalbuminuria or proteinuria is a major risk factor for renal disease progression, and it is also a powerful marker of cardiovascular disease and all-cause mortality [51]. For early diagnosis and better management of albuminuria with the help of sensitive tests such as albumin-to-creatinine ratio (UACR) is recommended for the avoidance of these complications [52].
- UACR is the key to early diagnosis of chronic kidney disease (CKD) in patients with diabetes mellitus because the patient is generally asymptomatic at this

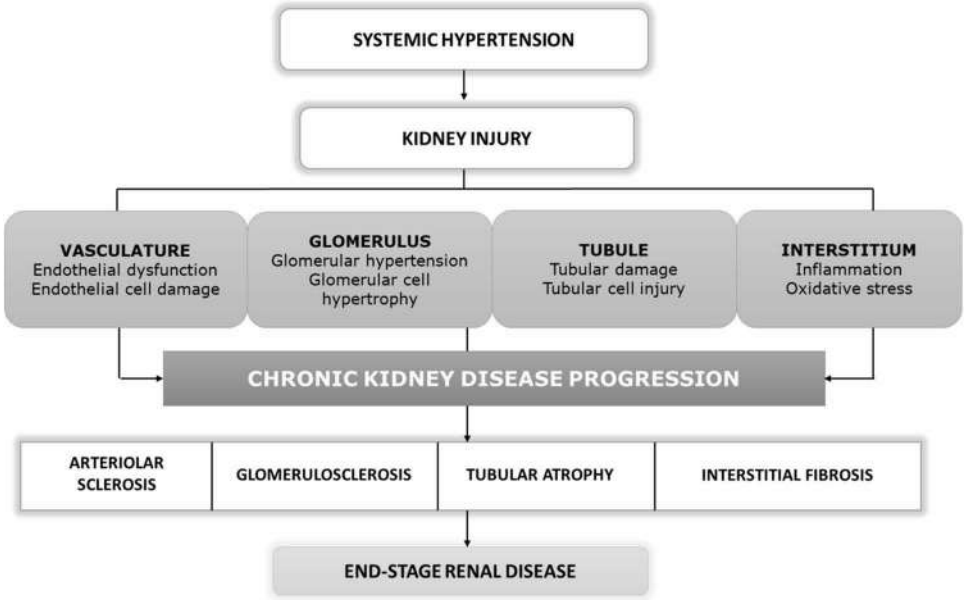

Fig. 4 Pathophysiological relationship between hypertension and chronic kidney disease [49]

stage, and their glomerular filtration rate (GFR) is also close to normal [52].

- American Diabetes Association (ADA), Kidney Disease Improving Global Outcomes (KDIGO), and US Kidney Disease Outcomes Quality Initiative (KDOQI) recommend at least annual screening for UACR in patients with diabetes mellitus [52].
- Indian guidelines recommend that all patients with hypertension must be screened for the presence of kidney disease at the time of their diagnosis and regularly thereafter [53]. Along with UACR, serum creatinine measurement and calculation of eGFR are also recommended [54].
- Urine protein-to-creatinine ratio (UPCR) is an accurate method to quantify proteinuria for the diagnosis of preeclampsia (PE), the onset of hypertension accompanied by significant proteinuria after 20 weeks of gestation [55].

Hypertension and coronary/peripheral artery disease

Globally, peripheral arterial disease (PAD), coronary artery disease (CAD), and cerebrovascular disease (CVD) are leading causes of morbidity and mortality [56]. Patients with cerebrovascular complications, including ischemic and hemorrhagic stroke, observed increased systolic hypertension more frequently. Post hoc analysis of the INternational VErapamil-SR/Trandolapril STudy (INVEST) demonstrated that among hypertensive CAD patients, concomitant PAD indicates a worse prognosis for adverse cardiovascular outcomes than CAD over a mean follow-up of 2.7 years [57].

Hypertension-associated erectile dysfunction

Hypertension and erectile dysfunction (ED) are related diseases with a common denominator, i.e. endothelial dysfunction. Changes in the endothelium-derived factors can lead to an increase in vascular smooth muscle (VSM) contraction [58]. Hypertension induces vascular changes that affect pudendal arteries and penile vasculature leading to reduced blood circulation to the penis [59]. High blood pressure or antihypertensive treatment can also lead to ED. Antihypertensive drugs like diuretics, beta-blockers, and centrally acting agents negatively affect erectile function. At the same time, calcium antagonists and ACE inhibitors are neutral [60].

Hypertension and heart failure

There is a direct correlation between increased blood pressure and the risk of developing heart failure. Patients with blood pressure greater than or equal to 160/100 mmHg have a doubled risk of heart failure than those with blood pressure less than 140/90 mmHg [61]. High blood pressure can also lead to high prevalence of atrial fibrillation [62], ventricular arrhythmias [63], and a sixfold greater risk of myocardial infarction [64], and subsequent heart failure with reduced ejection fraction (HFrEF). High BP increases the left ventricular (LV) afterload and peripheral vascular resistance, which causes diastolic dysfunction followed by concentric or eccentric LV hypertrophy (Fig. 5) [65].

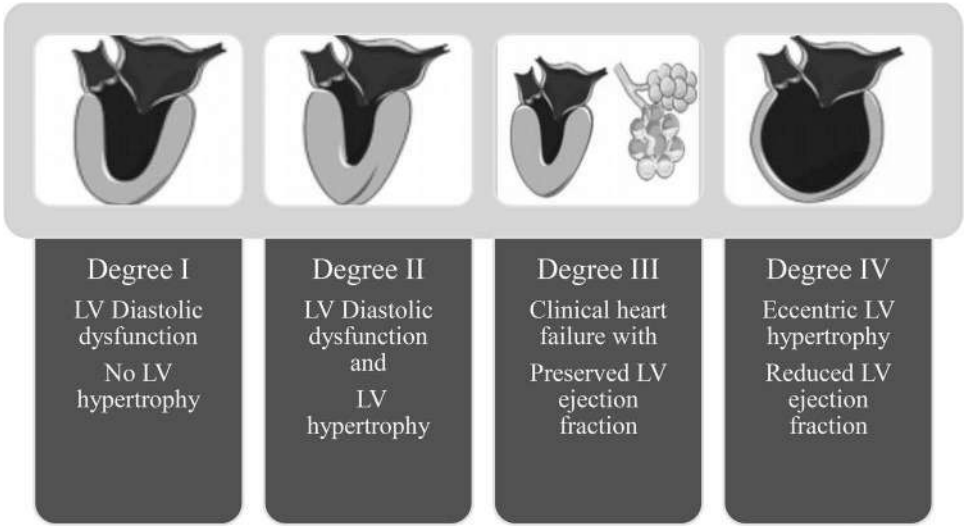

Fig. 5 Different stages of hypertensive heart disease

Hypertensive retinopathy

Choroidopathy, retinopathy, and optic neuropathy are hypertension-related ocular diseases [66]. Hypertensive retinopathy occurs because the retinal vessels are damaged due to elevated blood pressure. Angiotensin-converting enzyme allele deletion increases the risk of hypertensive retinopathy [67]. Higher plasma leptin level was reported to be associated with hypertensive retinopathy and vascular endothelium damage [68]. A study showed that serum uric acid (SUA) concentration and hypertensive retinopathy are significantly associated. For every 1 mg/dL increase in SUA, there was a significant 6% higher probability of hypertensive retinopathy [69].

Communities study of atherosclerosis risk showed that the incidence of stroke was two- to fourfold higher in patients with moderate hypertensive retinopathy, independent long-term hypertension, cigarette smoking, and dyslipidemia [70].

Long-term effects of hypertension

Patient with hypertension having abnormal BP is at high risk of transient ischemic attack (TIA) [71]. Not only first TIA, but hypertension is also a risk factor for recurrent TIA and stroke. A study in TIA patients ($N=1707$) showed that 58% of patients had a history hypertension while 75% of them had SBP > 140 mmHg following the onset of TIA [72, 73].

Normotensive people after stroke can have high blood pressure—acute hypertensive response perhaps due to autonomic nervous system dysfunction and/or abnormal cerebrovascular reactivity [74].

Hypertension might increase the risk of Alzheimer's disease (AD). The pathology linking hypertension to Alzheimer's disease is intracranial atherosclerosis, possibly limiting cerebral blood flow and/or dampening perivascular clearance [75]. A cross-sectional study in < 60 years of age group individuals showed all-cause dementia, mixed Alzheimer's/vascular dementia, and Alzheimer's disease with elevated SBP and those on antihypertensive medication [76].

For all the above disease situations, recommended BP thresholds for treatment are shown in Table 6.

Hypertension: risk factors

Several factors predisposing hypertension vary from country to country and between urban and rural region of same place. An Indian community-based cross-sectional study reported tobacco and alcohol consumption, overweight, obesity, and abdominal obesity as risk factors associated with HTN [78]. Old age and physical inactivity are independent risk factors for hypertension. Different epidemiologic and clinical studies showed sleep-related breathing disorders (SRBD) (obstructive sleep apnea (OSA) and habitual snoring) as independent risk factors for essential hypertension [79].

Table 7 [80] shows the cardiovascular risk assessment based on risk factors. Patient with grade 1 hypertension can be at low risk to high risk depending upon risk factors. Men above 50 years, non-smoking, and non-obese with grade 1 HTN may be at low risk, whereas smoking men are at moderately to higher risk of HTN. Diabetic patients irrespective of other factors are at high risk of HTN.

Table 6 Summary of office blood pressure thresholds for treatment [32]

Age group	Office SBP treatment threshold (mmHg)					Office DBP treatment threshold (mmHg
	Hypertension	+diabetes	+CKD	+CAD	+Stroke/TIA	
18-65years	≥140	≥140	≥140	≥140	≥140	≥90
65-79 years	≥140	≥140	≥140	≥140	≥140	≥90
≥80 years	≥160*	≥160	≥160	≥160	≥160	≥90
Office DBP treatment threshold (mmHg	≥90	≥90	≥90	≥90	≥90	

*As per NICE guideline (2019) ≥ 150 mmHg [77]

Table 7 Assessment of cardiovascular risk in patients with hypertension based on the number of risk factors

Hypertension disease staging	Other riskfactors, HMOD, or disease	BP (mmHg) grading			
		High normal SBP 130-139 DBP 85-89	Grade 1 SBP 140-159 DBP 90-99	Grade 2 SBP 160-179 DBP 100-109	Grade 3 SBP ≥ 180 DBP ≥ 110
Stage 1 (uncomplicated)	No other risk factor	**Low risk**	**Low risk**	**Moderate risk**	**High risk**
	1 or 2 risk factors	**Low risk**	**Moderate risk**	**Moderate to high risk**	**High risk**
	≥ 3 risk factors	**Low to moderate risk**	**Moderate to high risk**	**High risk**	**High risk**
Stage 2 (asymptomatic disease)	HMOD, CKD grade 3, or diabetes mellitus without organ damage	**Moderate to high risk**	**High risk**	**High risk**	**High to very high risk**
Stage 3 (Established disease)	Established CVD, CKD grade ≥ 4, or diabetes mellitus with organ damage	**Very High Risk**	**Very High Risk**	**Very High Risk**	**Very High Risk**

Methodology

The RSSDI guidelines for the management of hypertension in diabetics have been formulated in consultation with expert endocrinologists and diabetologists in India and Southeast Asia for making recommendations for the management of hypertension, along with strategies to reduce the risks for HMOD and cardiovascular complications. These recommendations were supported by literature evidence and clinical overview obtained from existing Indian and international guidelines. Literature evidence included data and recommendations from Indian, international, and South Asian journals, gathered based on extensive literature research, primarily conducted in PubMed and Cochrane libraries. After a thorough quality assessment, published RCTs, systematic reviews, meta-analysis papers, cross-sectional studies, cohort studies, and expert opinion papers were considered and included. The first draft having recommendations was

prepared and circulated among RSSDI panellists to gather suggestions for improvements. All the authors provided written recommendations for improvements in each section following the rigorous review of the document based on their expertise in the field (Tables 8 and 9). The draft was revised to address the identified gaps and was sent out to the authors for further review and feedback. Since all the expert authors approved the recommendations made in the second draft, it was finalized and sent out for publication.

Management/treatment

RSSDI recommendations for the management of hypertension in patients with diabetes mellitus

Summary of evidence

Dietary and lifestyle recommendations A nutrition education program is recommended for patients with DM and hypertension to reduce the risk of metabolic syndrome complications [81].

- In the RCT of 51 participants, it was found that the knowledge of food portion control for weight reduction, education about healthier food choices, individualized meal planning, understanding of the glycemic index and glycemic loads of different food items and their importance in blood glucose control, recognition of the food pyramid, and its use in meal planning for BP control assisted in the improvement of metabolic factors in patients with DM [81].
- In a systematic review of 198 studies for BP management in diabetic patients in low- and middle-income countries, it was stated that self-management and control through patient education are crucial for managing CVD risk factors [82]. Nutritional interventions that facilitate glycaemic and blood pressure control are recommended [81, 82].
- In the RCT of 40 patients with DM and hypertension, it was noted that Dietary Approaches to Stop Hypertension (DASH) diet and increased walking duration helped reduce ABPM values [83]. This dietary plan promotes

Table 8 Levels of recommendation based on the type of literature evidence

Level	Type of evidence
I	Systematic review (with homogeneity) of RCTs OR RCTs with a large sample size depicting significant results
II	Systematic review (with homogeneity) of cohort studies OR small-scale RCTs with unclear results OR consistent recommendations from multiple consensus guidelines (more than 2 national/international guidelines) OR randomized observational studies
III	Individual cohort studies or clinical studies without randomization OR "outcomes" research OR cross-sectional studies OR evidence gathered from existing consensus guidelines
IV	Systematic review (with homogeneity) of case–control studies OR individual case–control studies OR guidelines with improper evidence/lack of consensus OR retrospective analysis of patient data
V	Case series OR independent case study observations OR expert opinion without explicit critical appraisal based on standard principles or narrative reviews or literature reviews without systematic analysis

Table 9 Grades of recommendation for guiding practice implications for the physicians

Grade	Descriptor	Quantifying evidence	Implications for practice
A	Strong recommendation	Level I evidence with consistent findings from multiple studies of levels II, III, and IV	Clinicians should follow grade A recommendations unless a clear and compelling rationale for an alternative approach is defined
B	Recommendation	Levels II, III, and IV evidence with consistent findings but lack of level I evidence	Clinicians should follow grade B recommendation while remaining alert to newly published evidence and sensitive to patient preferences
C	Option	Levels II, III, and IV evidence with inconsistent findings	While considering grade C evidence for individual practice, clinicians should be flexible in their decision-making approach, patient preferences and peer opinions should have a substantial influencing role
D	Option	Level V evidence: little or no systematic empirical evidence	For grade D evidence, the physician must consider all options in their decision making and be alert to newly published evidence that clarifies the benefit versus harm of the selected approach; patient preference should have a substantial influencing role

higher consumption of whole grains, fat-free or low-fat dairy products, fruits, vegetables, poultry, fish, and nuts, along with reduced intake of saturated fat, total fat, cholesterol, and sodium and high intake of potassium, calcium, magnesium, fibre, and protein [79, 84]. DASH diet, in consideration of the taste preferences of Indian patients, is recommended for hypertensive control in DM patients.

- For the prevention of CVDs in patients with DM and hypertension, it is recommended that physicians must work closely with the patients to identify potential barriers and support them in reaching their target BP and HbA1c goals [84].
- Regular exercise or walking is recommended along with dietary control in patients with hypertension and DM [83, 85]. In the RCT of 94 Indian participants, it was affirmed that physical activity had a greater impact on BP control when compared with dietary salt restriction [86]. Therefore, brisk walking for 50 to 60 min, three to four times a week was recommended for effective BP management [86]. Yoga and salt restriction were also effective for Indian patients but had a lower impact than physical activity [86]. Alcohol intake was to be decreased or avoided [54]. Smoking cessation should be advised to all patients. Cessation therapies should be provided for patients who wish to quit smoking [54]. Combining these approaches would achieve maximal benefits for patients with co-existing disease [86].
- Worksite interventions are effective for reducing SBP, diastolic blood pressure (DBP), and blood glucose levels in obese Asian subjects at risk of metabolic conditions [87]. These interventions must be planned in the form of multidisciplinary sessions by including physicians, nutritionists, and physical trainers to guide the patient [87].

Recommendations

- Pharmacological therapy with lifestyle modifications should be initiated in patients with confirmed office-based BP > 140/90 mmHg
- A target value of 120 to 130 mmHg must be achieved in patients with co-existing DM and hypertension through a combination of dietary and lifestyle interventions, including a low sodium diet, plenty of fresh fruits, vegetables, and whole grains along with regular physical activity (grade A)
- Brisk walking as a physical form of activity, having sessions for 50–60 min three to four times a week, is more effective than dietary salt restriction and yoga and must be recommended in patients (grade B)
- Nutrition education about the role of diet and knowledge of healthy food choices is crucial for self-management of BP in diabetic patients (grade A)

Overview of treatment options for the management of hypertension in patients with diabetes mellitus

- Initial treatment for diabetes depends on the severity of hypertension with a regimen that includes calcium channel blockers (CCBs), angiotensin II receptor blocker (ARB)/ACE inhibitors, and diuretics, beta-blockers for compelling indications [54]. Real-world studies consider ACE inhibitors or ARBs as the first-line treatment agents for diabetic hypertensive patients depending on their treatment response and tolerance profiles [88].
- Clinical evidence suggests that single-pill combination (SPC) containing two or more antihypertensive agents (with complementary MOA) offers potential advantages over free drug combinations [89].
- Thiazides may also be used for the first-line treatment, but these must be administered alongside ACEi or ARBs [90].
- The drug's clinical effects must be evaluated before the selection of any treatment agents, particularly ARBs [91]. The selection of treatment agents must be based on the patient's profile, especially in those at the risk of end organ damage due to multiple comorbidities [91].

Antihypertensive drug therapy for the management of hypertension in diabetic patients

1. Blockers of the renin–angiotensin–aldosterone system (RAAS): angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs)

    - ARBs and ACEi are the most widely used antihypertensive drugs because of their similar effects on cardiovascular outcomes.
    - ARBs help in reducing the cardiovascular and cerebrovascular risks and renal complications, hence minimizing the morbidity and mortality risks in patients with hypertension [92].
    - ARBs are safer, tolerant, and more efficacious than ACEi for BP control [89, 92, 93]. In the RCT of 1600 patients, telmisartan facilitated greater BP reduction than ramipril [92]. ARBs have been found to have maximal reno-protective effects when compared with other classes of drugs [26]. The risk towards side effects is also lower with these agents compared with ACEi that induce cough [89]. Therapy having a combination of an ARB and an ACE inhibitor is not recommended as it is associated with an excess of adverse renal events [93].

- ARBs are administered in combination with other agents such as calcium channel blockers (CCBs) or thiazide diuretics to reduce the risk of CVDs and renal disorders in patients with DM [89, 93].
- In diabetic hypertensive patients, telmisartan and losartan are the most effective choice of ARBs to reduce cardiovascular risk factors [91]. Telmisartan must be selected as the first-line ARB agent in diabetic patients because of its beneficial impacts on fasting blood glucose and insulin levels [91]. In those at a higher risk of stroke, losartan must be preferred, whereas telmisartan is recommended in patients having a history of atrial fibrillation [91].
- German registry "EARLY" reported that a significantly greater proportion of patients in the azilsartan group achieved the target blood pressure of < 140/90 mmHg compared to ACEi [94].
- In RCT of 204 Indian patients, it was found that the efficacy and safety of azilsartan are like telmisartan. Thus, it can be selected as an alternative drug in patients based on its availability [92].
- ACEi may be preferred as first-line treatment agents in patients with diabetic hypertension as an alternative to ARBs [95]. Both these agents effectively reduce the risk of CVDs in high-risk patients, although ARBs are more efficacious [92–96].
- Both ARBs and ACEi effectively reduce the risk of end-stage renal disease [97].
- Azilsartan is a suitable agent for antihypertensive therapy in CKD patients. A 20-mg dose of azilsartan has demonstrated potent antiproteinuric effects compared with other agents such as candesartan [98]. An 80-mg dose is effective for controlling SBP and DBP in office and ambulatory settings [99]. Azilsartan improved diastolic function of the left ventricle in patients with heart failure with preserved ejection fraction (HFpEF) [100].
- While ACEi also achieved this clinical benefit, a more beneficial impact was observed with ARBs [101].
- A single-centre study including 133 hypertensive subjects diagnosed with COVID-19 infection showed a lower rate of admission to semi-intensive/intensive care units when patients were treated with RAAS inhibitor (32% using ARBs and 30% using ACEi) [102]. Suppose target blood pressure values are not achieved with either of the therapies. In that case, the addition of a thiazide diuretic is indicated as a second-line agent [95].
- A meta-analysis containing 7 studies compared ACEi/ARB alone and in combination therapy with sodium–glucose cotransporter 2 (SGLT2) inhibitors in T2DM patients. The analysis reported that combination therapy with SGLT2 inhibitors could achieve better control of blood pressure and estimated glomerular filtration rate (eGFR) [103].

2. Beta-blockers

- Beta-blockers are used for the initial management of BP in diabetic patients in cases with a previous history of CVDs such as myocardial infarction, heart failure, coronary artery disease, or stable angina [95].
- Beta-blockers and CCBs are not used independently. They are only indicated as a part of combination therapy with ARBs or ACEi in patients with DM [95].
- Recent clinical trial showed that the use of beta-blockers increases the risk of cardiovascular events in patients with DM due to the promotion of a hypoglycemic state. Hence, its use is not recommended in patients with co-existing DM and hypertension unless absolutely indicated [104].
- Beta-blockers are combined with other classes of antihypertensive drugs for treating hypertension in specific situations like heart rate control, symptomatic angina, post-myocardial infarction, HFrEF, and as an alternative to RAAS in young hypertensive women with a pregnancy plan or of child-bearing potential [105].

3. Calcium channel blockers

- Several RCTs have confirmed that CCBs can reduce cardiovascular morbidity and mortality.
- CCBs are potent, first-line blood pressure-lowering drugs with minimal contraindications [106].
- A meta-analysis including 147 RCTs involving 464,164 participants confirmed a significant reduction in risk of coronary events (20–25%) and stroke (30–45%) with all the five BP lowering agents. CCBs had a more pronounced preventive effect on stroke [107].
- CCBs can be used alongside ARBs as a part of combination therapy for the management of hypertension in diabetic patients.
- The vasodilatory effect of CCBs on vascular smooth muscle cells is attributed to their inhibitory effect on calcium entry through L-type calcium channels. Recently, novel CCBs are preferred due to their added pleiotropic benefits above their antihypertensive action. Certain CCBs block the activity on N- and L-type calcium channels and hence show additional benefits of lowering cardiovascular events and renal injury [106].

The role of the novel CCB — cilnidipine in diabetic hypertension:

- Cilnidipine is a novel and unique dihydropyridine calcium channel blocker that has inhibitory actions on both L-type and N-type calcium channels. Intracellular Ca^{2+} overload is associated with atrial fibrillation. Cilnidipine restricts this overload and activates eNOS which regulates cardiac function [108].
- CCBs are used alongside ARBs as a part of combination therapy for the management of hypertension in diabetic patients.
- As per a prospective observational trial, single-pill combination treatment with ARB plus cilnidipine helps in reducing morning home systolic blood pressure (MHSBP), in elderly patients [109].
- Cilnidipine is preferred in hypertensive patients with DM because it improves insulin sensitivity through its vasodilator effects [110]. Besides its antihypertensive effect, cilnidipine has improved insulin sensitivity along with various reno-protective and cardioprotective benefits, thus making it the choice of DHP-CCB in hypertension with DM [111]. Refer to Fig. 6 for the mechanism of action of cilnidipine.
- Amlodipine and cilnidipine are equally efficacious in reducing blood pressure; however, the incidences of pedal edema are lower with cilnidipine associated than amlodipine [112].
- When administered at a dosage of 5–20 mg/day, based on the patient's clinical profile, cilnidipine facilitates BP reduction and helps reduce heart rate and serum triglyceride levels in Indian patients, suggestive of cardioprotective benefit [113].
- Independent of blood pressure reduction, 8-week treatment with cilnidipine 5–10 mg/day improved left-ventricular systolic function [114].
- Cilnidipine is more tolerable in Indian patients and must be preferred in those with proteinuria or pedal edema [115, 116].

Thus, cilnidipine should be the preferred CCB in diabetic hypertensives due to its reno-protective and cardioprotective benefits and better safety and tolerability profile (pedal edema) over other CCBs.

4. Diuretics

- Low-dose thiazides have demonstrated their success in mild to moderate cases of hypertension, but its function is depleted if the sodium intake of the patient is above 8 g/day, indicating the relevance of dietary salt restriction and its use [118].
- Low doses, i.e. 12.5 to 25 mg/day of chlorthalidone or hydrochlorothiazide, or 1.25 mg/day of indapamide, minimize metabolic complications and their antihypertensive effects [119].
- Hydrochlorothiazide lowers pulse pressure by 4 to 6 mmHg due to the greater effect on systolic than on diastolic blood pressure [120].
- Thiazide-like diuretics such as chlorthalidone demonstrated superior blood pressure reduction in patients with resistant hypertension [121, 122].
- Diuretics such as hydrochlorothiazide are not recommended in patients with diabetic hypertension

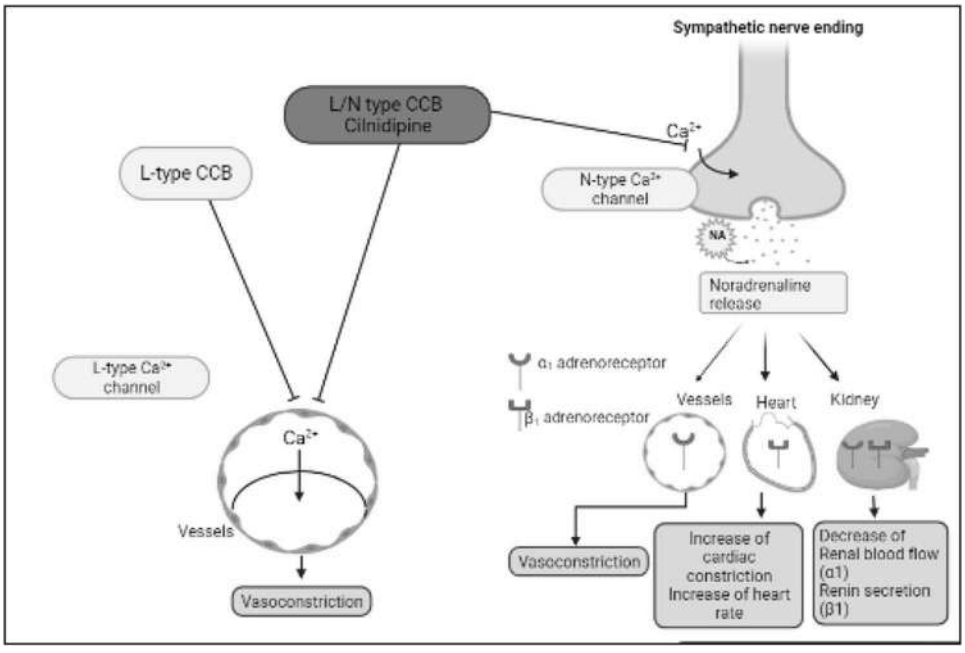

Fig. 6 Mechanism of action of cilnidipine in the lowering of blood pressure in hypertensive patients [117]

Table 10 Guideline comparison for hypertension treatment [129]

Guideline	Population	Goal BP mmHg	Initial management
2014 hypertension guideline	Diabetes	< 140/90	ACEi, ARB or CCB, thiazide-type diuretics
	CKD	< 140/90	ACEi or ARB
ESH/ESC 2013	Diabetes	< 140/85	ACEi or ARB
	CKD no proteinuria	< 140/90	
	CKD + proteinuria	< 130/90	
CHEP 2013	Diabetes	< 130/80	ACEi or ARB with additional CVD risk, ACEi, ARB, thiazide
	CKD	< 140/90	ACEi or ARB
ADA 2013	Diabetes	< 140/80	ACEi or ARB
ESC/ESH 2018	CAD, CKD, diabetes	≤ 140/90	ACEi or ARB + CCB or diuretic-dual combination for CAD) ACEi/ARB + CCB OR ACEi/ARB + diuretic (or loop diuretic-dual combination for CKD) ACEi/ARB + CCB/diuretic-dual combination for diabetes
ISH 2020	CKD	< 130/80 mmHg (< 140/80 in elderly patients)	RAS inhibitors as first line; CCBs and diuretics can be added
KDIGO 2012	CKD no proteinuria	≤ 140/90	ACEi or ARB
	CKD + proteinuria	≤ 130/80	
KDIGO 2021	RRT (CKD G1T-G5T)	≤ 130/80	CCB or ARB

because of its potential to elevate fasting blood glucose and HbA1c levels [123].

- A systematic review of 26 RCTs indicated that low doses of thiazide might avoid glycemic changes [124]. However, the strength of this finding is low since evidence against low-dose hydrochlorothiazide has been stated by another meta-analysis of 368 studies [125].
- While diuretics are not commonly used to manage hypertension in patients with DM, their use is indicated in certain specialized cases [126], such as elderly patients with existing CVDs [118].
- In a crossover trial, the use of potassium-sparing diuretics such as spironolactone reduced BP by − 8.7 mmHg for reaching the target levels in patients with resistant hypertension, compared to other two treatments, i.e. doxazosin and bisoprolol [127].
- A low dose of spironolactone is recommended in those whose serum potassium is < 4.5 mmol/L and

Table 11 Major drug combinations with efficacy results

Combination	Type of patients	% Change in relative risk
Two RAS blockers/ACE inhibitor 1 ARB or RAS blocker 1 renin inhibitor [131]	High-risk diabetic patients	More renal events
ACE inhibitor and diuretic	Stroke or TIA [132]	− 28% strokes ($p < 0.001$)
	Diabetes [133]	− 9% micro-/macrovascular events ($p = 0.04$)
	Hypertensive; > 80 years [134]	− 34% CV events ($p < 0.001$)
ARB and diuretic	Hypertensive; ≥ 70 years [135]	− 28% non-fatal strokes ($p = 0.04$)
CCB and diuretic	Hypertensive [136]	− 27% CV events ($p < 0.001$)
ACE inhibitor and CCB	Older with isolated systolic hypertension (ISH) [137]	− 37% CV events ($p < 0.004$)
Beta-blocker and diuretic	Older with ISH [138]	− 36% strokes ($p < 0.001$)
	Older hypertensive [139]	− 40% CV events ($p = 0.003$)
ARB and CCB	Older with ISH [140]	14% reduction in BP
	Hypertensive [141]	Lower incidences of CV events (risk ratio [RR], 0.80; 95% confidence interval [CI], 0.70–0.91; $p < .001$)

eGFR is > 45 mL/min/1.73 m^2 to achieve BP targets [127, 128].

Guideline comparison of blood pressure goals and recommended drug options in different populations has been mentioned in Table 10.

5. Combination therapies

- Drug combinations including ARB plus cilnidipine or ARB plus hydrochlorothiazide effectively reduce nocturnal BP fluctuations [125]. However, due to the risk of glycemic variability, ARB plus cilnidipine must be considered even in patients with nighttime BP fluctuations, rarer in patients with co-existing diabetes and hypertension [123–125].
- A combination of ARB and CCB must also be preferred over beta-blockers because of the potential of the latter to cause adverse cardiovascular events (Table 11) [105].

Safety considerations for hypertensive management in diabetic patients

- When treatment agents such as blockers of the renin–angiotensin–aldosterone system (RAAS) are being used to manage BP in patients with DM, close monitoring of kidney functions and the levels of electrolytes are recommended [38].
- Frequent monitoring of potassium and creatinine is recommended in patients treated with aldosterone antagonists, such as spironolactone. Monitoring of serum potassium levels in patients on combination therapy is stated to reduce the risk of hyperkalemia [38].
- An observational study in diabetes patients concluded that CCB, when added to ACEi/ARBs, is associated with reno-protective and cardioprotective outcomes compared to thiazide diuretics [130].

Recommendations: which treatment therapy to use and when

- Individual profile of the patient and their response to the treatment must be evaluated for the selection of the most suitable treatment agent for hypertensive management (grade A)
- ARBs, either alone or in combination with CCBs, can be used for BP control in diabetic patients (grade A)
- Combination therapy of ARB and CCB is recommended to be initiated in hypertensive patients for better BP control, reducing risks of complications, and better patient adherence (grade B)
- ARBs must be preferred over ACEi in diabetic patients with hypertension, telmisartan or azilsartan being selected as the first-line agent (grade B)
- In patients at the risk of CVDs, renal disorders, or cerebrovascular disorders, combination therapy must be preferred for the reduction of patient mortality (grade B)
- Calcium channel blockers must be preferred over beta-blockers and thiazides in combination therapy with ARBs. Cilnidipine is a comparatively more effective and safer novel molecule as compared to conventional CCBs for Indian diabetic hypertensive patients (grade A)
- The use of beta-blockers and thiazide diuretics must be avoided in patients with DM and hypertension because of their potential to cause cardiovascular events and hyperglycaemia, respectively (grade A)
- Monitoring of electrolyte levels, serum potassium, and creatinine levels, as well as regular evaluation of kidney function, is recommended for patients with diabetic hypertension based on the choice of treatment agents and their risk profile (grade B)

Data on the global approvals of molecules of each class For the selection of the treatment agent necessary on the individual clinical profile of the patient, it is essential to understand the treatment indications of various FDA-approved drug labels, which have been summarized in Tables 11 and 12.

Management of hypertension in patients with CKD

- In patients at the risk of CKD, it is recommended to maintain blood pressure values below 130/80 mmHg for renal and cardioprotection in patients under 60 years [52].
- National Institute for Health 2014 and Care Excellence guideline recommends a goal blood pressure of < 140/90 in a patient with CKD while < 130/80 mmHg in patients with an albumin creatinine ratio of ≥ 70 mg/mmol [146].
- 2012 KDIGO guideline recommendation for blood pressure goals in diabetic and non-diabetic patients with non-dialysis dependent CKD is mentioned in Fig. 7 [147].

  Combination therapy is mostly recommended for achieving these target BP levels in these patients [115]. It is useful for managing patients who are unresponsive to the use of a single drug agent [148].
- SPC with cilnidipine (10 mg) and ARB (80 mg) was seen to be effective in reducing BP values in patients with sympathetic hyperactivity [39]. Control of sympathetic activity is one of the treatment goals in hypertensive patients with DM and CKD to reduce cardiovascular risks. This SPC was also effective in reducing diurnal and nocturnal blood pressure fluctuations in patients with DM. Thus, SPCs are recommended [109, 125]. Cilnidipine has been clinically proven effective for morning hypertension and white-coat hypertension, closely associated with sympathetic overdrive.

Table 12 FDA- and DCGI-approved drug labels for hypertension management in diabetic patients

	Indications
FDA-approved drug	
Telmisartan	For hypertensive management in patients with CVD risk factors and diabetes patients with end-organ damage [142]
Azilsartan	In patients with DM and hypertension for BP control [93]
Olmesartan	Management of hypertension in diabetic patients with other comorbidities such as chronic kidney disease, cerebrovascular events, heart failure, and ischaemic heart disease [143]
Captopril	Management of hypertension in patients with impaired renal function, presence of diabetic nephropathy, myocardial infarction, and left ventricular dysfunction [144]
DGCI-approved drug [145]	
Cilnidipine tabs. 5 mg, 10 mg	For treatment of mild to moderate hypertension
Amlodipine besylate IP Eq. to amlodipine 10 mg + indapamide SR 1.5 mg tablet	For the treatment of mild to moderate hypertension
Combination drug containing - Cilnidipine:10 mg Olmesartan medoxomil IP (20 mg/40 mg) Chlorthalidone IP (12.5 mg)	For the treatment of essential hypertension
Combination drug containing - Losartan potassium IP (50 mg) Amlodipine besylate IP Eq. to amlodipine (5 mg) Hydrochlorothiazide IP (12 mg)	For the treatment of hypertension in patients who are not managed with the help of dual therapy
Combination drug containing - Olmesartan medoxomil (20 mg) Amlodipine besylate (5 mg)	For mild to moderate hypertension
Combination drug containing - Olmesartan medoxomil (20 mg) Ramipril (5 mg)	For treatment of essential hypertension

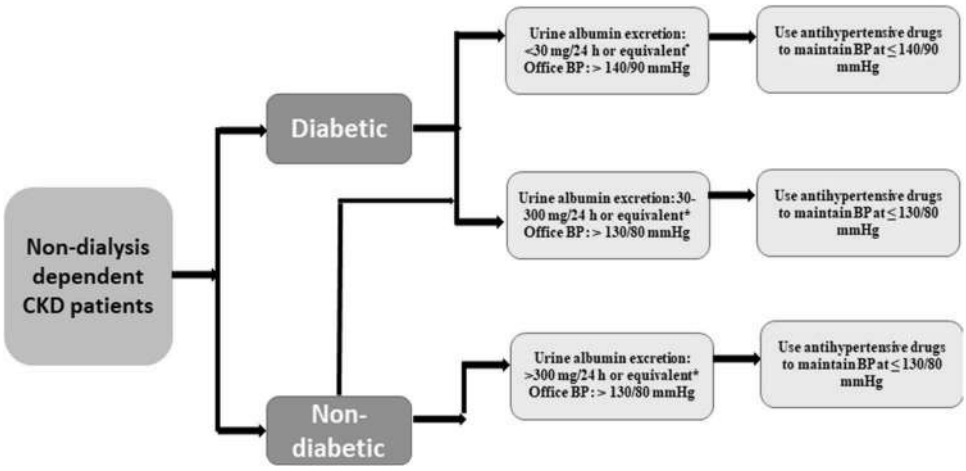

Fig. 7 2012 KDIGO guidelines on the management of hypertension in diabetic/non-diabetic CKD patients

- Due to its N-type calcium channel blockade and unique sympatholytic activity, cilnidipine offers cardiovascular benefits apart from its antihypertensive action. In a study involving hypertensive patients ($n = 2920$), treatment with cilnidipine and angiotensin receptor blocker showed significant reductions in heart rate, particularly in those with a higher baseline heart rate [149] (Tables 13 and 14).
- In patients with microalbuminuria/proteinuria, treatment with cilnidipine is recommended when CCBs are used in combination therapy [150, 151]. Compared to other drugs, such as amlodipine, cilnidipine facilitates UACR reduction and helps in decreasing albumin excretion in hypertensive patients [149, 150]. It is a preferred agent in patients with proteinuria, sympathetic overactivity, and pedal edema. It is a better treatment agent than amlodipine for hypertensive patients [109, 150].
- In a clinical trial of 50 patients with diabetic nephropathy, it was found that 12-week treatment with cilnidipine

Table 13 Indications of individual drug classes based on guidelines and clinical studies [6]

Compelling indication*	Recommended drugs^						Clinical trials basis#
	Diuretic	BB	ACEI	ARB	CCB	ALDO ANT	
Heart failure	■	■	■	■		■	ACC/AHA Heart failure Guidelines, MERIT-HF, COPERNICUS, CIBIS, SOLVD, AIRE, TRACE, ValHEFT, RALES
Postmyocardial infraction		■	■	■[2]	■[1]	■	ACC/AHA Post-MI Guideline, BHAT, SAVE, Capricorn, EPHESUS
High coronary disease risk	■	■	■		■		ALLHAT, HOPE, $ANBP_2$, LIFE, CONVINCE
Diabetes	■	■	■	■	■		NKF-ADA, Guideline, UKPDS, ALLHAT
Chronic kidney disease			■	■	■[3]		KDIGO 2021, NKF Guideline, Captopril Trial, RENAAL, IDNT, REIN, AASK
Recurrent stroke prevention	■		■		■[4]		PROGRESS

[1]Option for patients without heart failure or impaired LV function in patients with contraindications to beta-blockers (Danish Verapamil Infarction Trial II–DAVIT)[153]

[2]If ACEi not tolerated

[3]First-line antihypertensive agent in adult kidney transplant recipients

[4]A meta-analysis of 13 studies with 1789 subjects randomized to CCBs

*Compelling indications for antihypertensive drugs are based on benefits from outcome studies or existing clinical guidelines; the compelling indication is managed in parallel with the BP

^Drug abbreviations: *ACEI* angiotensin-converting enzyme inhibitor, *BB* beta-blockers, *ARB* angiotensin receptor blocker, *Aldo ANT* aldosterone antagonist, *CCB* calcium channel blocker

#Conditions for which clinical trials demonstrate the benefit of specific classes of antihypertensive drugs

led to a significant reduction in estimated GFR values and serum creatinine levels [125]. Six months of treatment with the drug helped significantly control albumin excretion [150, 151].

- Thiazide diuretics can be used if GFR is greater than or equal to 40 mL per minute per 1.73 m^2, while loop diuretics are used in GFR ≤ 40 to 50 mL per minute per 1.73 m^2 [115].
- A combination of ACEi and ARBs reduces urinary albumin excretion compared to monotherapy; however, they are associated with a further risk to the kidney and hence are not recommended [152].

Oral antidiabetic agents that exert reno-protection:

- Combining SGLT2 with ACEi + ARB inhibitor reduced composite kidney outcome (CKO) among T2DM patients with CKD [161].
- Evidence suggests that DPP-4 inhibitors and SGLT2 inhibitors exert reno-protective effects in patients with diabetes.

Management of resistant hypertension

- Early diagnosis of resistant hypertension with the help of ABPM is recommended to avoid end-organ damage in patients [32, 126].
- Initial treatment with ARBs along with CCBs is recommended [126]. In patients with resistant hypertension, additional treatment agents such as diuretics are recommended since patients may be unresponsive to standard combination therapies [5, 126]. Refer to Fig. 8 for treatment selection and management in patients with resistant hypertension.

Novel concepts in hypertension and future treatment molecules

- Growing significance of central aortic blood pressure:

In the pathogenesis of cardiovascular disease, central (aortic and carotid) pressures are gaining more relevance than peripheral pressures. The left ventricle encounters the aortic systolic pressure during systole (afterload), while the aortic pressure during diastole determines coronary perfusion. Ideally, central aortic pressures should be measured directly using invasive devices, but there are numerous methods available currently to derive the central pressures by analysing the applanated radial and carotid pulses or carotid distension waves. Higher augmentation index (AI) is linked with coronary artery disease (CAD).

Central pressure correlates with cardiovascular risk in apparently healthy subjects as well. Carotid systolic BP is an independent determinant of left-ventricular wall thickness, and late systolic augmentation of the central pressure

Table 14 Indian evidence on the use of antihypertensive agents [154–160]

Drug	Efficacy data	Safety data	Indications	Adverse events/contraindications	Corresponding Indian study
Azilsartan	• Effective for lowering BP levels by 26–29 mmHg as per a 6-week RCT of 303 patients • Non-inferior to telmisartan	• Non-inferior to telmisartan in terms of safety in Indian patients • Well-tolerated with only mild treatment-related side effects	First-line therapy in essential hypertension	Headache and dizziness	[154]
Telmisartan	• Telmisartan 40 mg is efficacious in reducing DBP by 18.1% compared to Losartan (14.3%) as reported in adult hypertensive male and non-pregnant female patients between 18 and 65 years of age	No serious adverse events were reported in this study	Patients with clinic blood pressure (BP) levels of systolic BP (SBP) of 140–200 mmHg and diastolic BP (DBP) of 95–114 mmHg	No reports	[155]
	• Telmisartan reduces proteinuria in hypertensive patients with chronic kidney disease	Well tolerated with no adverse events	Patients with CKD (96.36% hypertensive; 63.61% diabetic)	No reports	[156]
Olmesartan	Olmesartan 20/40 mg helped in reducing by 34/18 mmHg in 6 months as per the results of an open-label observational study of 8940 Indian patients	No serious adverse events were reported in this study	Patients with BP values above 140/99 mmHg	Few patients complained of dizziness, vertigo, and oedema	[157]
Cilnidipine	Comparable efficacy to amlodipine in lowering BP levels as per the results of an Indian cross-sectional study of 140 mild to moderate hypertensive patients	• Superior safety profile than amlodipine • No significant impacts on the heart rate	Patients with CVDs	No adverse events Previous studies have reported headache, dizziness, and GI symptoms as adverse events	[158]
	Cilnidipine helped in lowering the heart rate and uric acid levels of the patient in addition to their BP management over 24 weeks as per a clinical study of 100 Indian patients	Higher safety profile than amlodipine No reflex tachycardia	Patients with high uric acid levels and those with CVDs/tachycardia	Not reported	[159]
	Cilnidipine helps in reducing the heart rate along with lowering BP levels of patients, as depicted by an Indian RCT of 63 participants Amlodipine also lowered BP but significantly increased heart rate	Higher safety over amlodipine due to cardiovascular benefit through heart rate reduction	Patients with proteinuria, pedal oedema, and sympathetic overactivity	Nausea, decreased appetite, headache, insomnia, and palpitation	[160]

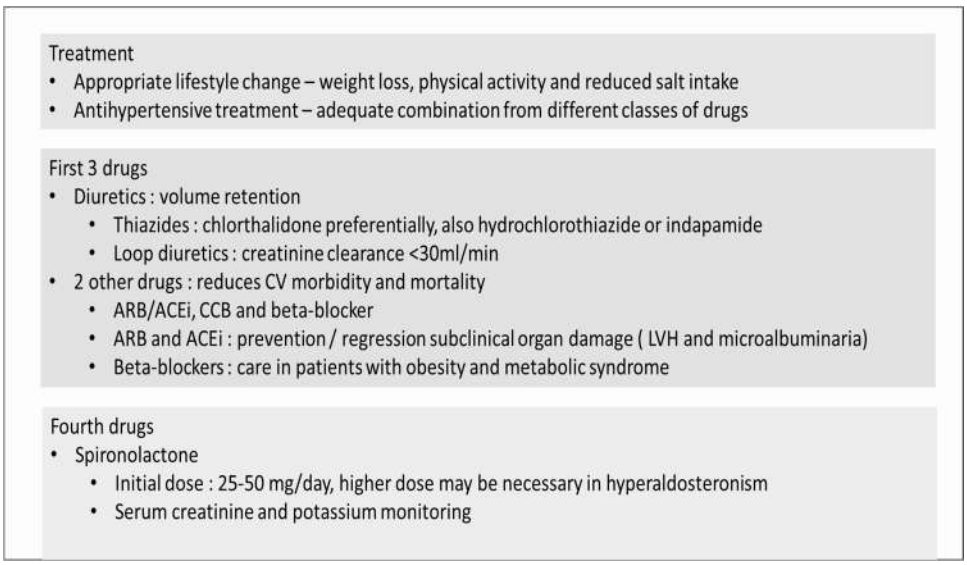

Fig. 8 Therapeutic approach in resistant hypertension [162]

waveform may denote an increase in left ventricular mass index, independent of age and BP.

Study suggests that non-invasively determined central pulse pressure is a better predictor of incident cardiovascular disease than does the corresponding brachial pulse pressure, which may be because of a more accurate representation of the vascular load on the left ventricle. There is growing evidence that central BP may provide incremental value over and above peripheral BP in firmly confirming the cardiovascular risk. The CAFE Study was the first randomized, prospective event-based study which mentioned that central BP and related indices may be a useful guide to treatment [163].

- Future treatment molecules:

Several novel drugs such as peptide- and non-peptide-based therapeutic agents that may function as RAAS inhibitors have been emerging to manage hypertension in diabetic patients. For personalized treatment of hypertension in patients with DM, the use of artificial intelligence technologies such as gene sequencing mechanisms, genomics, transcriptomics, proteomics, and metabolomics is increasing in clinical practice towards understanding the disease pathogenesis for early recognition of possible end-organ damage, detection of the treatment response of the patient, and their monitoring [164, 165].

Currently, among the existing CCBs, cilnidipine is a promising molecule, effective in BP reduction and its multiple pleiotropic benefits, and a good choice for use as a combination therapy for hypertension management in patients with DM [150, 151, 166–168]. As anticipated in the future, the use of other conventional CVD risk reduction drugs such as statins and immunosuppressants such as mycophenolate mofetil may also expand in clinical practice [169]. While new drugs shall continue to emerge, the use of CCBs, ARBs, and ACEi will persist in the future. At the same time, beta-blockers and thiazide agents may need more studies to understand their usage in this sub-set of patients [170].

Summary

Key messages: For the management of hypertension in patients with DM (Fig. 9), it is essential to understand the clinical profile of the patient to select the most suitable treatment agents that do not add to any risks.

1. Definition of hypertension. As per the Indian Guideline of Hypertension IV (IGH IV), hypertension is defined as systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg.
2. Type of hypertension. Primary hypertension is mostly asymptomatic, while secondary hypertension is due to various underlying pathologies. Uncontrolled BP, despite the usage of 3 antihypertensive drugs, is referred to as "resistant hypertension".
3. Epidemiology and risks. Over 1 billion people suffer from hypertension globally, which is expected to rise up to 1.5 billion by 2025. Up to 50% of cases of hypertension are also diagnosed with type 2 diabetes mellitus (T2DM). Hypertension presents as a major risk factor for heart failure, CVD, CKD, PAD, ED, and end-organ damage.
4. Blood pressure measurement. For patients with DM and hypertension, 24-h ambulatory blood pressure monitoring is recommended to maintain BP targets of ~ 120–130 mmHg.

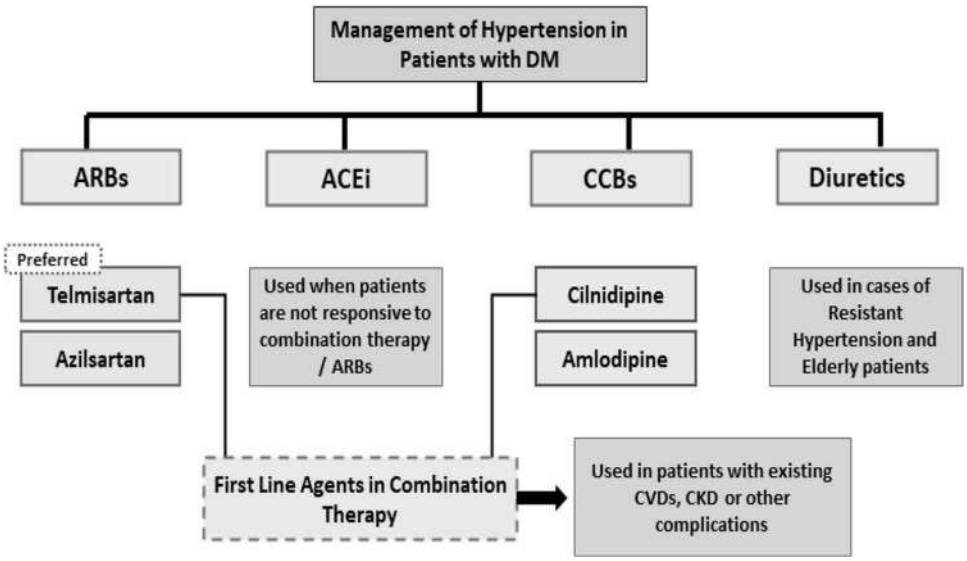

Fig. 9 Summary of guideline

The use of an aneroid sphygmomanometer must be preferred over digital devices; however, digital machines may be preferred for home-based measurement. Blood pressure thresholds vary with age and comorbid conditions.

5. Hypertension and diabetes: the relationship. There is a complex cause–effect relationship between hypertension and diabetes, which predisposes the patients to increased risks of cardiovascular complications.
6. Non-pharmacological management of hypertension in diabetic patients. Lifestyle modifications may delay the need for pharmacological interventions or can complement the BP lowering effect of drugs. A low sodium diet, a physical activity, and a healthy diet are recommended to manage hypertension in diabetic patients.
7. Pharmacological management of hypertension in diabetic patients. ARBs are recommended as the choice of therapy preferably in combination with CCBs to manage hypertension and its resulting complications. For combination therapy, newer CCBs (e.g. cilnidipine) along with ARBs are recommended. Cilnidipine is a novel, effective, and safe CCB, which is established for its reno-protective benefits. Combination therapy of ARBs with thiazide-like diuretics also reduces the risk of renal disorders. While telmisartan can be the first-line ARB for treatment of diabetic hypertensives due to its beneficial effects on fasting blood glucose and insulin levels, alternatively, azilsartan, with a similar safety and efficacy profile, is also recommended. Thiazide-like diuretics can be preferred in elderly patients with existing CVDs. ACE inhibitors may be used as an alternative to ARBs for CVD risk reduction in high-risk patients. Beta-blockers may be preferred in patients with a previous history of CVDs and as an alternative to RAS blockers in pregnant women. Hypertension is a strong, modifiable risk factor for the macrovascular and microvascular complications of diabetes. Strong evidence from clinical trials and meta-analyses supports targeting blood pressure reduction to at least140/90 mmHg in most adults with diabetes. Lower blood pressure targets may be beneficial for selected patients with high cardiovascular disease risk if they can be achieved without undue burden, and such lower targets may be considered on an individual basis. In addition to lifestyle modifications, multiple medication classes are often needed to attain blood pressure goals.

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References

1. Gupta R, Yusuf S. Towards better hypertension management in India. Indian J Med Res. 2014;139(5):657.
2. Geldsetzer P, Manne-Goehler J, Theilmann M, Davies JI, Awasthi A, Vollmer S, Jaacks LM, Bärnighausen T, Atun R. Diabetes and hypertension in India: a nationally representative study of 1.3 million adults. JAMA Int Med. 2018;178(3):363–72.
3. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABS/ACPM/AGS/APHA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation and management of high blood pressure in adults: executive summary. J Am Coll Cardiol. 2018;71:2199–269.
4. Bhattacharya S, Thakur JS, Singh A. Knowledge attitude, and practice regarding dietary salt intake among urban slum population of North India. J Fam Med Prim Care. 2018;7(3):526.
5. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cifkova R, Dominiczak AF, Grassi G, Jordan J, Poulter NR, Rodgers A, Whelton PK. Hypertension Nat Rev Dis Primers. 2018;4:18014.

6. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42(6):1206–52.
7. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. Lancet. 2017;389(10064):37–55.
8. Standard treatment guidelines of hypertension; MoHFW https://nhm.gov.in/images/pdf/guidelines/nrhm-guidelines/stg/Hypertension_full.pdf.
9. Choi HY, Park HC, Ha SK. Salt sensitivity and hypertension: a paradigm shift from kidney malfunction to vascular endothelial dysfunction. Electrolyte Blood Press. 2015;13(1):7–16.
10. Balafa O, Kalaitzidis RG. Salt sensitivity and hypertension. J Hum Hypertens. 2021;35(3):184–92.
11. Indian hypertension guidelines II 2007, Association of Physicians of India https://www.hsindia.org/images/indian_hypertension_guidelines.pdf.
12. Unger T, et al. 2020 International Society of Hypertension global hypertension practice guidelines. Hypertension. 2020;75:1334–57.
13. Rossi GP, Bisogni V, Rossitto G, Maiolino G, Cesari M, Zhu R, et al. Practice recommendations for diagnosis and treatment of the most common forms of secondary hypertension. High Blood Press Cardiovasc Prev. 2020;27(6):547–60.
14. Trinquart L, Mounier-Vehier C, Sapoval M, Gagnon N, Plouin PF. Efficacy of revascularization for renal artery stenosis caused by fibromuscular dysplasia: a systematic review and meta-analysis. Hypertension. 2010;56(3):525–32.
15. Tan JL, Thakur K. Systolic hypertension. StatPearls; 2020.
16. Midha T, Idris M, Saran R, Srivastava A, Singh S. Isolated systolic hypertension and its determinants - a cross-sectional study in the adult population of Lucknow District in North India. Indian J Community Med. 2010;35(1):89–93.
17. Gupta R, Sharma AK. Prevalence of hypertension and subtypes in an Indian rural population: clinical and electrocardiographic correlates. J Hum Hypertens. 1994;8(11):823–9.
18. Park JB, Kario K, Wang JG. Systolic hypertension: an increasing clinical challenge in Asia. Hypertens Res. 2015;38(4):227–36.
19. Luger RK, Kight BP. Hypertension in pregnancy. InStatPearls. StatPearls Publishing; 2020.
20. Ahmeda AF, Alzoghaibi M. Factors regulating the renal circulation in spontaneously hypertensive rats. Saudi J Biol Sci. 2016;23(4):441–51.
21. Gorostidi M, Vinyoles E, Banegas JR, de la Sierra A. Prevalence of white-coat and masked hypertension in national and international registries. Hypertens Res. 2015;38(1):1–7.
22. Aronow WS. Masked hypertension. Ann Transl Med. 2017;5(23):456–456.
23. Kaul U, Omboni S, Arambam P, Rao S, Kapoor S, Sawhney JPS, Sharma K, Nair T, Chopda M, Hiremath J, Ponde CK, Oomman A, Srinivas BC, Suvarna V, Jasuja S, Borges E, Verberk WJ. Blood pressure related to age: the India ABPM study. J Clin Hypertens (Greenwich). 2019;21(12):1784–94.
24. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020;16(4):223–37.
25. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. Circulation. 2016;134(6):441–50.
26. Gupta R, Gaur K, S Ram CV. Emerging trends in hypertension epidemiology in India. J Hum Hypertens. 2019;33(8):575–587.
27. Anchala R, Kannuri NK, Pant H, Khan H, Franco OH, Di Angelantonio E, et al. Hypertension in India: a systematic review and meta-analysis of prevalence, awareness, and control of hypertension. J Hypertens. 2014;32(6):1170–7.
28. Lastra G, Syed S, Kurukulasuriya LR, Manrique C, Sowers JR. Type 2 diabetes mellitus and hypertension: an update. Endocrinol Metab Clin. 2014;43(1):103–22.
29. Katon WJ, Rutter C, Simon G, Lin EH, Ludman E, Ciechanowski P, Kinder L, Young B, Von Korff. The association of co-morbid depression with mortality in patients with type 2 diabetes. M Diabetes Care. 2005;28(11):2668–72.
30. Viswanathan V, Smina TP. Blood pressure control in diabetes - the Indian Perspective. J Hum Hypertens. 2019;33(8):588–93.
31. Grossman E. Ambulatory blood pressure monitoring in the diagnosis and management of hypertension. Diabetes Care. 2013;36(Supplement 2):S307–11.
32. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, et al. 2018 ESC/ESH guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Cardiology and the European Society of Hypertension. J Hypertens. 2018;36:1953–2041.
33. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Hypertension. 2018;71:1269–324.
34. Stergiou GS, Palatini P, Asmar R, Bilo G, de la Sierra A, Head G, et al. Blood pressure monitoring: theory and practice. European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability Teaching Course Proceedings. Blood Press Monit. 2018;23(1):1–8.
35. Kario K, Shin J, Chen CH, Buranakitjaroen P, Chia YC, Divinagracia R, et al. Expert panel consensus recommendations for ambulatory blood pressure monitoring in Asia: the HOPE Asia Network. J Clin Hypertens (Greenwich). 2019;21(9):1250–83.
36. De Boer IH, Bangalore S, Benetos A, Davis AM, Michos ED, Muntner P, Rossing P, Zoungas S, Bakris G. Diabetes and hypertension: a position statement by the American Diabetes Association. Diabetes Care. 2017;40(9):1273–84.
37. Grossman E, Messerli FH. Management of blood pressure in patients with diabetes. Am J Hypertens. 2011;24(8):863–75.
38. Aronow WS, Shamliyan TA. Blood pressure targets for hypertension in patients with type 2 diabetes. Ann Transl Med. 2018;6(11):199.
39. Logan AG, Irvine MJ, McIsaac WJ, Tisler A, Rossos PG, Easty A, Feig DS, Cafazzo JA. Effect of home blood pressure telemonitoring with self-care support on uncontrolled systolic hypertension in diabetics. Hypertension. 2012;60(1):51–7.
40. India Hypertension Control Initiative training manual. 2019. https://linkscommunity.org/assets/PDFs/htn-draft-training-module_simple-version_05122019-for-print.pdf.
41. Shahbabu B, Dasgupta A, Sarkar K, Sahoo SK. Which is more accurate in measuring the blood pressure? A digital or an aneroid sphygmomanometer. J Clin Diagn Res. 2016 Mar;10(3):LC11–14.
42. Bruno RM, Taddei S. New-onset diabetes in hypertensive patients and mortality: timing is Everything. Eur Heart J. 2016;37(12):975–7.
43. Cheung BMY, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep. 2012;14(2):160–6.

44. Yki-Järvinen H. Nonglycemic effects of insulin. Clin Cornerstone. 2003;Suppl 4:S6–12.
45. Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Can J Cardiol. 2018;34(5):575–84.
46. Arauz-Pacheco C, Parrott MA, Raskin P; American Diabetes Association. Treatment of hypertension in adults with diabetes. Diabetes Care. 2003;26 Suppl 1:S80–2.
47. Oh JS, Lee CH, Park J il, Park HK, Hwang JK. Hypertension-mediated organ damage and long-term cardiovascular outcomes in Asian hypertensive patients without prior cardiovascular disease. J Korean Med Sci. 2020;35(48):e400.
48. Velasquez, M. T. Management of hypertension in chronic kidney disease. Chronic Renal Disease, 2015;634–645.
49. Cha R hui, Lee H, Lee JP, Kim YS, Kim SG. The influence of blood pressure patterns on renal outcomes in patients with chronic kidney disease. Medicine (Baltimore). 2020;99(8):e19209.
50. Judd E, Calhoun DA. Management of hypertension in CKD: beyond the guidelines. Adv Chronic Kidney Dis. 2015;22(2):116–22.
51. Christofides EA, Desai N. Optimal early diagnosis and monitoring of diabetic kidney disease in type 2 diabetes mellitus: addressing the barriers to albuminuria testing. J Prim Care Community Health. 2021;12:21501327211003684.
52. Sarafidis PA, Khosla N, Bakris GL. Antihypertensive therapy in the presence of proteinuria. Am J Kidney Dis. 2007;49(1):12–26.
53. Shah SN, Munjal YP, Kamath SA, Wander GS, Mehta N, Mukherjee S, Kirpalani A, Gupta P, Shah H, Rohatgi R, Billimoria AR. Indian Guidelines on Hypertension-IV (2019). J Hum Hypertens. 2020;34(11):745–58.
54. Chawla R, Madhu SV, Makkar BM, Ghosh S, Saboo B, Kalra S, et al. RSSDI-ESI clinical practice recommendations for the management of type 2 diabetes mellitus 2020. Indian J Endocrinol Metab. 2020;24(1):1–122.
55. Stefańska K, Zieliński M, Zamkowska D, Adamski P, Jassem-Bobowicz J, Piekarska K, et al. Comparisons of dipstick test, urine protein-to-creatine ratio, and total protein measurement for the diagnosis of preeclampsia. Int J Environ Res Public Health. 2020;17(12):E4195.
56. Steg G, Bhatt DL, Wilson PWF, D'Agostino R, Ohman EM, Rother J et al., for the REACH Registry Investigators. One year cardiovascular event rates in outpatients with atherothrombosis. JAMA 2007;297:1197–1206.
57. Bavry AA, Anderson RD, Gong Y, Denardo SJ, Cooper-Dehoff RM, Handberg EM, et al. Outcomes Among hypertensive patients with concomitant peripheral and coronary artery disease: findings from the INternational VErapamil-SR/Trandolapril STudy. Hypertension. 2010;55(1):48–53.
58. Nunes KP, Labazi H, Webb RC. New insights into hypertension-associated erectile dysfunction. Curr Opin Nephrol Hypertens. 2012;21(2):163–70.
59. Hale TM, Hannan JL, Carrier S, deBlois D, Adams MA. Targeting vascular structure for the treatment of sexual dysfunction. J Sex Med. 2009;6(Suppl 3):210–20.
60. Doumas M and Boutari C. Arterial hypertension and erectile dysfunction: an under-recognized duo. Eur soc of cardiology, 2016. 14(4).
61. Lloyd-Jones DM, Larson MG, Leip EP, Beiser A, D'Agostino RB, Kannel WB, et al. Lifetime risk for developing congestive heart failure: the Framingham Heart Study. Circulation. 2002;106(24):3068–72.
62. Kannel WB, Wolf PA, Benjamin EJ, Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based Estimates. Am J Cardiol. 1998;82:2N-9N.
63. McLenachan JM, Henderson E, Morris KI, Dargie HJ. Ventricular arrhythmias in patients with hypertensive left ventricular hypertrophy. N Engl J Med. 1987;317(13):787–92.
64. Levy D, Larson MG, Vasan RS, Kannel WB, Ho KK. The progression from hypertension to congestive heart failure. JAMA. 1996;275(20):1557–62.
65. Messerli FH, Rimoldi SF, Bangalore S. The transition from hypertension to heart failure: contemporary update. JACC Heart Fail. 2017;5(8):543–51.
66. Henderson AD, Bruce BB, Newman NJ, Biousse V. Hypertension-related eye abnormalities and the risk of stroke. Rev Neurol Dis. 2011;8(1–2):1–9.
67. Pontremoli R, Sofia A, Tirotta A, Ravera M, Nicolella C, Viazzi F, Bezante GP, Borgia L, Bobola N, Ravazzolo R, Sacchi G, Deferrari G. The deletion polymorphism of the angiotensin I-converting enzyme gene is associated with target organ damage in essential hypertension. J Am Soc Nephrol. 1996;7(12):2550–8.
68. Uckaya G, Ozata M, Sonmez A, Kinalp C, Eyileten T, Bingol N, Koc B, Kocabalkan F, Ozdemir IC. Is leptin associated with hypertensive retinopathy? J Clin Endocrinol Metab. 2000;85(2):683–7.
69. Chen X, Meng Y, Li J, She H, Zhao L, Zhang J, et al. Serum uric acid concentration is associated with hypertensive retinopathy in hypertensive chinese adults. BMC Ophthalmol. 2017;17(1):83.
70. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. Lancet. 2001;358(9288):1134–40.
71. Zhang WW, Cadilhac DA, Donnan GA, O'Callaghan C, Dewey HM. Hypertension and TIA. Int J Stroke. 2009;4(3):206–14.
72. Johnston SC, Gress DR, Browner WS, Sidney S. Short-term prognosis after emergency department diagnosis of TIA. JAMA. 2000;284(22):2901–6.
73. Johnston SC, Rothwell PM, Nguyen-Huynh MN, Giles MF, Elkins JS, Bernstein AL, et al. Validation and refinement of scores to predict very early stroke risk after transient ischaemic attack. Lancet. 2007;369(9558):283–92.
74. Qureshi AI. Acute hypertensive response in patients with stroke pathophysiology and management. Circulation. 2008;118:176–87.
75. Graham M L Eglit, Alexandra J Weigand, Daniel A Nation, Mark W Bondi, Katherine J Bangen. Hypertension and Alzheimer's disease: indirect effects through circle of Willis atherosclerosis. Brain Commun. 2020;2(2):fcaa114,
76. Gabin JM, Tambs K, Saltvedt I, Sund E, Holmen J. Association between blood pressure and Alzheimer disease measured up to 27 years prior to diagnosis: the HUNT Study. Alzheimers Res Ther. 2017;9(1):37.
77. Hypertension in adults: diagnosis and management. NICE guideline [NG136]. August 2019. https://www.nice.org.uk/guidance/ng136.
78. Singh S, Shankar R, Singh GP. Prevalence and associated risk factors of hypertension: a cross-sectional study in Urban Varanasi. Int J Hypertens. 2017;2017:5491838.
79. Silverberg DS, Oksenberg A. Are sleep-related breathing disorders important contributing factors to the production of essential hypertension? Curr Hypertens Rep. 2001;3(3):209–15.
80. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31(7):1281–357.
81. Thuita AW, Kiage BN, Onyango AN, Makokha AO. Effect of a nutrition education programme on the metabolic syndrome in type 2 diabetes mellitus patients at a level 5 hospital in Kenya: "a randomized controlled trial." BMC nutrition. 2020;6(1):1–4.

82. Correia JC, Lachat S, Lagger G, Chappuis F, Golay A, Beran D. Interventions targeting hypertension and diabetes mellitus at community and primary healthcare level in low-and middle-income countries: a scoping review. BMC Public Health. 2019;19(1):1–20.
83. Paula TP, Viana LV, Neto AT, Leitao CB, Gross JL, Azevedo MJ. Effects of the DASH diet and walking on blood pressure in patients with type 2 diabetes and uncontrolled hypertension: a randomized controlled trial. J Clin Hypertens. 2015;17(11):895–901.
84. Campbell AP. DASH eating plan: an eating pattern for diabetes management. Diabetes Spectrum. 2017;30(2):76–81.
85. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, Michos ED. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019;74(10):e177-232.
86. Subramanian H, Soudarssanane MB, Jayalakshmy R, Thiruselvakumar D, Navasakthi D, Sahai A, Saptharishi LG. Non-pharmacological interventions in hypertension: a community-based cross-over randomized controlled trial. Indian J Community Med. 2011;36(3):191.
87. Shrivastava U, Fatma M, Mohan S, Singh P, Misra A. Randomized control trial for reduction of body weight, body fat patterning, and cardiometabolic risk factors in overweight worksite employees in Delhi. India J Diabetes Res. 2017;29:2017.
88. Zhang Y, Ding X, Hua B, Liu Q, Chen H, Zhao XQ, Li W, Li H. Real-world use of ACEI/ARB in diabetic hypertensive patients before the initial diagnosis of obstructive coronary artery disease: patient characteristics and long-term follow-up outcome. J Transl Med. 2020;18(1):150.
89. Mallat SG. What is a preferred angiotensin II receptor blocker-based combination therapy for blood pressure control in hypertensive patients with diabetic and non-diabetic renal impairment? Cardiovasc Diabetol. 2012;10(11):32.
90. Ganesh J, Viswanathan V. Management of diabetic hypertensives. Indian J Endocrinol Metab. 2011;15(Suppl4):S374.
91. Dézsi CA. The different therapeutic choices with ARBs. Which one to give? When? Why?. American Journal of Cardiovascular Drugs. 2016;16(4):255–66
92. Kumar A, Khrime D. Efficacy and safety of azilsartan and telmisartan in hypertensive patients. Saudi J Med Med Sci. 2021;9(1):82.
93. Aalbers J. Angiotensin receptor blockers (ARBs) in hypertension patients: earlier use of these better tolerated medications is warranted. Cardiovasc J Afr. 2010;21(2):124.
94. Gitt AK, Bramlage P, Potthoff SA, Baumgart P, Mahfoud F, Buhck H, et al. Azilsartan compared to ACE inhibitors in antihypertensive therapy: one-year outcomes of the observational EARLY registry. BMC Cardiovasc Disord. 2016;8(16):56.
95. Whalen K, Stewart RD. Pharmacologic management of hypertension in patients with diabetes. Am Fam Physician. 2008;78(11):1277–82.
96. Fried LF, Emanuele N, Zhang JH, Brophy M, Conner TA, Duckworth W, et al. Combined angiotensin inhibition for the treatment of diabetic nephropathy. N Engl J Med. 2013;369(20):1892–903.
97. Thomopoulos C, Parati G, Zanchetti A. Effects of blood-pressure-lowering treatment on outcome incidence. 12. Effects in individuals with high-normal and normal blood pressure: overview and meta-analyses of randomized trials. J Hypertens. 2017;35(11):2150–2160.
98. Suehiro T, Tsuruya K, Yoshida H, Tsujikawa H, Yamada S, Tanaka S, Tsuchimoto A, Eriguchi M, Fujisaki K, Torisu K, Nakano T. Stronger effect of azilsartan on reduction of proteinuria compared to candesartan in patients with CKD: a randomized crossover trial. Kidney Blood Press Res. 2021;46(2):173–84.
99. Wang JG, Zhang M, Feng YQ, Ma CS, Wang TD, Zhu ZM, Kario K. Is the newest angiotensin-receptor blocker azilsartan medoxomil more efficacious in lowering blood pressure than the older ones? A systematic review and network meta-analysis. J Clin Hypertens. 2021;23(5):901–14.
100. Sakamoto M, Asakura M, Nakano A, Kanzaki H, Sugano Y, Amaki M, et al. Azilsartan, but not candesartan improves left ventricular diastolic function in patients with hypertension and heart failure. Int J Gerontol. 2015;1:9.
101. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, Xie J, Liu YM, Zhao YC, Huang X, Lin L, Xia M. Association of inpatient use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. Circ Res. 2020;126(12):1671–81.
102. Felice C, Nardin C, Di Tanna GL, Grossi U, Bernardi E, Scaldaferri L, et al. Use of RAAS inhibitors and risk of clinical deterioration in COVID-19: results from an Italian cohort of 133 hypertensives. Am J Hypertens. 2020;33(10):944–8.
103. Tian B, Deng Y, Cai Y, Han M, Xu G. Efficacy and safety of combination therapy with sodium-glucose cotransporter 2 inhibitors and renin-angiotensin system blockers in patients with type 2 diabetes: a systematic review and meta-analysis. Nephrol Dial Transplant. 2022;37(4):720–9.
104. Tsujimoto T, Sugiyama T, Shapiro MF, Noda M, Kajio H. Risk of cardiovascular events in patients with diabetes mellitus on β-blockers. Hypertension. 2017;70(1):103–10.
105. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V, Desormais I; ESC Scientific Document Group. 2018 ESC/ESH guidelines for the management of arterial hypertension. Eur Heart J. 2018;39(33):3021–3104.
106. Ozawa Y, Hayashi K, Kobori H. New generation calcium channel blockers in hypertensive treatment. Curr Hypertens Rev. 2006;2(2):103–11.
107. Law MR, Morris JK, Wald NJ. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. BMJ. 2009;19(338):b1665.
108. Voigt N, Heijman J, Wang Q, Chiang DY, Li N, Karck M, et al. Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. Circulation. 2014;129(2):145–56.
109. Kario K, Matsuda S, Nagahama S, Kurose Y, Sugii H, Teshima T, Suzuki N. Single-pill combination of cilnidipine, an l-/n-type calcium channel blocker, and valsartan reduces the day-by-day variability of morning home systolic blood pressure in patients with treated hypertension: a sub-analysis of the HOPE-combi survey. J Clin Hypertens. 2021;23(2):392–7.
110. Yagi S, Goto S, Yamamoto T, Kurihara S, Katayama S. Effect of cilnidipine on insulin sensitivity in patients with essential hypertension. Hypertens Res. 2003;26(5):383–7.
111. Shete MM. Cilnidipine: next generation calcium channel blocker. J Assoc Physicians India. 2016;64(4):95–9.
112. Adake P, Somashekar HS, Mohammed Rafeeq PK, Umar D, Basheer B, Baroudi K. Comparison of amlodipine with cilnidipine on antihypertensive efficacy and incidence of pedal edema in mild to moderate hypertensive individuals: a prospective study. J Adv Pharm Technol Res. 2015;6(2):81–5.
113. Kumar P, Das A, Chandra S, Gari M, Keshri USP, Kumari K. Serum triglyceride lowering effect of cilnidipine in patients with essential hypertension. Cardiol Res. 2016;7(5):173–7.

114. Jang Y, Kim M, Jang Y, Cho EY, Lee S, Yu J, et al. PS 17–54 effects of cilnidipine versus atenolol on left ventricular diastolic function and hypertrophy in essential hypertension - CANDLE Trial. J Hypertens. 2016;34:e489.
115. Pugh D, Gallacher PJ, Dhaun N. Management of hypertension in chronic kidney disease. Drugs. 2019;79(4):365–79.
116. Currie G, Delles C. The future of "omics" in hypertension. Can J Cardiol. 2017;33(5):601–10.
117. Sharma S. Hypertension therapeutics update: a brief clinical summary on azilsartan, cilnidipine and nebivolol. Hypertens J. 2015;1(2):111–7.
118. Shah SU, Anjum S, Littler WA. Use of diuretics in cardiovascular disease: (2) hypertension. Postgrad Med J. 2004;80(943):271–6.
119. Psaty BM, Lumley T, Furberg CD, Schellenbaum G, Pahor M, Alderman MH, et al. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. JAMA. 2003;289(19):2534–44.
120. Musini VM, Nazer M, Bassett K, Wright JM. Blood pressure-lowering efficacy of monotherapy with thiazide diuretics for primary hypertension. Cochrane Database Syst Rev. 2014;(5):CD003824.
121. Khosla N, Chua DY, Elliott WJ, Bakris GL. Are chlorthalidone and hydrochlorothiazide equivalent blood-pressure-lowering medications? J Clin Hypertens (Greenwich). 2005;7(6):354–6.
122. Kumar N, Calhoun DA, Dudenbostel T. Management of patients with resistant hypertension: current treatment options. Integr Blood Press Control. 2013;22(6):139–51.
123. Lin JJ, Chang HC, Ku CT, Chen HY. Hydrochlorothiazide hypertension treatment induced metabolic effects in type 2 diabetes: a meta-analysis of parallel-design RCTs. Eur Rev Med Pharmacol Sci. 2016;20(13):2926–46.
124. Zhang X, Zhao Q. Association of thiazide-type diuretics with glycemic changes in hypertensive patients: a systematic review and meta-analysis of randomized controlled clinical trials. J Clin Hypertens. 2016;18(4):342–51.
125. Fujiwara T, Hoshide S, Tomitani N, Kanegae H, Kario K. Comparative effects of valsartan plus cilnidipine or hydrochlorothiazide on nocturnal home blood pressure. J Clin Hypertens. 2021;23(3):687–91.
126. Yaxley JP, Thambar SV. Resistant hypertension: an approach to management in primary care. J Family Med Prim Care. 2015;4(2):193–9.
127. Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, Ford I, Cruickshank JK, Caulfield MJ, Salsbury J, Mackenzie I, Padmanabhan S, Brown MJ, British Hypertension Society's PATHWAY Studies Group. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. Lancet 2015;386:2059–2068.
128. Williams B, MacDonald TM, Morant SV, Webb DJ, Sever P, McInnes GT, et al. Endocrine and haemodynamic changes in resistant hypertension, and blood pressure responses to spironolactone or amiloride: the PATHWAY-2 mechanisms sub-studies. Lancet Diabetes Endocrinol. 2018;6(6):464–75.
129. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC Jr, Svetkey LP, Taler SJ, Townsend RR, Wright JT Jr, Narva AS, Ortiz E. 2014 Evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014;311(5):507–20. Erratum in: JAMA. 2014 May 7;311(17):1809.
130. Schroeder EB, Chonchol M, Shetterly SM, Powers JD, Adams JL, Schmittdiel JA, Nichols GA, O'Connor PJ, Steiner JF. Add-on antihypertensive medications to angiotensin-aldosterone system blockers in diabetes: a comparative effectiveness study. Clin J Am Soc Nephrol. 2018;13(5):727–34.
131. Parving HH, Brenner BM, McMurray JJV, de Zeeuw D, Haffner SM, Solomon SD, et al. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. N Engl J Med. 2012;367(23):2204–13.
132. Williams B, Lindholm LH, Sever P. Systolic pressure is all that matters. Lancet. 2008;371(9631):2219–21.
133. Patel A, ADVANCE Collaborative Group, MacMahon S, Chalmers J, Neal B, Woodward M, et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. Lancet. 2007;370(9590):829–40.
134. Beckett NS, Peters R, Fletcher AE, Staessen JA, Liu L, Dumitrascu D, et al. Treatment of hypertension in patients 80 years of age or older. N Engl J Med. 2008;358(18):1887–98.
135. Lithell H, Hansson L, Skoog I, Elmfeldt D, Hofman A, Olofsson B, et al. The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial. J Hypertens. 2003;21(5):875–86.
136. Liu L, Zhang Y, Liu G, Li W, Zhang X, Zanchetti A, et al. The Felodipine Event Reduction (FEVER) Study: a randomized long-term placebo-controlled trial in Chinese hypertensive patients. J Hypertens. 2005;23(12):2157–72.
137. Wang JG, Staessen JA, Gong L, Liu L. Chinese trial on isolated systolic hypertension in the elderly. Systolic Hypertension in China (Syst-China) Collaborative Group. Arch Intern Med. 2000;160:211–220.
138. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. JAMA. 1991;265(24):3255–64.
139. Dahlöf B, Lindholm LH, Hansson L, Scherstén B, Ekbom T, Wester PO. Morbidity and mortality in the Swedish Trial in Old Patients with Hypertension (STOP-Hypertension). Lancet. 1991;338(8778):1281–5.
140. Morgan T, Anderson A. A comparison of candesartan, felodipine, and their combination in the treatment of elderly patients with systolic hypertension. Am J Hypertens. 2002;15(6):544–9.
141. Chi C, Tai C, Bai B, Yu S, Karamanou M, Wang J, et al. Angiotensin system blockade combined with calcium channel blockers is superior to other combinations in cardiovascular protection with similar blood pressure reduction: a meta-analysis in 20,451 hypertensive patients. J Clin Hypertens (Greenwich). 2016;18(8):801–8.
142. Galle J, Schwedhelm E, Pinnetti S, Böger RH, Wanner C; VIVALDI investigators. Antiproteinuric effects of angiotensin receptor blockers: telmisartan versus valsartan in hypertensive patients with type 2 diabetes mellitus and overt nephropathy. Nephrol Dial Transplant. 2008;23(10):3174–83.
143. Ono T, Sanai T, Miyahara Y, Noda R. Olmesartan is more effective than other angiotensin receptor antagonists in reducing proteinuria in patients with chronic kidney disease other than diabetic nephropathy. Curr Ther Res Clin Exp. 2013;74:62–7.
144. Marte F, Sankar P, Cassagnol M. Captopril. [Updated 2021 May 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535386/.
145. https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadApprovalMarketingFDC/approvfdcfeb19.pdf.
146. Abraham G, Arun KN, Gopalakrishnan N, Renuka S, Pahari DK, Deshpande P, Isaacs R, Chafekar DS, Kher V, Almeida AF, Sakhuja V, Sundar S, Gulati S, Abraham A, Padmanaban R. Management of hypertension in chronic kidney disease: consensus statement by an expert panel of Indian nephrologists. J Assoc Physicians India. 2017;65(2 Suppl):6–22.
147. Kidney Disease: Improving Global Outcomes. KDIGO clinical practice guideline for the management of blood pressure in chronic kidney disease. Kidney Int Supplements (2012) 2:337.

148. Sharma T, Tausif A, Ahmad S. Evaluating the antiproteinuric efficacy of cilnidipine in diabetic kidney disease. Int J Basic Clin Pharmacol. 2019;24(9):129.
149. Chandra KS, Ramesh G. The fourth-generation calcium channel blocker: cilnidipine. Indian Heart J. 2013;65(6):691–5.
150. Kawabata Y, Soeki T, Ito H, Matsuura T, Kusunose K, Ise T, Yamaguchi K, Tobiume T, Yagi S, Fukuda D, Yamada H. Effects of L-/N-type calcium channel blockers on angiotensin ii–renin feedback in hypertensive patients. Int J Hypertens. 2020;22:2020.
151. Dharpur P, Kavitha VC. A study at BRIMS hospital for comparing the tolerability of cilnidipine and amlodipine in the treatment of hypertension. Indian J Pharm Pharmacol. 2019;6(1):11–3.
152. Nakao N, Yoshimura A, Morita H, Takada M, Kayano T, Ideura T. Combination treatment of angiotensin-II receptor blocker and angiotensin-converting-enzyme inhibitor in non-diabetic renal disease (COOPERATE): a randomised controlled trial. Lancet. 2003;361(9352):117–24.
153. Effect of verapamil on mortality and major events after acute myocardial infarction (the Danish Verapamil Infarction Trial II--DAVIT II). Am J Cardiol. 1990;66(10):779–85.
154. Sinha S, Chary S, Reddy Bandi M, Thakur P, Talluri L, Reddy VK, Agarwal M, Naik K S, Azilsartan Study In Indian Adult Patients Of Essential Hypertension Investigators Azilehi Group. Evaluation of the efficacy and safety of azilsartan in adult patients with essential hypertension: a randomized, phase-iii clinical study in India. J Assoc Physicians India. 2021;69(2):35–39.
155. Desai A, Samra S, Gawde A, Baliga V, Jain M. Comparison of the efficacy, safety, and tolerability of Telmisartan with Losartan in Indian patients with mild to moderate hypertension-a pilot study. Am J Hypertens. 2005;18(5):A86–7.
156. Agrawal A, Kamila S, Reddy S, Lilly J, Mariyala MS. Effect of telmisartan on kidney function in patients with chronic kidney disease: an observational study. J Drug Assess. 2016;5(1):24–8.
157. Kumbla DK, Kumar S, Reddy YV, Trailokya A, Naik M. WIN OVER study: Efficacy and safety of olmesartan in Indian hypertensive patients: results of an open label, non-comparative, multi-centric, post marketing observational study. Indian Heart J. 2014;66(3):340–4.
158. Shetty K, et al. A comparative study of clinical and biochemical parameters in amlodipine and cilnidipine treated hypertensive patients. J Clin Diagn Res. 2017; 11(5):FC01-FC05.
159. Das A, Kumar P, Kumari A, Chandra S, Gari M, Singh N, et al. Effects of cilnidipine on heart rate and uric acid metabolism in patients with essential hypertension. Cardiol Res. 2016;7(5):167–72.
160. Harlalka S, Roy UK, Majumdar G, Das K, Mandal P. An open label prospective study on evaluation of safety and efficacy of cilnidipine over amlodipine in stage 1 hypertensive patients. Kathmandu Univ Med J (KUMJ). 2020;18(69):42–48.
161. Zhao LM, Zhang M, Zhan ZL, Qiu M. Does combination therapy with SGLT2 inhibitors and renin-angiotensin system blockers lead to greater reduction in cardiorenal events among patients with type 2 diabetes? Front Cardiovasc Med. 2021;8:679124. Published 2021 May 5.
162. Muxfeldt ES, de Souza F, Salles GF. Resistant hypertension: a practical clinical approach. J Hum Hypertens. 2013;27(11):657–62.
163. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, Wang J-G, Wilkinson IB. Bryan Williams and Charalambos Vlachopoulos; Central blood pressure measurements and antihypertensive therapy - a consensus document. Hypertension. 2007;50:154–60.
164. Ghatage T, Goyal SG, Dhar A, Bhat A. Novel therapeutics for the treatment of hypertension and its associated complications: peptide- and nonpeptide-based strategies. Hypertens Res. 2021;44(7):740–55.
165. Mueller FB. AI (artificial intelligence) and hypertension research. Curr Hypertens Rep. 2020;22(9):1–7.
166. Shiraki A, Nakashima K, Node K. Does cilnidipine, a dual L-and N-type Ca 2+ blocker, shows promise in drug repositioning approaches? Hypertens Res. 2020;43(7):726–8.
167. Harlalka S, Roy UK, Majumdar G, Das K, Mandal P. An open label prospective study on evaluation of safety and efficacy of cilnidipine over amlodipine in stage 1 hypertensive patients. Kathmandu Univ Med J. 2020;18(1):42–8.
168. R R, Om S, Kaladharan A. Evaluation of renoprotective effect of cilnidipine in patients with mild to moderate hypertension and type 2 diabetes mellitus – a prospective study. Asian J Pharm Clin Res. 2021;144–6.
169. Tanase DM, Gosav EM, Radu S, Ouatu A, Rezus C, Ciocoiu M, Costea CF, Floria M. Arterial hypertension and interleukins: potential therapeutic target or future diagnostic marker. Int J Hypertens. 2019;2:2019.
170. Phillips RA. Current and future treatment of hypertension in the SPRINT Era. Methodist Debakey Cardiovasc J. 2015;11(4):206–13.

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REVIEW ARTICLE

The prevalence of gestational diabetes mellitus in Bangladesh: a systematic review and meta-analysis

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Abstract

Background Currently, gestational diabetes mellitus (GDM) is a major public health concern with a higher risk of adverse pregnancy outcomes for both the mother and the fetus. The present study was aimed to estimate the pooled prevalence of GDM in Bangladesh in order to assist public health policymakers.

Methods We systematically searched different electronic databases like PubMed, Scopus, Embase, Google Scholar, and the national journal databases for studies on the prevalence of GDM in Bangladesh published up to May 2021. We used a random-effects model to estimate the pooled prevalence of GDM and the odds ratio (OR) with the corresponding 95% confidence interval (CI).

Results This meta-analysis included eight studies with a total of 6948 pregnant women participants. Six studies were conducted in an urban setting, one in a rural setting, and one in both settings. The pooled estimated prevalence of GDM in Bangladesh was 13% (95% CI: 7.0–21). The results of subgroup analysis revealed that the prevalence was significantly higher in older pregnant women than in the younger pregnant women (OR: 2.37, 95% CI: 1.22–4.60, p=0.01), and women who were overweight or obese had a significantly higher prevalence than women with normal body weight (OR: 1.88, 95% CI: 1.18–2.99, p=0.007).

Conclusion The prevalence of GDM in Bangladesh was higher than the global prevalence of GDM as well as the prevalence of GDM in South Asian countries.

Keywords Bangladesh · Gestational diabetes mellitus · Meta-analysis · Prevalence

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy and occurs when the pancreatic functions of a woman are insufficient to overcome the diabetogenic environment of pregnancy [1, 2]. GDM is one of the foremost causes of mortality and morbidity for both the mother and the fetus across the world, and it is increasing at an alarming rate [3]. Pregnant women with GDM are at risk of developing gestational hypertension, cesarean section, premature rupture of fetal membranes, infection, and premature birth [4, 5]. Furthermore, mothers with a history of GDM are more likely to develop type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs) later in life [6]. The newborn babies of GDM mothers are at risk of having macrosomia, suffering from congenital anomalies, and developing neonatal hypoglycemia, and T2DM later in life [7]. As a result, the rising trend in the prevalence of GDM in recent years has become a worldwide public health burden.

In a report by the International Diabetes Federation in 2017, about 21.3 million (16.2%) of live births had hyperglycemia and the majority of these (86.4%) were due to GDM [8]. The estimated worldwide prevalence of GDM is 10.13% [9] and varies from 1 to 28% in different areas of the world based on the characteristics of the population studied and the different diagnostic criteria of GDM [10]. The prevalence of GDM in Europe is 5.4% [11] and 14.0% in high-risk women of Africa [12], whereas the prevalence of GDM in Asia varies from 0.7 to 51.0% [13–15], and the vast difference in prevalence rates could be due to differences in ethnicity [15], diagnostic criteria [16], screening approaches [17], and population characteristics such as maternal age, socioeconomic status, and body composition [18].

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Bangladesh, a densely populated developing country in South Asia, has a high birth rate, as well as a high infant mortality rate and a high low birthweight rate [19]. This makes it important to determine the prevalence of GDM in Bangladeshi women. Like other Asian countries, the prevalence of GDM has been increasing in Bangladesh. Some population-based studies conducted in Bangladesh revealed an increasing trend of GDM prevalence ranging between 6.8 and 12.9% [19, 20]. According to recent studies conducted at a tertiary level hospital in Bangladesh, the prevalence of GDM in Bangladesh is 36.6% and 35.5% based on WHO 1990 and WHO 2013 diagnostic criteria, respectively [21, 22]. The history of abortion, neonatal death, and stillbirth were found to be higher among GDM mothers than among non-GDM mothers [23]. There are individual studies in this area, but so far, no comprehensive analysis has been conducted to report the pooled prevalence of GDM in Bangladesh. Therefore, the present study was carried out to explore the current prevalence trend of GDM in Bangladesh, which may help health policymakers in implementing appropriate measures for better screening and control of GDM.

Methods

Literature search strategy

This systematic review study was conducted on the prevalence of GDM in Bangladeshi pregnant women according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [24] (Supplementary file 1). Different electronic databases like PubMed, Scopus, Embase, Google Scholar, and the national journal databases of Bangladesh Journals Online were comprehensively searched to find relevant studies on Bangladeshi women up to May 2021. The keywords like 'prevalence', 'epidemiology', 'gestational diabetes mellitus', 'gestational diabetes', 'pregnancy-induced diabetes', 'diabetes in pregnancy', 'hyperglycemia in pregnancy', 'diabetic pregnancy', and 'Bangladesh' were used to get the relevant studies. Moreover, the native journals that could not be accessed online were searched manually, and unpublished studies were retrieved from the library of Noakhali Science and Technology University, Bangladesh. The complete lists of diagnostic criteria for GDM are listed in Table 1.

Inclusion and exclusion criteria

The listed inclusion criteria were as follows: (1) studies with pregnant women living in Bangladesh; (2) studies reporting the prevalence of GDM; (3) studies conducted on pregnant women regardless of gestational age, sample size, and study setting; (4) standard diagnostic guidelines were followed for the diagnosis of GDM; (5) study must be published in a peer-reviewed journal in English; and (6) reported studies were available in full text (not editorial, commentary, or abstract for conferences). A reported study published in another language other than English and containing only qualitative data was excluded from this systematic analysis.

Data extraction

Based on our study protocol, the extraction of data was done systematically and independently by two investigators (RB and SR) from eligible studies after screening the titles and abstracts, and removing duplicated studies according to the eligibility criteria. The following information was extracted from each study: author(s) name, study design, study setting, publication year, year of data collection, sample size, gestational age, age of pregnant women, screening method (one and/or two steps), GDM screening criteria, and prevalence of GDM.

Quality assessment

The Newcastle-Ottawa Scale was used to assess the quality of the included studies, which is mainly based on the following three major components: (1) a clear and appropriate study title with selective objectives/aim/research question; (2) a valid methodology with a comprehensive description of sample size, collection of data, and screening method of GDM; and (3) applicability of outcomes. The studies that met the above-mentioned criteria were labelled as 'high quality' with low risk of bias. The quality assessment process was carried out by two independent reviewers. Any kind of disagreements was solved by the team.

Data analysis

Meta-analysis was performed by the application of the statistical software ProMeta 3. A random-effects model (DerSimonian-Laird) was used to pool the prevalence and odds ratio (OR) assessed from individual studies with a 95% confidence interval (CI). The existence of heterogeneity among the studies was evaluated using both the Cochrane chi-square Q-test and I^2 index with its resultant p-value. A value of I^2 which is 25%, 50%, and 75% was used to define the heterogeneity of low, medium, and high, respectively. Finally, the funnel plot was used to assess the possible publication bias graphically and formally by Begg's and Egger's test (significant at $p<0.05$).

Table 1 Screening criteria for the diagnosis of GDM

Organization	Year	OGTT	No. of abnormal values for diagnosis (≥1)	Fasting mmol/l (mg/dl)	1 H mmol/l (mg/dl)	2 H mmol/l (mg/dl)	3 H mmol/l (mg/dl)
WHO	2013	75 g	≥ 1	5.1 (92)	10.0 (180)	8.5 (153)	-
WHO	1999	75 g	≥ 1	7.0 (126)	-	7.8 (140)	-
ADA	2010	75/100 g	≥ 2	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)
IADPSG	2010	75 g	≥ 1	5.1 (92)	10.0 (180)	8.5 (153)	-
CC	1982	100 g	≥ 2	5.3 (95)	10.0 (180)	8.6 (153)	7.8 (140)
EASD	1996	75 g	≥ 1	7.0 (126)	11.0 (198)	9.0 (172)	-
NDDG	1979	100 g	≥ 2	5.9 (105)	10.6 (190)	9.2 (165)	8.1 (145)
O'Sullivan	1964	100 g	≥ 2	5 (90)	9.1 (165)	8.05 (145)	6.9 (125)

ADA American Diabetes Association, *CC* Carpenter-Coustan, *EASD* European Association for the Study of Diabetes, *H* hour, *IADPSG* International Association of the Diabetes and Pregnancy Study Groups, *NDDG* National Diabetes Data Group, *OGTT* Oral Glucose Tolerance Test, *WHO* World Health Organization

Results

Search result

A total of 185 studies were initially identified through various database searching. After the removal of duplicate records, additional screening, and analysis of the titles and abstracts, 25 articles were retrieved for further assessment. Twenty-five studies as full text were evaluated, and 10 articles were included for quantitative review. Among 10 studies, eight studies were selected for systematic review and meta-analysis, and the remaining two studies were excluded since they only looked at risk factors for GDM in Bangladesh. Fig. 1 demonstrated the overall process of the literature search, screening, and eligibility assessment of the study articles.

Characteristics of included studies

A total of eight studies reported the prevalence of GDM in Bangladesh, and the features of the included studies are presented in Table 2. A total of 6948 pregnant women from the rural and urban settings of Bangladesh were included in the analysis. The included studies were conducted throughout 2001–2017. Among the studies, the smallest sample size

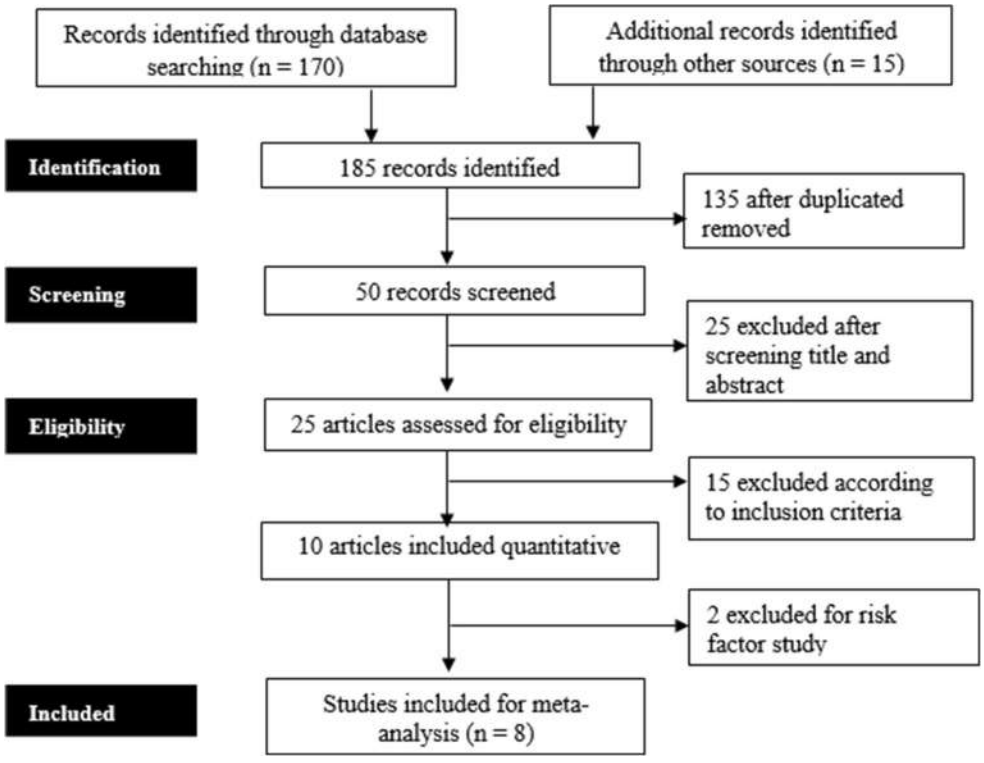


Fig. 1 Flowchart depicting the literature search and study selection according to PRISMA guidelines

was 100 [25] and the largest sample size was 3447 [20]. The age range of the participants was 15–45. Studies were conducted on pregnant women in their 12th to 40th gestational weeks. Six studies were conducted in urban areas [25–30], whereas only one study was reported in a rural area [19], and one study covered both rural and urban areas [20]. Regarding the study design, four were cross-sectional [20, 25, 27, 28], one was cross-sectional followed by cohort type [29], one was prospective analytical [30], one was population-based [19], and one was hospital-based observational study [26]. It was observed that the settings of the majority of studies were in the hospitals where the sampling frame was either antenatal clinics/centres or outdoor departments, and only one study was community-based. In terms of diagnostic criteria, five studies [19, 25, 26, 28, 30] followed World Health Organization (WHO) criteria 1999, one used American Diabetes Association (ADA) criteria [27], one used modified Carpenter-Coustan (CC) criteria [29], and one used both WHO 1999 and new ADA criteria [20]. Out of eight studies, six studies [19, 25–30] employed the most commonly used one-step screening strategy, whereas only one study [20] used a two-step screening approach. In terms of quality, all studies were rated as high quality and had a total quality score of not less than 7 based on the Newcastle-Ottawa Scale (Table 2).

Meta-analysis

The pooled estimated prevalence of GDM in Bangladesh was 13% (95% CI: 7.0–21) (Fig. 2). The results of subgroup analysis among the included studies based on age and body mass index (BMI) are presented in Fig. 3. In a subgroup analysis, older pregnant women (age >30 years) had a significantly higher prevalence of GDM than younger pregnant women (age <30 years) (18.31% vs. 11.56%; OR: 2.37, 95% CI: 1.22–4.60, p=0.01). The prevalence of GDM in overweight or obese pregnant women was 20.45%, which was significantly higher than that of women who had a normal body weight (13.84%, OR: 1.88, 95% CI: 1.18–2.99, p=0.007). The visually observed funnel plot shows no evidence of an asymmetrical pattern and indicates no publication bias (Fig. 4). Finally, this was confirmed by the quantitative analysis using Begg's test (p=0.998) and Egger test (p=0.535).

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis to summarize the current prevalence of GDM in Bangladesh. This review included a total of eight studies with 6948 participants from the rural and urban areas and we found that the overall pooled prevalence of GDM in Bangladesh was 13%.

Table 2 Characteristics of the included studies, order by year of publication and alphabetically within the same year

Authors, published year	Year of data collection	Study area	Study settings	Sample size	Age (mean/range) (years)	Mean gestational age (weeks)	Diagnostic criterion	Prevalence of GDM (%)	Quality score
Sayed et al., 2005	2001–2002	Rural	Community	147	18–44	24–28th	WHO (1999)	8.2	7
Mamun et al., 2012	2006–2007	Urban	Hospital	960	15–45	24–28th	CC (1982)	7.5	8
Begum IA, 2014	2007	Urban	Hospital	117	21.1	All trimesters	WHO (1999)	33.3	7
Jesmin et al., 2014	2012–2013	Rural and urban	Hospital	3447	20–30	26th	WHO (1999) and ADA (2010)	18.1	8
Mustafa FN, 2015	2010–2011	Urban	Hospital	1489	20–30	1st trimester	WHO (1999)	6.9	8
Sultana et al., 2016	2011–2013	Urban	Hospital	385	26.4	All trimesters	WHO (1999)	40.3	8
Begum et al., 2017	2015–2016	Urban	Hospital	303	18–38	Not available	ADA (2010)	7.3	7
Debnath et al., 2018	2017	Urban	Hospital	100	21–31	13–40th	WHO (1999)	5	7

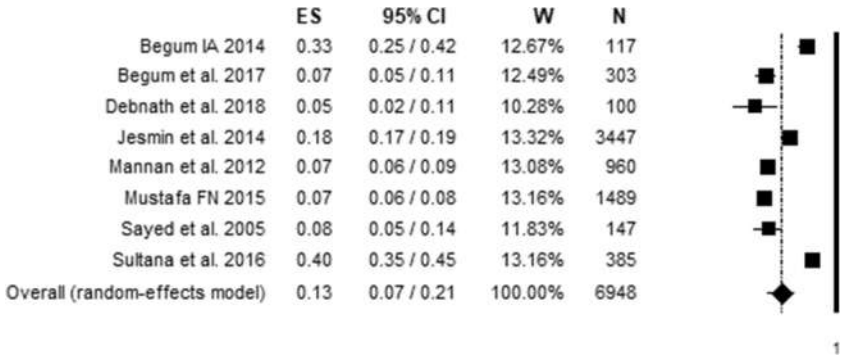
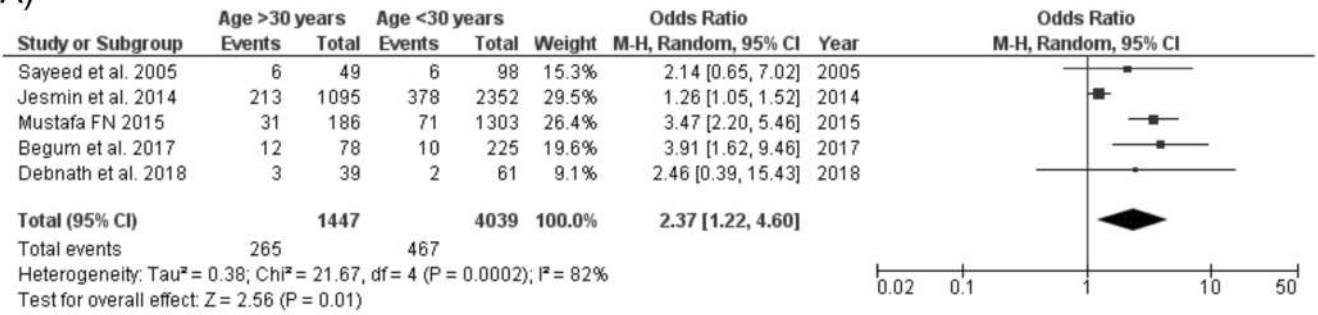


Fig. 2 Forest plot of pooled prevalence of GDM in Bangladesh

The pooled prevalence of GDM in Bangladesh was higher than the global pooled prevalence of GDM (10.13%) [9]. The outcomes of meta-analysis from the relevant studies of other South Asian countries reported that the prevalence of GDM was 11.4%, 8.8%, and 7.7% for Sri Lanka, India, and Pakistan, respectively [10]. The reported prevalence rates of GDM in Eastern Asian countries like China, Japan, South Korea, and Taiwan were 12.6%, 2.8%, 10.5%, and 6.51%, respectively [31]. This prevalence of GDM in Bangladesh was also found to be higher than that in European countries (5.4%) but lower than that in African countries (14.0%) [32, 33]. Therefore, it is confirmed that the prevalence of GDM varies significantly by region and is higher in developing countries. This could be because developed countries have better facilities and adequate screening for GDM compared to the huge population, lack of proper health care facilities, and poor economic and social status in Bangladesh, and also because of the use of various diagnostic criteria for GDM, presented in Table 2. Age, body mass index (BMI), poor dietary habits, and lifestyle conditions were identified as major risk factors for the rising trend of GDM in Bangladesh and other South Asian countries.

The findings of subgroup analysis showed that the prevalence of GDM was significantly higher in older pregnant women (18.31%) than in women with overweight and

A)



B)

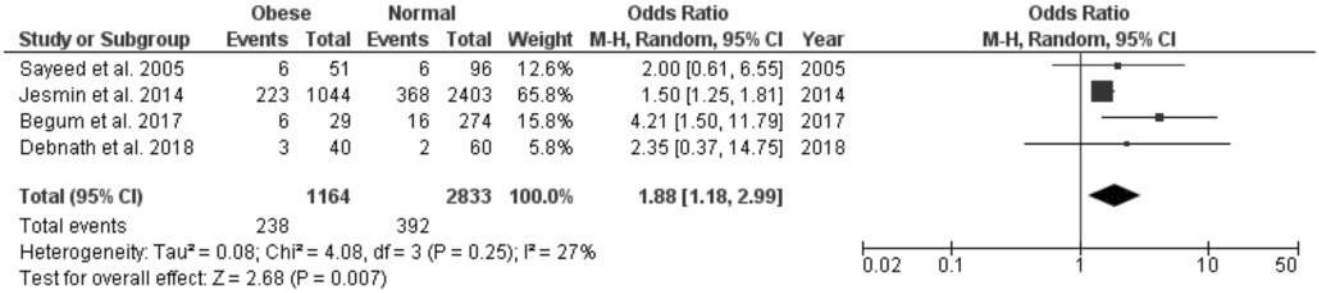


Fig. 3 Random-effects analysis of GDM in subgroups using the forest plot (**A**) between age groups and (**B**) between normal weight and overweight/obese groups

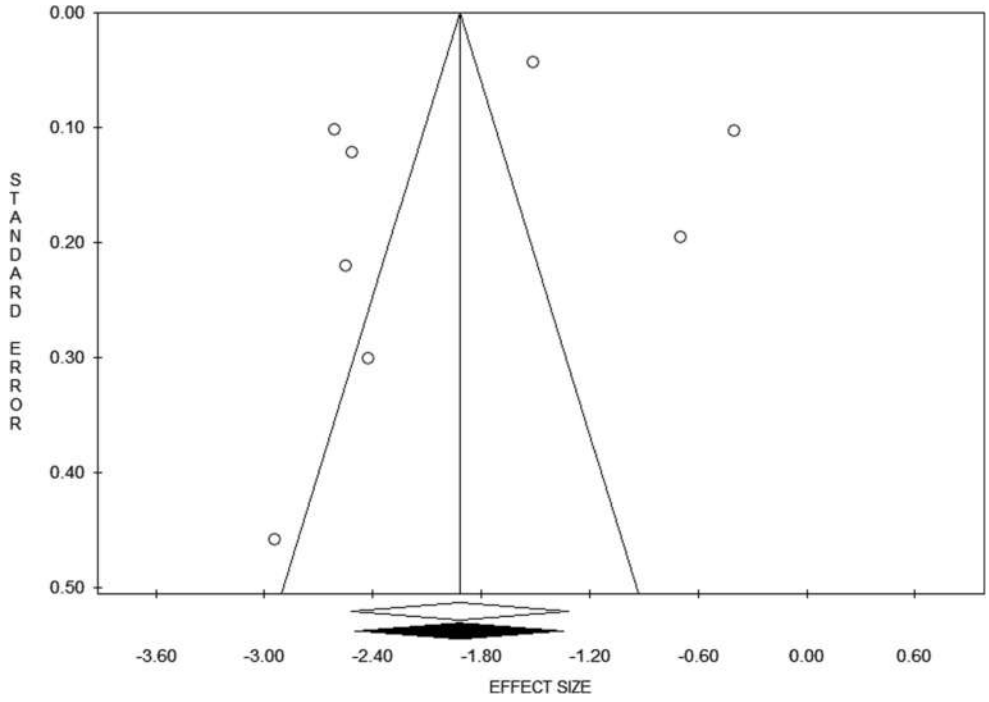


Fig. 4 Funnel plot to detect publication bias

obesity (20.45%). Consistent with this study, a study from China reported a 2-fold higher prevalence of GDM among older pregnant women than younger women, and a 2.1-fold higher incidence of GDM in pregnant women with overweight or obese than that of women with normal body weight [34]. Obesity is widely recognized as a major risk factor for the development of diabetes and GDM [35, 36]. According to Saldana et al. [37], the prevalence of GDM was significantly higher in women with a higher BMI and higher pre-pregnancy weight than in women with normal body weight. A recommended weight increase during pregnancy is 6–11 kg for women with normal body weight. Furthermore, it is now known that maternal age has a significant influence on the increased risk of GDM [38]. According to the ADA, the lowest prevalence of GDM was observed among pregnant women over the age of 25 [39], and pregnancy in young age groups below 16 years old is considered a high-risk pregnancy with adverse outcomes such as anemia, hypertensive disorders, and GDM [40].

However, despite the merits of our study, there are several limitations to this meta-analysis. Firstly, only eight studies were included in the meta-analysis, and they only contributed to 6948 patients out of the 165 million population of Bangladesh. Secondly, the study participants were mostly from urban areas, and the reported prevalence of GDM in pregnant women in rural areas is rare, which will have an impact on the total prevalence of GDM in Bangladesh. Thirdly, most of the studies were limited to the hospital-based setting, not to nationwide studies. Finally, we have no information on tribal or ethnic communities in our study as their food habits and cultures are different from native.

Conclusion

This study provides an estimation of the prevalence of GDM in Bangladesh. Our study shows that the pooled estimation of the prevalence of GDM was 13%, indicating that Bangladesh has a higher prevalence of GDM than the worldwide prevalence of GDM. Therefore, more attention should be paid to the prevention and control of GDM.

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Availability of data and material Not applicable

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Author contribution *Rahima Begum*: literature search, data analysis/interpretation, and manuscript writing. *Sourav Roy*: literature search, and manuscript writing. *Sujan Banik*: research idea and study design, data acquisition, statistical analysis, and revision of the manuscript.

Declarations

Ethics approval Not required

Informed consent No informed consent was necessary.

Conflict of interest The authors declare no competing interests.

References

1. Wendland EM, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, et al. Gestational diabetes and pregnancy outcomes-a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. BMC Pregnancy Childbirth. 2012;12:23.
2. Gilmartin AB, Ural SH, Repke JT. Gestational diabetes mellitus. Rev Obstet Gynecol. 2008;1:129.
3. Kanguru L, Bezawada N, Hussein J, Bell J. The burden of diabetes mellitus during pregnancy in low- and middle-income countries: a systematic review. Glob Health Action. 2014;7:23987.
4. Marchetti D, Carrozzino D, Fraticelli F, Fulcheri M, Vitacolonna E. Quality of life in women with gestational diabetes mellitus: a systematic review. J Diabetes Res. 2017;2017:1–12.
5. Yogev Y, Xenakis EM, Langer O. The association between preeclampsia and the severity of gestational diabetes: the impact of glycemic control. Am J Obstet Gynecol. 2004;191:1655–60.
6. Retnakaran R, Qi Y, Connelly PW, Sermer M, Zinman B, Hanley AJ. Glucose intolerance in pregnancy and postpartum risk of metabolic syndrome in young women. J Clin Endocrinol Metab. 2010;95:670–7.
7. Langer O, Yogev Y, Most O, Xenakis EM. Gestational diabetes: the consequences of not treating. Am J Obstet Gynecol. 2005;192: 989–97.
8. Federation ID. IDF diabetes atlas 8th edition. Int Diabetes Fed. 2017:905–11.
9. Gyasi-Antwi P, Walker L, Moody C, Okyere S, Salt K, Anang L, et al. Global prevalence of gestational diabetes mellitus: a systematic review and meta-analysis. New Am J Med. 2020;1
10. Jiwani A, Marseille E, Lohse N, Damm P, Hod M, Kahn JG. Gestational diabetes mellitus: results from a survey of country prevalence and practices. J Matern Fetal Neonatal Med. 2012;25:600–10.
11. Eades CE, Cameron DM, Evans JM. Prevalence of gestational diabetes mellitus in Europe: a meta-analysis. Diabetes Res Clin Pract. 2017;129:173–81.
12. Mwanri AW, Kinabo J, Ramaiya K, Feskens EJ. Gestational diabetes mellitus in sub-Saharan Africa: systematic review and metaregression on prevalence and risk factors. Trop Med Int Health. 2015;20:983–1002.
13. Alfadhli EM, Osman EN, Basri TH, Mansuri NS, Youssef MH, Assaaedi SA, Aljohani BA. Gestational diabetes among Saudi women: prevalence, risk factors and pregnancy outcomes. Ann Saudi Med. 2015;35:222–30.
14. Nguyen CL, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of gestational diabetes mellitus in eastern and southeastern Asia: a systematic review and meta-analysis. J Diabetes Res. 2018;2018:1–10.
15. Wahi P, Dogra V, Jandial K, Bhagat R, Gupta R, Gupta S, Wakhloo A, Singh J. Prevalence of gestational diabetes mellitus (GDM) and its outcomes in Jammu region. J Assoc Physicians India. 2011;59: 227–30.
16. Lauring JR, Kunselman AR, Pauli JM, Repke JT, Ural SH. Comparison of healthcare utilization and outcomes by gestational diabetes diagnostic criteria. J Perinat Med. 2018;46:401–9.
17. Corrado F, Pintaudi B. Diagnosis of gestational diabetes mellitus: Italian perspectives on risk factor-based Screening. In: Rajendram R, Preedy V, Patel V, editors. Nutrition and Diet in Maternal Diabetes. Nutrition and Health. Humana Press; 2018. p. 87–97.
18. Xiao Y, Chen R, Chen M, Luo A, Chen D, Liang Q, Cai Y, Chen L, Zhao X. Age at menarche and risks of gestational diabetes mellitus: a meta-analysis of prospective studies. Oncotarget. 2018;9:17133–40.
19. Sayeed MA, Mahtab H, Khanam PA, Begum R, Banu A, Azad Khan AK. Diabetes and hypertension in pregnancy in a rural community of Bangladesh: a population-based study. Diabet Med. 2005;22:1267–71.
20. Jesmin S, Akter S, Akashi H, Al-Mamun A, Rahman MA, Islam MM, et al. Screening for gestational diabetes mellitus and its prevalence in Bangladesh. Diabetes Res Clin Pract. 2014;103:57–62.
21. Sultana N, Hasanat MA, Jahan S, Panthi S, Hasan M, Aktar Y, Fariduddin M. Screening for gestational diabetes mellitus (GDM): comparison between WHO 1999 and modified O'Sullivan Criteria. JCD. 2015;2:21–6.
22. Panthi S, Hasanat MA, Aktar Y, Sultana N, Jahan S, Rahman MA, Fariduddin M. Frequency of gestational diabetes mellitus in Bangladesh impact of WHO 2013 screening criteria: efficiency DIPSI and WHO 1999 criteria. JCD. 2015;2:13–20.
23. Biswas RT. Risk factors and pregnancy outcomes among gestational diabetic mothers: a hospital based study in Bangladesh. 2006. https://www.duo.uio.no/handle/10852/30121. Accessed Jan 2021
24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol. 2009;62:e1–34.
25. Debnath J, Talukder S, Islam MS, Khan MSI, Sabuj SS, Jhorna DE. Prevalence of gestational diabetes and associated risk factors. Asian J Med Biol Res. 2018;4:274–8.
26. Begum IA. Gestational diabetes mellitus in Primi Gravida of Bangladesh in different trimesters. Int J Biol. 2014;6:18.
27. Begum P, Shaha DR, Walid KM. Gestational diabetes mellitus and associated risk factors in patients attending diabetic association medical college hospital in Faridpur. J Enam Med Coll. 2017;7: 126–33.
28. Hasanat MA, Atiqur-Rahman M, Fariduddin M. Association of risk factors in gestational diabetes mellitus among pregnant mothers attending at a tertiary care hospital in Bangladesh. J Bioinforma Diabetes. 2016;1:54–60.
29. Mannan MA, Rahman MH, Ara I, Afroz H. Prevalence and pregnancy outcome of gestational diabetes mellitus among Bangladeshi urban pregnant women. J Med. 2012;13:147–51.
30. Mustafa FN. Pregnancy profile and perinatal outcome in gestational diabetes mellitus: a hospital based study. J Bangladesh Coll Physicians Surg. 2015;33:79–85.
31. Tamayo T, Tamayo M, Rathmann W, Potthoff P. Prevalence of gestational diabetes and risk of complications before and after initiation of a general systematic two-step screening strategy in Germany (2012–2014). Diabetes Res Clin Pract. 2016;115:1–8.
32. Badakhsh M, Daneshi F, Abavisani M, Rafiemanesh H, Bouya S, Sheyback M, Rezaie Keikhaie K, Balouchi A. Prevalence of gestational diabetes mellitus in Eastern Mediterranean region: a systematic review and meta-analysis. Endocrine. 2019;65:505–14.
33. Casagrande SS, Linder B, Cowie CC. Prevalence of gestational diabetes and subsequent type 2 diabetes among US women. Diabetes Res Clin Pract. 2018;141:200–8.
34. Gao C, Sun X, Lu L, Liu F, Yuan J. Prevalence of gestational diabetes mellitus in mainland China: a systematic review and meta-analysis. J Diabetes Investig. 2019;10:154–62.
35. Lavery JA, Friedman AM, Keyes KM, Wright JD, Ananth CV. Gestational diabetes in the United States: temporal changes in prevalence rates between 1979 and 2010. BJOG Int J Obstet Gynaecol. 2017;124:804–13.
36. Leung MYM, Carlsson NP, Colditz GA, Chang S-H. The burden of obesity on diabetes in the United States: medical expenditure panel survey, 2008 to 2012. Value Health. 2017;20:77–84.

37. Saldana TM, Siega-Riz AM, Adair LS, Suchindran C. The relationship between pregnancy weight gain and glucose tolerance status among black and white women in central North Carolina. Am J Obstet Gynecol. 2006;195:1629–35.
38. Lao TT, Ho L-F, Chan BC, Leung W-C. Maternal age and prevalence of gestational diabetes mellitus. Diabetes Care. 2006;29:948–9.
39. World Health Organization expert committee on diabetes mellitus: second report. World Health Organ Tech Rep Ser. 1980. https://apps.who.int/iris/handle/10665/41399. Accessed Jan 2021.
40. Demirci O, Yılmaz E, Tosun Ö, Kumru P, Arınkan A, Mahmutoğlu D, et al. Effect of young maternal age on obstetric and perinatal outcomes: results from the tertiary center in Turkey. Balkan Med J. 2016;33:344–9.

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ORIGINAL ARTICLE

Evaluation of the circulating serum endotrophin in women with and without gestational diabetes mellitus during second trimester

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Abstract

Purpose Endotrophin is a newly discovered adipokine, and its clinical utility in diagnosing gestational diabetes mellitus (GDM) remains unclear. We aimed to investigate the possible association between endotrophin and GDM and determine its correlation with maternal metabolic parameters.

Methods Screening and diagnosis for GDM were carried out between the 24th and 28th weeks of gestation. The study included 50 patients with GDM and 37 healthy pregnant women. Endotrophin was assayed by a commercially available enzyme-linked immunosorbent assay kit.

Results The endotrophin level was significantly higher in the GDM group compared to uncomplicated pregnancies (121.98 ± 104.08 vs 72.5 ± 76.2, $p < 0.05$). Furthermore, the fasting glucose, fasting insulin, and HOMA-IR values of and plasma glucose levels obtained at 1 and 2 h after 75 g oral glucose administration were significantly higher in the GDM group than in the control group ($p < 0.05$). The Spearman analysis showed a statistically significant correlation between the endotrophin level and the fasting glucose, first-hour glucose, fasting insulin, HOMA-IR, and pre-pregnancy and current body mass indexes (BMI) in the GDM group ($p < 0.05$).

Conclusion Although oral glucose tolerance test (OGTT) is still the gold standard in the diagnosis of GDM, considering the technical difficulties and disadvantages of OGTT, endotrophin measurement at 24–28 weeks of pregnancy in healthy risk-free pregnant women seems to be an appropriate approach to limit the use of OGTT.

Keywords Endotrophin · Gestational diabetes mellitus · Adipokine · Hyperglycemia

Introduction

Although adipose tissue was considered to be a static amount of fat for a long period of time, it is now recognized as the largest endocrine organ secreting a large number of bioactive proteins called "adipokines" [1]. Adipokines are involved in different metabolic processes from insulin sensitivity and insulin secretion to appetite control, fat distribution, energy expenditure, inflammation, regulation of adipogenesis, and chemoattraction of immune cells into adipose tissue. Impaired adipokine secretion causes gestational diabetes mellitus (GDM) by contributing to changes in glucose homeostasis during pregnancy [2, 3]. Since adipokines influence key pathways involved in the pathophysiology of GDM, such as insulin resistance, pancreatic β-cell dysfunction, and body weight gain, researchers have recently focused on the effects of various adipokines on the development of this metabolic disorder [4, 5].

Endotrophin is a newly discovered adipokine, a soluble proteolytic fragment of the α3 chain of microfilamentous interstitial type VI collagen, which is composed of α1, α2, and α3 constituent chains and expressed in connective tissues and prominently in adipose tissue [6]. Endotrophin is associated with various biological processes. This cleavage product of collagen VI has been shown to be effective both

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in the pathways of cancer development, such as fibrosis, angiogenesis, inflammation, and epithelial-mesenchymal transition, and in various metabolic functions, including insulin sensitivity, energy balance, food intake, and adipose tissue inflammation [7–9]. Different studies have demonstrated that endotrophin causes increased insulin resistance by triggering fibrosis and inflammation in pathologically relevant pathways in an "unhealthy" adipose tissue environment [8–12]. In a study conducted to investigate the different metabolic effects of endotrophin, higher endotrophin levels were observed in patients with high insulin resistance [12]. However, it has been reported that high fasting glucose levels do not induce an increase in endotrophin levels independent of insulin resistance. Similarly, obese patients with insulin resistance have been shown to have higher levels of endotrophin than obese individuals with normal insulin sensitivity. In an experimental model, blocking endotrophin with a neutralizing antibody improved metabolic adverse effects and effectively reversed metabolic dysfunction. It was also observed that overexpression of endotrophin led to abnormal lipid metabolism and hence ectopic steatosis [10].

GDM, defined as the onset or initial recognition of abnormal glucose tolerance during pregnancy, is considered a pre-diabetic condition characterized by an enormous insulin resistance and insufficient insulin compensation [13, 14]. Although the global prevalence of hyperglycemia in pregnancy is estimated at 17%, this rate is predicted to increase over time, possibly due to the accelerating frequency of obesity [15]. While the crucial functions of endotrophin in metabolic disorders have been characterized, its direct effects on GDM, the metabolic disturbance of pregnant women, remains to be elucidated. To our knowledge, there is a lack of data on serum endotrophin levels in GDM. To address this gap, this study aimed to investigate the possible association between endotrophin and GDM and to determine the correlation of this adipokine with maternal metabolic parameters.

Material and methods

Study design

This cross-sectional study was conducted at Medipol Mega University Hospital with 87 pregnant women aged 22 to 42 years, who were referred to the internal medicine outpatient clinic from the obstetrics and gynecology department between December 2019 and July 2020. During our study, a total of 278 pregnant women were examined, and all pregnant underwent 75 gr oral glucose tolerance test (OGTT) at a routine visit between the 24th and 28th weeks of pregnancy. The diagnosis of GDM was made based on the abnormality of one or more of the following plasma glucose parameters: (1) fasting plasma glucose $\geq$ 92 mg/dl, (2) first-hour blood sugar $\geq$ 180 mg/dl in OGTT, and (3) second-hour blood sugar $\geq$ 153 mg/dl in OGTT were obtained according to the criteria of the International Association of Diabetes and Pregnancy Study Groups [16]. Patients with a history of pregestational diabetes mellitus; documented chronic diseases such as malignancy, liver disease, chronic inflammatory diseases, hypertension, hypothyroidism, and acute medical conditions (pulmonary embolism, pneumonia, trauma, surgery, etc.); multiple pregnancies; malformed fetuses; and those abusing drugs or alcohol were not included in the study. At least, the patient group consisted of 50 pregnant women diagnosed with GDM. Thirty-seven healthy pregnant women with normal OGTT results were included in the study as a control group.

The height and weight of the participants were measured, and their body mass indexes (BMI) were calculated by dividing body weight by the square of height (kg/m^2) and recorded as current BMI. The pre-pregnancy BMIs of the participants were calculated by obtaining the height and weight data from the hospital database and recorded. In all cases, the blood pressure was also measured with a sphygmomanometer after 10 min of rest and recorded.

Ethical approval and patient consent

All patients were given detailed information that the study was not part of their treatment and their informed consent was obtained. The study was approved by the Ethics Committee of the university (10,840,098–604.01.01-E.3374 number: 76) and conducted in accordance with the principles of the Declaration of Helsinki.

Blood sample test

Blood samples were taken from the patients after 10 to 12 h of fasting to assess biochemical parameters on the day of OGTT screening. Laboratory data, including the levels of serum insulin, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were recorded. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula:

$$\text{fasting glucose (mg/dl)} \times \text{fasting insulin (uIU/ml)} / 405 \quad (17)$$

During routine blood tests, an extra tube of EDTA blood was collected from the participants and centrifuged at $1000 \times g$ for 15 min, and the obtained sera were stored at – 80 °C until endotrophin analyses.

Measurement of serum endotrophin

Serum endotrophin levels were measured using a commercially available human double-antibody sandwich enzyme-linked immunosorbent assay kit (catalog number 201–12-9305, Sunred Biological Technology, Shanghai, China). The results were expressed as nanograms per milliliter (ng/ml). The intra-assay coefficient of variation (CV) of endotrophin is < 10%, and the inter-assay CV is < 12%. The sensitivity of endotrophin is 1.398 ng/ml. The standard curve range for the endotrophin ELISA kit is 1.5–300 ng/ml. These values are based on the human endotrophin ELISA kit instruction of the manufacturer.

Statistical analysis

Data were collected by a relevant clinical physician, transferred to Microsoft Excel program, edited, and prepared for statistical analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software v. 26.0. In the patient group, age, gestational age, first- and second-hour glucose variables, HOMA-IR, fasting insulin, and current BMI were normally distributed ($p > 0.05$), while other variables were not normally distributed ($p < 0.05$). In the control group, while age, gestational age, fasting and second-hour glucose, TC, LDL-C, HDL-C, fasting insulin, and current BMI variables were normally distributed ($p > 0.05$), other variables were not normally distributed ($p < 0.05$). Parametric tests were used in the analysis of normally distributed variables, and non-parametric tests were used in the analysis of non-normally distributed variables. The chi-square and Shapiro–Wilk tests were used to check the normality assumption. Differences between the study groups were accessed using the Mann–Whitney *U* test, *T* test, Wilcoxon test, and correlations with Spearman's test. The receiver operating characteristics (ROC) curve analysis was performed to identify the cut-off values of the studied parameters. Differences of $p < 0.05$ were considered to be statistically significant.

Results

The demographical and clinical characteristics of the participants are shown in Table 1. Fifty patients diagnosed with GDM and 37 healthy pregnant women were included in the study. No significant difference was found between the GDM and control groups in terms of age, gestational age, systolic or diastolic blood pressure, and current BMI ($p > 0.05$). On the other hand, the pre-pregnancy BMI was significantly higher in the GDM group compared to the control group ($p < 0.05$).

The biochemical laboratory data of the study participants are summarized in Table 2. The serum endotrophin levels of the GDM group were statistically significantly higher than those of the controls ($p < 0.05$). Furthermore, there was a significant difference between the GDM and control groups in terms of the fasting, first-hour and second-hour glucose levels at 75 gr OGTT, HOMA-IR, and fasting insulin levels. Fasting, first-hour and second-hour glucose levels at 75 gr OGTT, HOMA-IR, and fasting insulin levels of the GDM group were significantly higher than those of the control group ($p < 0.05$).

Table 3 demonstrates the relationships between the serum endotrophin level and other variables. When we analyzed the correlation between the variables and endotrophin in the control group, the HOMA-IR and fasting insulin values had a moderate degree of positive correlation with the endotrophin levels ($p < 0.05$). As for the GDM group, there was a very strong positive correlation between the endotrophin level and the fasting glucose level and HOMA-IR ($p < 0.05$). In addition, there was a weak degree of positive correlation of endotrophin with the first-hour glucose level, diastolic blood pressure, pre-pregnancy BMI, current BMI, and fasting insulin level ($p < 0.05$).

Table 1 Clinical characteristics of the GDM and control groups (mean ± SD/median-range)

	Control group $n = 37$ (42.5%)	GDM group $n = 50$ (57.5%)	Total	*p*
Age (years)	30.57 ± 4.93/31–19	31.1 ± 4.73/31–17	30.87 ± 4.8/31–20	0.612
Gestational age at sampling (weeks)	25.8 ± 1.49/25.86–5.57	26.21 ± 1.48/26.07–6	26.04 ± 1.49/26–6.57	0.197
SBP (mmHg)	110.27 ± 10.65/114–36	110.24 ± 7.38/110–30	110.25 ± 8.86/110–36	0.549
DBP (mmHg)	69.89 ± 6.49/70–25	69.62 ± 5.62/70–20	69.74 ± 5.97/70–25	0.788
BMI (kg/m^2)				
Pre-pregnancy	24.75 ± 5.55/22.8–22.1	26.28 ± 4.08/25.3–17.36	25.63 ± 4.79/24.98–22.1	0.025*
Current, at sampling	27.41 ± 5.26/26.56–20.81	28.72 ± 4.26/28.18–19.94	28.16 ± 4.73/27.47–20.81	0.206

*Statistically significant at 0.05. *GDM*, gestational diabetes mellitus; *SBP*, Systolic blood pressure; *DBP*, diastolic blood pressure; *BMI*, body mass index

Table 2 Biochemical characteristics of the GDM and control groups (mean ± SD/median-range)

	Control group	GDM group	Total	*p*
75 gr OGTT (mg/dl)				
Fasting glucose	85.66 ± 6.22/85.6–24.2	97.25 ± 12.46/96–72	92.32 ± 11.74/91–72	0.000*
First hour	135.46 ± 31.35/146–111.3	197.83 ± 24.76/193.5–123	171.31 ± 41.5/175–187.6	0.000*
Second hour	106.54 ± 20.24/102.7–78	157.96 ± 29.57/156–137	136.09 ± 36.38/135–180.5	0.000*
TC (mg/dl)	254.86 ± 48.99/257–206	234.22 ± 56.97/225.5–234	243 ± 54.4/241–237	0.080
LDL-C (mg/dl)	144.24 ± 39.22/138–181.4	126.6 ± 44.25/115.5–215	134.1 ± 42.86/126–215	0.057
TG (mg/dl)	169.08 ± 54.38/159–204	198.8 ± 89.54/176.5–491	186.16 ± 77.61/165–491	0.135
HDL-C (mg/dl)	74.49 ± 15.7/72–72	68.99 ± 16.57/69–74	71.33 ± 16.34/70.9–83	0.085
HOMA-IR	2.80 ± 1.53/2.61–6.24	4.13 ± 1.85/3.80–8.72	3.56 ± 1.84/3.18–8.78	0.000*
Fasting insulin (mIU/ml)	13.19 ± 6.73/12.00–27.00	17.06 ± 6.91/16.00–29.00	15.41 ± 7.07/14.00–29.00	0.007*
Endotrophin (ng/ml)	72.5 ± 76.2/29.16–269.96	121.98 ± 104.08/115.72–404.51	100.94 ± 95.96/53.29–414.91	0.003*

*Statistically significant at 0.05. *GDM*, gestational diabetes mellitus; *OGTT*, oral glucose tolerance test; *TC*, total cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *TG*, triglyceride; *HDL-C*, high-density lipoprotein cholesterol; *HOMA-IR*, homeostasis model assessment of insulin resistance

In the ROC analysis to determine the serum endotrophin level of the GDM group, the area under the curve (AUC) was calculated as 0.688 (95% CI 0.574–0.803) (Fig. 1). For the GDM group, at the cut-off value of ≥ 25.99 (calculated by the Youden index), the sensitivity, specificity, positive predictive, and negative predictive values of endotrophin were 88%, 43.2%, 67.7%, and 72.7%, respectively, with an accuracy of 69%.

Discussion

With the groundbreaking discovery of the first adipokines over two decades ago, adipose tissue began to be recognized as an endocrine organ. Since then, many more adipokines have been identified, and considerable progress has been made on the knowledge of how adipose tissue communicates with other organs of the body to

Table 3 Relationship of the endotrophin values with the investigated variables and results of the Spearman-Pearson correlation analysis (*r* (*p*))

	Total	Control group	GDM group
Age (years)	0.16 (*p* = 0.146)	0.11 (*p* = 0.502)	0.22 (*p* = 0.121)
Gestational age (weeks)	0.08 (*p* = 0.474)	−0.10 (*p* = 0.556)	0.21 (*p* = 0.140)
75 gr OGTT (mg/dl)			
Fasting glucose	0.50 (*p* = 0.000*)	0.25 (*p* = 0.144)	0.91 (*p* = 0.000*)
First-hour glucose	0.11 (*p* = 0.315)	−0.01 (*p* = 0.964)	0.33 (*p* = 0.021*)
Second-hour glucose	0.13 (*p* = 0.216)	0.08 (*p* = 0.647)	0.24 (*p* = 0.096)
TC (mg/dl)	−0.10 (*p* = 0.356)	0.05 (*p* = 0.759)	−0.20 (*p* = 0.156)
LDL-C (mg/dl)	−0.07 (*p* = 0.494)	0.11 (*p* = 0.531)	−0.21 (*p* = 0.134)
TG (mg/dl)	0.13 (*p* = 0.219)	0.14 (*p* = 0.396)	0.17 (*p* = 0.228)
HDL-C (mg/dl)	−0.19 (*p* = 0.078)	−0.18 (*p* = 0.280)	−0.23 (*p* = 0.115)
SBP (mmHg)	0.15 (*p* = 0.170)	0.24 (*p* = 0.149)	0.06 (*p* = 0.677)
DBP (mmHg)	0.24 (*p* = 0.027)	0.18 (*p* = 0.279)	0.34 (*p* = 0.017*)
BMI ($kg(m^2)$)			
Pre-pregnancy	0.21 (*p* = 0.049*)	0.23 (*p* = 0.168)	0.30 (*p* = 0.033*)
Current	0.22 (*p* = 0.040*)	0.18 (*p* = 0.282)	0.33 (*p* = 0.021*)
HOMA-IR	0.59 (*p* = 0.000*)	0.52 (*p* = 0.001*)	0.85 (*p* = 0.000*)
Fasting insulin (mIU/ml)	0.40 (*p* = 0.000*)	0.50 (*p* = 0.002*)	0.37 (*p* = 0.008*)

*Statistically significant at 0.05. *GDM*, gestational diabetes mellitus; *TC*, total cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *TG*, triglyceride; *HDL-C*, high-density lipoprotein cholesterol; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *BMI*, body mass index; *HOMA-IR*, homeostasis model assessment of insulin resistance

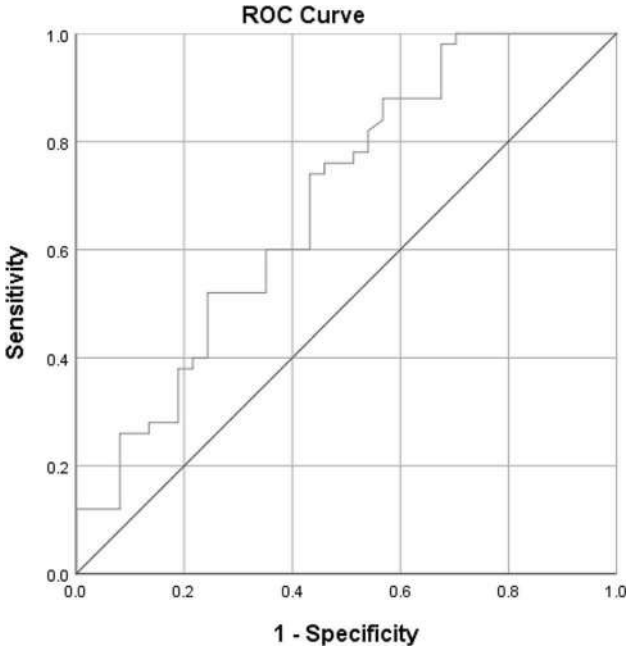

Fig. 1 ROC curve graph of the endotrophin level

maintain systemic homeostasis. Some of the adipokines contribute to insulin resistance, especially in the last half of pregnancy. Recent research has suggested that metabolic mediators from adipose tissue may play a role in the development of GDM. In this study, we aimed to investigate the potential alteration of endotrophin in pregnant women with GDM. We primarily showed that the serum endotrophin levels in pregnant women with GDM were clearly increased compared to the healthy pregnant controls. Our second aim was to determine the possible relationship between endotrophin and metabolic parameters. In the GDM group, endotrophin was positively correlated with the fasting glucose level and plasma glucose levels obtained at 1 h after 75 g oral glucose administration, fasting insulin levels, HOMA-IR values, and pre-pregnancy and current BMI scores. To our knowledge, this is the first study investigating endotrophin levels in pregnancies complicated by GDM.

Extracellular matrix is a dynamic structure that provides stability for tissues and organisms and transmits differentiation, growth, and migration signals to neighboring cells [17]. Collagen type VI is one of the major extracellular matrix proteins in various connective tissues such as skin, cartilage, skeletal muscle, and adipose tissue. Collagen type VI is a triple-helix molecule composed of α1, α2, and α3 constituent chains and has a microfilamentous structure. During the formation of microfilaments, the triple helical nucleus of this type VI collagen is released proteolytically from the propeptide. Finally, by the cleavage of the C-terminal propeptide of the α3 chain, a newly identified adipokine, endotrophin, arises [11].

Endotrophin is involved in various biological processes [18]. In the field of tumor physiopathology, endotrophin has been identified as a potent tumor promoting factor that is produced and released in abundance from adipocytes [18–20]. Besides the vital function of endotrophin in tumor development, as an adipocyte-derived and obesity-related factor, endotrophin seems to act as a driver of metabolic degradation since it has crucial effects on adipose tissue dysfunction. In experimental studies, endotrophin has been demonstrated to negatively modulate various metabolic functions, such as energy balance, food intake, and insulin sensitivity [12, 21]. Collagen type VI-deficient ob/ob mice and mice fed a high-fat diet exhibit improved glucose tolerance and insulin sensitivity [21]. It has been reported that the overexpression of endotrophin, especially in adipose tissue, disrupts the proper function of adipocytes, and thus leads to systemic metabolic dysfunction, while the suppression of endotrophin by a neutralization antibody ameliorates insulin sensitivity and adipose tissue inflammation [12]. This correlates well with our findings indicating that the endotrophin levels were higher in the GDM group compared to the healthy pregnant women [11, 12, 14]. In another human study, it was reported that diabetic patients had higher endotrophin levels. However, this increase in endotrophin levels was found to be associated with higher insulin resistance rather than fasting glucose levels [12]. Taking together the current evidence on the role of endotrophin in glucose homeostasis and higher plasma endotrophin concentrations in patients with GDM, it can be suggested that endotrophin may play a role in the GDM pathogenesis.

When we analyzed the correlation between endotrophin and HOMA-IR within the two groups, although there was a much stronger correlation in the GDM group compared to the control group, we concluded that endotrophin was correlated with HOMA-IR in both groups. This result is not surprising in light of the fact that endotrophin serves as a powerful co-stimulator of existing pathological processes, leading to increased insulin resistance. It has also been reported that plasma endotrophin can be used as a biomarker in predicting the effectiveness of thiazolidinedione therapy that increases insulin sensitivity [11]. More importantly, when the correlation table obtained from this study was examined, it was observed that fasting blood glucose and first-hour blood glucose, which are two of the three OGTT parameters, were correlated with endotrophin only in the GDM group. Although there is no consensus among healthcare professionals on screening methods for GDM worldwide, and glycemic thresholds have not been standardized across different guidelines, OGTT remains the "gold standard" for the diagnosis of GDM. However, OGTT has disadvantages such as not being reproducible, being expensive,

time consuming, and quite laborious and inconvenient for patients. In addition, it has not been adjusted for body weight and its differential predictive value varies due to the variable prevalence of GDM by ethnicity [22]. Recently, there has been discussion to avoid OGTT as a diagnostic test for GDM in pregnant women. According to our results, the correlation of endotrophin with fasting and first-hour blood glucose levels suggests that, given the numerous problems of the OGTT, it may be a suitable candidate for reducing the use of OGTT, at least in healthy pregnant women without risk factors for GDM.

Obesity is spreading worldwide, and it is estimated that nearly 50% of women at reproductive age are overweight or obese [23]. Among pregnant women, the prevalence of obesity exceeds 16% [24]. Maternal obesity is associated with short- and long-term negative health consequences for both the mother and the child [16]. Obesity and GDM are often coexisting conditions. In previous studies, it was demonstrated that pre-pregnancy weight gain increased the risk of GDM, and compared to women with a normal BMI, the risk of developing GDM was three times higher in obese women [25–28]. We indicated that the pre-pregnancy BMI was significantly higher in the GDM group compared to non-diabetic pregnant women. Moreover, we showed a positive correlation between endotrophin levels and pre-pregnancy and current BMI in the GDM group. From this point of view, increased endotrophin levels secondary to the growing adipose tissue before and during pregnancy seem to contribute to the development of GDM.

In the current literature, despite numerous studies evaluating the role of adipokines in GDM [6, 29], the association between endotrophin, GDM, and BMI remains elusive. One of the additional contributions of the current study to the literature on GDM is that we demonstrated a statistically significant increase in endotrophin levels in parallel with the increased BMI values found in pre-pregnancy and the 24–28th weeks of the gestation. Given this argument, we can address two possibilities to explain this situation. The first is that high amounts of endotrophin are secreted from accreted adipose tissue in patients with a high BMI. This possibility becomes even more likely with the detection of increased endotrophin levels in the non-pregnant population with a high BMI [12]. On the other hand, this positive correlation being significantly higher in the GDM group compared to the non-diabetic pregnant group leads to a second hypothesis, which suggests that the significantly elevated endotrophin values of the patients with GDM with increased BMI levels may have been due to not only the increase in adipose tissue but also impaired glucose metabolism and increased insulin resistance. The most important indicator of this situation is that increased endotrophin values showed a much higher positive correlation with fasting blood glucose and HOMA-IR in the GDM group compared to the control group. Considering that increased BMI, which is accepted as an indicator of increased adiposity, is an important and preventable risk factor for GDM development in pregnant women, understanding the role of adipose tissue in GDM can provide new information about its pathophysiology and may even offer a novel strategy for the treatment of impaired glucose metabolism and insulin resistance in obese GDM patients based on anti-endotrophin therapy in future.

Another important data obtained from the results of our study is that while the endotrophin values had a sensitivity of up to 88% in the differentiation between the healthy and GDM groups, the specificity values were lower. This shows that endotrophin is very effective in detecting GDM, but it may produce false-positive results in some healthy individuals. Therefore, considering the technical difficulties of OGTT that we have pointed before, the need for OGTT applied to all pregnant women at 24–28 weeks may be eliminated for risk-free pregnant who are below the cut-off value we found for endotrophin.

Some limitations of our study that should be considered are the small sample size and assessment of maternal endotrophin levels only in the second trimester of pregnancy. Furthermore, another limitation of the current study is the lack of adequate scientific evidence on endotrophin in the literature to compare with our results.

In conclusion, the most significant contribution of this study to the literature is that it clearly demonstrated significantly increased serum endotrophin levels are in pregnancies complicated with GDM and revealed the positive correlation of endotrophin with fasting blood glucose and HOMA-IR. Taking together the results of our study, it can be suggested that endotrophin may play a role in the GDM pathogenesis. In addition, although OGTT is still the gold standard in the diagnosis of GDM, considering the technical difficulties and disadvantages of OGTT, endotrophin measurement at 24–28 weeks of pregnancy in healthy risk-free pregnant women seems to be an appropriate approach to limit the use of OGTT. Further studies in larger patient groups are required to improve our insights into the relationship of this mysterious adipokine with the pathophysiology of GDM and to increase our knowledge of its clinical use in patients with GDM.

Author contribution Concept – D.A., E.C.E.; Design – D.A, E.C.E.; Supervision – D.A.; Resources – D.A.; Data Collection and/or processing –D.A.; Analysis and/ or interpretation – D.A.; Literature search – D.A., E.C.E.; Writing manuscript – D.A.; Critical review – D.A.

Data availability Derived data supporting the findings of this study are available from the corresponding author [D.A.] on request.

Code availability Not applicable.

Declarations

Ethics approval The study was approved by the Medipol University Ethics Committee (10840098–604.01.01-E.3374 number:76).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Proceedings of the 4th International Workshop-Conference on Gestational Diabetes Mellitus Chicago, Illinois, USA. Diabetes Care. 1998; 21 Suppl 2:B1.
2. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational diabetes. Lancet Diabetes Endocrinol. 2014;2:488–99.
3. Kim SY, Saraiva C, Curtis M, Wilson HG, Troyan J, Sharma AJ. Fraction of gestational diabetes mellitus attributable to overweight and obesity by race/ethnicity, California, 2007–2009. Am J Public Health. 2013;103:e65–72.
4. Fève B, Bastard C, Fellahi S, Bastard J-P. Capeau J New adipokines. Ann Endocrinol (Paris). 2016;77:49–56.
5. Kralisch S, Bluher M, Paschke R, Stumvoll M, Fasshauer M. Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. Mini Rev Med Chem. 2007;7:39–45.
6. de Gennaro G, Palla G, Battini L, Simoncini T, del Prato S, Bertolotto A, Bianchi C. The role of adipokines in the pathogenesis of gestational diabetes mellitus. Gynecol Endocrinol. 2019;35:737–51.
7. Brink HS, van der Lely AJ, van der Linden J. The potential role of biomarkers in predicting gestational diabetes. Endocr Connect. 2016;5:R26–34.
8. Eruzun H, Toprak İD, Arman Y, Yılmaz U, Özcan M, Kutlu Y, Irmak S, Kutlu O, Yoldemir ŞA, Altun Ö, Çil EÖ, Tükek T. Serum endotrophin levels in patients with heart failure with reduced and mid-range ejection fraction. Eur J Intern Med. 2019;64:29–32.
9. Park J, Scherer PE. Endotrophin in the tumor stroma: a new therapeutic target for breast cancer? Expert Rev Anticancer Ther. 2013;13:111–3.
10. Dankel SN, Svard J, Mattha S, Claussnitzer M, Klöting N, Glunk V, Fandalyuk Z, Grytten E, Solsvik MH, Nielsen H-J, Busch C, Hauner H, Blüher M, Skurk T, Sagen JV, Mellgren G. COL6A3 expression in adipocytes associates with insulin resistance and depends on PPARγ and adipocyte size. Obesity (Silver Spring). 2014;22:1807–13.
11. Karsdal MA, Henriksen K, Genovese F, Leeming DJ, Nielsen MJ, Riis BJ, Christiansen C, Byrjalsen I, Schuppan D. Serum endotrophin identifies optimal responders to PPARγ agonists in type 2 diabetes. Diabetologia. 2017;60:50–9.
12. Sun K, Park J, Gupta OT, Holland WL, Auerbach P, Zhang N, Marangoni RG, Nicoloro SM, Czech MP, Varga J, Ploug T, An Z, Scherer PE. Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. Nat Commun. 2014;5:3485.
13. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011;121:2094–101.
14. Sun K, Park J, Kim M, Scherer PE. Endotrophin, a multifaceted player in metabolic dysregulation and cancer progression, is a predictive biomarker for the response to PPARγ agonist treatment. Diabetologia. 2017;60:24–9.
15. Metzger BE, Gabbe SG. Persson B et al International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33:676–82.
16. Erol O, Ellidağ HY, Ayık H, Özel MK, Derbent AU, Yılmaz N. Evaluation of circulating betatrophin levels in gestational diabetes mellitus. Gynecol Endocrinol. 2015;31:652–6.
17. Scherer PE, Bickel PE, Kotler M, Lodish HE. Cloning of cell-specific secreted and surface proteins by subtractive antibody screening. Nat Biotechnol. 1998;16:581–6.
18. Park J, Scherer PE. Endotrophin - a novel factor linking obesity with aggressive tumor growth. Oncotarget. 2012;3:1487–8.
19. Iyengar P, Combs TP, Shah SJ, Gouon-Evans V, Pollard JW, Albanese C, Flanagan L, Tenniswood MP, Guha C, Lisanti MP, Pestell RG, Scherer PE. Adipocyte-secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene stabilization. Oncogene. 2003;22:6408–23.
20. Bu D, Crewe C, Kusminski CM, Gordillo R, Ghaben AL, Kim M, Park J, Deng H, Xiong W, Liu XZ, Lønning PE, Halberg N, Rios A, Chang Y, Gonzalez A, Zhang N, An Z, Scherer PE. Human endotrophin as a driver of malignant tumor growth. JCI Insight. 2019;5:e125094.
21. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, Zhang BB, Bonaldo P, Chua S, Scherer PE. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. Mol Cell Biol. 2009;29:1575–91.
22. Agarwal MM. Gestational diabetes mellitus: screening with fasting plasma glucose. World J Diabetes. 2016;25:279–89.
23. Kanagalingam MG, Forouhi NG, Greer IA, Sattar N. Changes in booking body mass index over a decade: retrospective analysis from a Glasgow Maternity Hospital. BJOG. 2005;112:1431–3.
24. Heslehurst N, Ells LJ, Simpson H, Batterham A, Wilkinson J, Summerbell CD. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. BJOG. 2007;114:187–94.
25. Leddy MA, Power ML, Schulkin J. The impact of maternal obesity on maternal and fetal health. Rev Obstet Gynecol. 2008;1:170–8.
26. Hauger MS, Gibbons L, Vik T, Belizán JM. Prepregnancy weight status and the risk of adverse pregnancy outcome. Acta Obstet Gynecol Scand. 2008;87:953–9.
27. Whiteman VE, Aliyu MH, August EM, McIntosh C, Duan J, Alio AP, Salihu HM. Changes in prepregnancy body mass index between pregnancies and risk of gestational and type 2 diabetes. Arch Gynecol Obstet. 2011;284:235–40.
28. Yong HY, Mohd Shariff Z, Mohd Yusof BN, Rejali Z, Tee YYS, Bindels J, van der Beek EM. Independent and combined effects of age, body mass index and gestational weight gain on the risk of gestational diabetes mellitus. Sci Rep. 2020;10:8486.
29. Franz M, Polterauer M, Springer S, Kuessel L, Haslinger P, Worda C, Worda K. Maternal and neonatal omentin-1 levels in gestational diabetes. Arch Gynecol Obstet. 2018;297:885–9.

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ORIGINAL ARTICLE

Comparison of biomarkers of oxidative stress, 8-isoprostane, advanced oxidation protein products, and 8-hydroxy-2′-deoxyguanosine and pro-apoptosis, cytokeratin 18 M30, in women with normal glucose tolerance and gestational diabetes mellitus

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Abstract

Aim We aimed to compare the values of selected biomarkers of oxidative stress, 8-isoprostane (8IsoP), advanced oxidation protein products (AOPP), and 8-hydroxy-2′-deoxyguanosine (8OHdG) and pro-apoptosis, cytokeratin 18 M30 (CK18 M30), in women with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM).

Methods The study included the following: NGT group, including pregnant women who were healthy and did not have a diabetic pregnancy (n=83) and GDM group, including pregnant women with GDM (n=69). The inclusion criteria were the following: being between the ages of 18–42, gestational age being 24–41 weeks, and the absence of labor. We collected perinatal clinical characteristics and measured serum 8IsoP, AOPP, 8-OHdG, and CK18 M30.

Results Prepregnancy body mass index and gestational weight gain and rate of cesarean delivery of the GDM group were significantly higher compared to the NGT group (p<0.05). Compared to the NGT group, the median values of serum 8IsoP, AOPP, 8OHdG, and CK18 M30 in the GDM group were found as increased significantly [8IsoP: 194.5 (121.8–255.9) vs. (153.1 (123.7–178.5); (p=0.023)]; [AOPP: 3.7 (1.7–8.9) vs. (2.1 (0.8–6.4); (p=0.036)]; [8OHdG: 11.8 (6.9–31.3) vs. (6.8 (1–28.2); (p=0.042)]; and [CK18 M30: 2.4 (1.4–5.4) vs. (1.8 (1.13.6); (p=0.044)].

Conclusions Women with GDM have increased oxidative stress presented by serum oxidized protein, lipid, and DNA biomarkers and pro-apoptosis demonstrated by CK18 M30. This supports the role of oxidized biomolecules in the pathogenesis of GDM. With piling data related to biomarkers changed into women with GDM, it will be possible to clarify the pathophysiology of GDM and its comorbidities.

Keywords Gestational diabetes mellitus · Oxidative stress · Pro-apoptosis · 8-Isoprostane · Advanced oxidation protein products · 8-Hydroxy-2′-deoxyguanosine · Cytokeratin 18 M30

Introduction

Diabetes is the most common medical complication of pregnancy that causes significant maternal and fetal morbidity [1]. There are several types of diabetes mellitus (DM) grouped on the basis of pathogenic process, including type 1 diabetes mellitus (T1DM), characterized with insulin requirement, type 2 DM (T2DM), characterized with insulin resistance; these are known as pregestational diabetes in perinatological perspective, and additionally, gestational diabetes mellitus (GDM), encountered during second half of pregnancy. It is predicted by the International Diabetes Federation that 2.2% of all

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pregnancies may be due to pregestational diabetes mellitus (PGDM) [2]. The most common complications in women with PGDM are macrosomia, cesarean delivery, preeclampsia, congenital anomaly, and stillbirth [3]. Gestational diabetes mellitus is a glucose intolerance condition that appears for the first-time during pregnancy and improves with delivery. The prevalence of GDM is increasing all over the world due to maternal obesity and advanced maternal age. The worldwide prevalence of GDM is estimated to be 9.3% and 25.5% [4].

Diabetic problems developing during pregnancy result in significant morbidity and mortality, leading to significant healthcare delivery costs concerning maternal and infant health. Although there are few studies to elucidate the molecular mechanisms underlying the development of diabetes complications, the exact pathophysiology of the events in pregnancies with DM has not been elucidated. In this context, one of the main mechanisms is the pathophysiological changes associated with oxidative stress. Oxidative stress develops when the rate of free radical formation exceeds the strength of antioxidant defense systems that combat the toxic effects of free radicals. Free radical species are physiological components that also have important beneficial effects on biological homeostasis, but when their production is excessive and increases beyond the body's antioxidant capacity, they evolve into a harmful state in the form of oxidative stress [5–8]. Oxidative stress causes an important upstream for the development of insulin resistance as well as diabetes complications and induces pathophysiological molecular mechanisms, leading to the operation of a number of deleterious pathways that lead to insulin resistance and aggravation of DM [9].

Various studies have shown that a diabetic state causes the development of a wide variety of oxidative stress–related processes [6–8] and proapoptotic changes [10, 11] in the human body. Studies have shown that women with DM have more oxidative stress products and less antioxidant products than normal pregnant women. In women with DM, excess glucose is auto-oxidized, which is involved in the generation of free radicals [12].

Understanding the pathophysiology of DM-related events during pregnancy, research activities depending on several biomarkers continue to increase for the development of more successful predictive, diagnostic, and prognostic biomarkers.

Massive cellular damage caused by excessive oxidative stress can be presented by the assessment of damaged proteins, lipids, and DNA. Lipid peroxidation leads to the production of the end products, including 8-isoprostane (8IsoP), which is derived from free radical-catalyzed, nonenzymatic oxidation of arachidonic acid and is considered to be an accurate, stable, and sensitive biomarker of endogenous lipid peroxidation [13].

Besides lipid peroxidation, protein oxidation is also presented under diabetic conditions. Oxidative stress can increase the development of protein cross-linking, fragmentation of the peptide in addition to formation of modified, denaturated, and non-functioning proteins revealed by intensified proteolysis reactions, leading to increased levels of advanced oxidation protein products (AOPP). Also, hyperglycemia in pregnant women can cause the development of oxidative DNA modification, which may be observed as an elevated level of 8-hydroxy-2′-deoxyguanosine (8-OhdG) [14].

Studies support that cytokeratin 18 (CK18) is an important protein involved in the cell death pathway. Types of cell death, apoptosis, and autophagy, are important physiological and pathophysiological processes used in the cell in response to abnormal cellular events such as endoplasmic reticulum (ER) stress and oxidative stress [15]. During cell death, the release of CK18 into the extracellular compartment takes place where they can be measured in body fluids. Both the whole CK18 protein (CK18 M65) and the caspase 3-cleaved fragment (CK18 M30) can be detected by biochemical methods. With CK18 M65, total cell death can be assessed, while with CK18 M30, specific caspase-3 dependent (apoptotic) cell death may also be determined. There is a potential of CK 18 M30 being a biomarker of disease states [16].

To achieve a better understanding of the clinical presentation and pathogenesis of diabetic states in pregnancy, it is necessary to select high-risk pregnant women with a potential for the development of severe DM-related morbidities and mortality, and examine the correlation among the concentrations of 8IsoP, AOPP, 8-OHdG, and CK18 M30 and the relationship of these biomarkers with clinical characteristics of women with GDM. The aim of this study was to compare the values of selected biomarkers of oxidative stress, 8IsoP, AOPP, and 8-OhdG and pro-apoptosis, CK18 M30, in women with or without GDM. We chose to assess a broader spectrum of oxidative stress biomarkers to cover different types of cellular damage under the influence of DM.

Material and methods

Study groups

The study groups were the following: normal glucose tolerance (NGT) group, including pregnant women who were healthy and did not have a diabetic pregnancy (n=83) and GDM group, including pregnant women with GDM (n=69). The inclusion criteria were the following: being between the ages of 18–42, gestational age being 24–41 weeks, and absence of labor. The exclusion criteria are multiple pregnancy, pregestational diabetes, preeclampsia, congenital fetal infections, fetal congenital malformations, pregnancy without an interval of 1-year, those with severe systemic disease and chronic drug use, and those having findings of infection and inflammation.

The diagnosis of GDM was made when any of the following at least one of the three criteria of 75g, 2-h oral glucose

tolerance test (OGTT) thresholds were met or exceeded: fasting 92 mg/dL, 1-h 180 mg/dL, or 2-h 153 mg/dL at 24–28 weeks of gestation [17].

Study population characteristics recorded included maternal age, gravidity, parity, ethnicity (native, emigrant), education levels (literate, primary school, secondary school, high school, higher education), family history of DM (yes or no), smoking status (yes or no), requiring assisted reproductive technology for conception (yes or no), pre-pregnancy body mass index, weight gain during pregnancy, single deepest pocket amniotic fluid, requirement of pharmacological treatment in GDM women (diet or insulin), mode of delivery (vaginal or cesarean), gestational age at delivery (week), the ratio of fetal gender (female or male), newborns birthweight, Apgar scores at 1 and 5 min, cord blood pH, and the ratio of NICU admission (yes or no).

Blood collection and analysis

From each study participant, fasting blood samples were collected when they attended for their second and third trimester antenatal visits. Maternal hematological and biochemical tests were performed. Serum AOPP, 8-OhdG, CK18 M30, and 8IsoP were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (BT LAB, China) according to the manufacturer's protocol. Serum samples were not diluted with those kits, and the standards were serially diluted from starting concentrations of 48 ng/mL down to 1.5 ng/mL for AOPP, from 1280 ng/L d to 40 ng/L for 8IsoP, from 128 to 4 ng/L for 8-OhdG, from 16 to 1ng/mL for CK18 M30, in the sample diluent supplied with the kit. The intra- and inter-assay coefficient of variation for the assays ranged between 8 and 10%.

Statistical analysis

Analysis of data was conducted with the IBM SPSS v23 (IBM, USA). Graphical presentations were prepared by using the GraphPad Prism v9 (GraphPad, San Diego, CA, USA). Descriptive analyses of numerical data as mean, standard deviation, median and interquartile range, and percentage were presented. After the normality test was done with Kolmogorov-Smirnov test, comparisons of parametric data with *t*-test and comparison of non-parametric data with Mann–Whitney *U* test were done. If the *p* value was below 0.05 after the analysis of the data, it was considered that there was a significant difference as a result of the comparison.

Results

The demographic and baseline clinical characteristics of the GDM and NGT groups are presented in the Table 1. The median ages of GDM group were significantly higher than that of the NGT group (31 (19–42) vs. 27 (18–42), respectively; $p<0.05$). There were no significant differences with regard to the gravidity, parity, the ratios of being literate and having secondary education, and the ratio of smoking between the study groups ($p>0.05$). The ratios of family history of DM, being native citizen, and the having natural pregnancies were significantly higher in the GDM group than that of the NGT group ($p<0.05$). We found significantly higher values in the preconception BMI, gestational weight gain, and single deepest pocket of amniotic fluid in the GDM group compared to the NGT group ($p<0.05$). In the GDM group, the ratio of insulin treatment was 46%. The ratio of cesarean delivery was higher in the GDM group than that of the NGT group ($p<0.05$). There were no significant differences between the study groups regarding the gestational age at delivery, the ratio of female newborns, Apgar scores at 1 and 5 min, cord blood pH, and the ratio of NICU admission ($p>0.05$). The mean birth weight in the GDM group was significantly higher than that of the NGT control (3549.1±591.0 vs. 3130.7 ±434.1, respectively; $p<0.05$).

Table 2 shows the maternal hematological and biochemical finding of the GDM and NGT groups. The mean white blood cell count value and the median neutrophil to lymphocyte ratio, aspartate aminotransferase, and lactate dehydrogenase values in the GDM group were significantly lower than that of the NGT group ($p<0.05$). The median values of fasting plasma glucose, HbA1c, and triglyceride were significantly higher than that of the NGT group ($p<0.05$). There were no significant differences between the study groups regarding the mean values of hemoglobin, hematocrit, platelet, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol ($p>0.05$). The median values of platelets to lymphocyte ratio, creatinine, and alanine aminotransferase were found as similar in the study groups ($p>0.05$).

Figures 1, 2, 3, 4 present the median values of serum 8IsoP, AOPP, 8OhdG, and CK18 M30 in the NGT and GDM groups. The median value of serum 8IsoP in the GDM group was significantly higher compared to the NGT group [194.5 (121.8–255.9) vs. (153.1 (123.7–178.5); ($p=0.023$)]. We found that the median value of serum AOPP in the GDM group was significantly higher than that of the NGT group [3.7 (1.7–8.9) vs. (2.1 (0.8–6.4); ($p=0.036$)]. The median value of serum 8OHdG in the women in GDM group was significantly higher compared to the NGT group [11.8 (6.9–31.3) vs. (6.8 (1–28.2); ($p=0.042$)]. We found that the median value of serum CK18 M30 in the GDM group was significantly higher than that of the NGT group [2.4 (1.4–5.4) vs. (1.8 (1.13.6); ($p=0.044$)].

Discussion

Women in the GDM group were older and they had more family history of DM in our study. Also, they had higher

Table 1 Maternal baseline clinical characteristics of the GDM and NGT groups

	NGT (*n*=83)	GDM (*n*=69)	*P* value
Maternal age (years)	27 (18–42)	31 (19–42)	0.001
Gravidity	3 (1–7)	3 (1–8)	0.751
Parity	2 (0–5)	2 (0–5)	0.880
Ethnicity, *n* (%)			0.001
Native	56 (67.5%)	64 (92.8%)	
Emigrant	27 (32.5%)	5 (7.2%)	
Education, *n* (%)			0.009
Literate	25 (30.1%)	7 (10.1%)	
Primary school	33 (39.8%)	29 (42%)	
Secondary school	14 (16.9%)	25 (36.2%)	
High school	10 (12%)	6 (8.7%)	
Higher education	1 (1.2%)	2 (2.9%)	
History of DM in family, *n* (%)			0.001
Yes	13 (15.7%)	34 (49.3%)	
No	70 (84.3%)	35 (50.7%)	
Smoking, *n* (%)			0.762
Yes	5 (6%)	5 (7.2%)	
No	78 (94%)	64 (92.8%)	
ART pregnancy, *n* (%)			0.026
Yes	0 (0%)	4 (5.8%)	
No	83 (100%)	65 (94.2%)	
Pre-pregnancy BMI (Kg/m^2)	26.6 (19–45.5)	34.4 (22.2–53.4)	0.001
Gestational weight gain (kg)	10 (2–25)	10 (0–29)	0.031
SDP amniotic fluid (mm)	30 (0–90)	46.5 (0–180)	0.001
Pharmacological treatment, *n* (%)			
Diet		39 (54%)	
Insulin		30 (46 %)	
Mode of delivery, *n* (%)			0.038
Vaginal	35 (42.2%)	18 (26%)	
Cesarean	48 (57.8%)	51 (74%)	
Gestational age at delivery (week)	39 (28–41)	38 (25–41)	0.847
Fetal gender, *n* (%)			0.704
Female	48 (57.8)	42.(60%)	
Male	35 (42.2%)	27 (40%)	
Birth weight (g)	3130.7±434.1	3549.1±591.0	0.001
Apgar score			
At 1 min	9 (5–9)	9 (2–9)	0.817
At 5 min	10 (6–10)	10 (6–10)	0.707
Cord blood pH	7.34 (6.96–7.49)	7.34 (7.2–7.5)	0.77
NICU admission, *n* (%)			0.69
Yes	10 (12%)	16 (23%)	
No	73 (88%)	53 (77%)	

Data were presented as mean with standard deviation, median with range, or number (%) as appropriate. *NGT*, normal glucose tolerance; *DM*, diabetes mellitus; *ART*, assisted reproductive technology; *BMI*, body mass index; *SDP*, single deepest pocket; *NICU*, neonatal intensive care unit

values of pre-pregnancy BMI, higher values of fasting plasma glucose, HbA1c, and triglyceride. The women in the GDM group had a higher gestational weight gain than that of the NGT group. We found significantly higher median values of serum 8IsoP, AOPP, 8OhdG, and CK18 M30 in the GDM group compared to the NGT group.

Table 2 Maternal hematological and biochemical finding of the GDM and NGT groups

	NGT (n=83)	GDM (n=69)	P value
WBC (10^3 uL)	12.7±4.3	10.9±3.1	0.006
Hb (g/dL)	11.1±1.4	11.4±1.3	0.202
Hct (%)	33.6±3.9	33.3±6.5	0.715
PLT (10^3 uL)	219±63.7	223±70	0.750
PLR	130 (1.2–322.5)	116 (0-260)	0.117
NLR	5.8 (0–35.3)	3.9 (0-18.6)	0.022
Fasting plasma glucose (mg/dL)	82 (60–126)	95 (70-225)	0.001
HbA1c (%)	5.2 (4.3–6.1)	5.8 (4.3-9.7)	0.001
Creatinine (mg/dL)	0.48 (0.3–0.72)	0.45 (0.3-0.8)	0.589
AST (IU/L)	17 (9–45)	16 (5-37)	0.013
ALT (IU/L)	10 (7–40)	11 (7-40)	0.914
LDH (IU/L)	253 (159–445)	187 (133-449)	0.001
Triglyceride (mg/dL)	198 (106–597)	239 (79-435)	0.002
HDL-C (mg/dL)	59.3±15.4	59.4±17.2	0.985
LDL-C (mg/dL)	114.7±37.1	105±46.8	0.215

Data were presented as mean with standard deviation or median with range as appropriate. *NGT*, normal glucose tolerance; *DM*, diabetes mellitus; *WBC*, white blood cell count; *Hb*, hemoglobin; *Hct*, hematocrit; *PLT*, platelet; *PLR*, platelets to lymphocyte ratio; *NLR*, neutrophil to lymphocyte ratio; *HbA1c*, hemoglobin A1C; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *LDH*, lactate dehydrogenase; *IU*, international unit; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol

In parallel with the increasing obesity rates worldwide, the frequency of all diabetes types in pregnancy continues to increase. While the gestational form of diabetes is more common during pregnancy, the T2DM form of the pregestational form has become more common than T1DM [18]. DM increases the perinatal risks faced by the mother and infant in general, proportionally to the degree of hyperglycemia, but also associated with the chronic complications and comorbidities of diabetes. In general, the specific risks of diabetes in pregnancy include a variety of serious events such as spontaneous abortion, fetal anomalies, preeclampsia, fetal death, macrosomia, birth injuries, increased cesarean delivery rates, neonatal hypoglycemia, hyperbilirubinemia, and neonatal respiratory distress syndrome, among others. In addition,

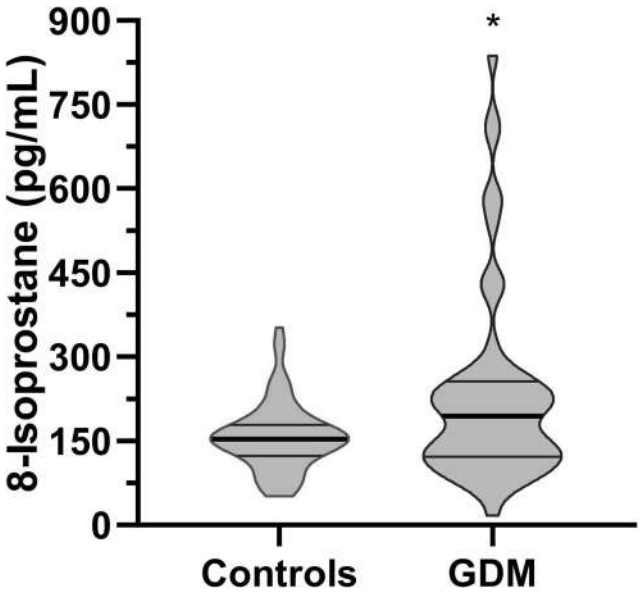

Fig. 1 Median values of serum 8IsoP in the NGT and GDM groups. Data are expressed as median with interquartile range (25–75%). *Mann–Whitney test revealed significantly higher median serum 8IsoP in the GDM group compared to NGT group (p=0.023)

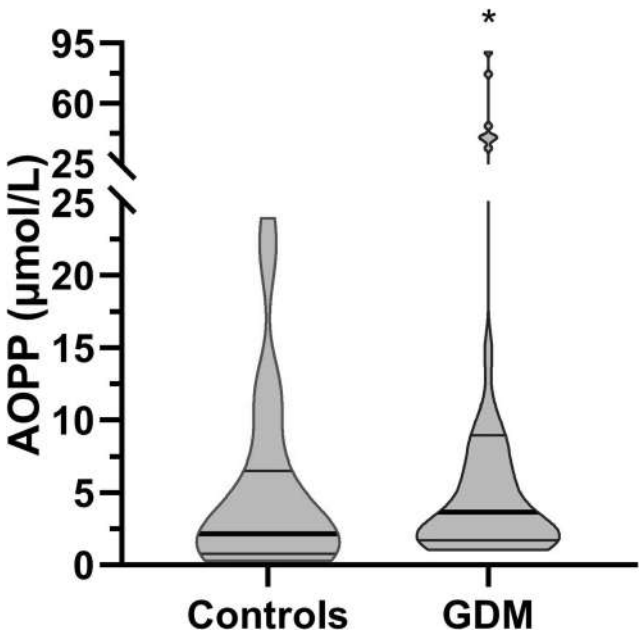

Fig. 2 Median values of serum AOPP in the NGT and GDM groups. Data are expressed as median with interquartile range (25–75%). *Mann–Whitney test revealed significantly higher median serum AOPP in the GDM group compared to NGT group (p=0.036)

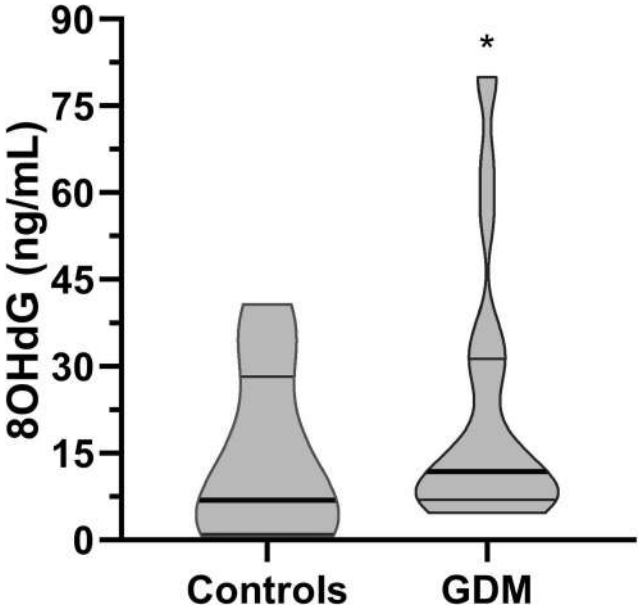

Fig. 3 Median values of serum 8OHdG in the NGT and GDM groups. Data are expressed as median with interquartile range (25–75%). *Mann–Whitney test revealed significantly higher median serum 8OHdG in the GDM group compared to NGT group (p=0.042)

when there is DM during pregnancy, obesity, hypertension, and T2DM can be seen more in babies later in life. Compared to PGDM, when good glucose control can be achieved with diet (and insulin if necessary), it is possible to reduce maternal and fetal risks to acceptable levels in GDM cases [18].

Hyperglycemia due to abnormal glucose metabolism plays a key role in the development of GDM and T2DM. Chronic persistent hyperglycemia is known to play a role in the development of micro- and macro-vascular complications, which are known to occur through a number of mechanisms including oxidative stress and inflammation. As a result of the oxidative stress caused by hyperglycemia, ROS are known to damage DNA, lipids and proteins, and the degree of damage or injury is related to the duration and severity of the hyperglycemia [7]. It is thought that oxidative stress, inflammation, and insulin resistance, which interact in the pathophysiology of T2DM, determine the clinical picture of the disease with the help of disorders in insulin secretion, glucose utilization, and hepatic glucose metabolism and increased pro-inflammatory cytokines [7].

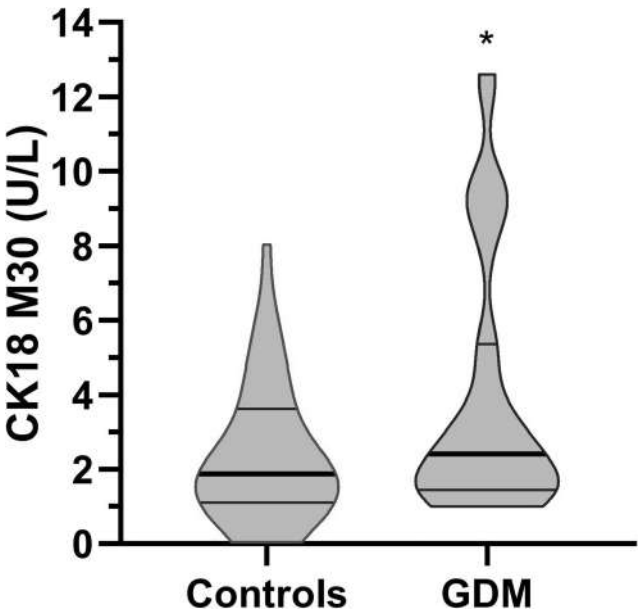

Fig. 4 Median values of serum CK18 M30 in the NGT and GDM groups. Data are expressed as median with interquartile range (25–75%). *Mann–Whitney test revealed significantly higher median serum CK18 M30 in the GDM group compared to NGT group (p=0.044)

Recently, the role of oxidative stress in diabetic pregnancies has attracted the attention of investigators [12], although their value for the prediction and diagnosis of diabetic pregnancies has not been systematically characterized. Various studies have supported that oxidative stress changes in parallel with insulin resistance and plays a central role in the short- and long-term effects of DM, with research results showing that biomarkers, including protein, lipid, and DNA damage products are high in body fluids taken from diabetic individuals [19].

8IsoP, a family of compounds produced from arachidonic acid in membrane phospholipids via a free radical–catalyzed mechanism, have been used as the most reliable markers of in vivo lipid peroxidation resulting from oxidative stress [20]. Kapustin et al. determined the serum levels of 8IsoP, nitrotyrosine, and total antioxidant capacity in pregnant women with different types of DM. They found that elevated 8-isoprostane levels were observed in all patients with DM, but this biomarker's maximum value had been seen in T1DM and T2DM on insulin groups. They concluded that DM activated oxidative stress might lead to the development of adverse perinatal outcomes [21]. In a prospective study conducted to compare the levels of oxidative stress biomarkers between pregnancies with GDM and pregnancies, they examined the maternal blood that was collected at gestational age 24–28 weeks and at early labor and they also collected fetal cord blood to analyze the levels of 8IsoP, TNF-α, and IL-10. They reported that maternal serum 8Isop and TNF-α levels were significantly higher in the GDM group at 24–28 weeks and at early labor. They suggested that GDM was significantly associated with inflammatory process when compared to normal pregnancies, but the biomarkers in the cord blood as well as maternal and neonatal outcomes in GDM were not significantly different [22].

AOPP is a group of oxidized proteins that occurs when oxidation overload is encountered and can be used as a biomarker of both oxidative stress and inflammation because proteins, being present and abundant in cells, plasma, and most tissues, are the major targets of oxidants. It is also more stable than biomarkers that measure lipid oxidation products [23]. In a study evaluating maternal circulating levels of

oxidant/antioxidant biomarkers in women with GDM and preeclampsia and with uncomplicated pregnancies between at gestational weeks of 24 and 36, the authors found that antioxidant defense biomarkers were decreased in women with GDM and preeclampsia that the levels of AOPP were increased in women with GDM, but not with preeclampsia [24]. Li et al. examined plasma markers of oxidative stress during the second and third trimester of pregnancy in patients with GDM with 8-iso-prostaglandin F2α, AOPP, protein carbonyl, glutathione peroxidase-3, and paraoxonase-1 at 16–20 weeks, 24–28 weeks, and 32–36 weeks of gestation [25]. Numerous studies indicate that AOPP are significantly increased in patients with DM with or without complications [24–27].

8-OHdG is frequently used in oxidative stress studies as it is a valuable biomarker for evaluating DNA oxidation [23]. Many studies have found a link between diabetes and oxidative stress by measuring various biomarkers including DNA damage and lipid peroxidation products. Free radicals are thought to play a key role in the initiation and progression of diabetes complications, as they damage lipids, proteins, and DNA. Qiu et al. conducted a study examining the predictive value of maternal urinary 8-OHdG in pregnant women with a gestational age of less than 20 weeks to determine the development of GDM. According to their findings, high levels of urine 8-OHdG in early pregnancy might be associated with increased GDM risk [28].

During cell death both in vitro and in vivo, the soluble fraction of CK18 can be released extracellularly [15]. A recent study has shown that CK18 is involved in the regulation of transcription of a number of genes in the NF-κB pathway and certain apoptosis pathways [29]. Besides caspase-induced apoptosis, CK18 can also be released in other types of cell death. For example, CK18 is known to increase during pyroptosis, non-lethal apoptosis processes independent of caspase activation, or secondary lysis following programmed cell death [30]. Karakus et al. investigated the serum levels of endothelin-1 (ET-1), CK 18 M30, and angiopoietins (Ang) 1-2 in patients with preeclampsia or HELLP syndrome, and normal controls in a cross-sectional study. They found that in HELLP syndrome, ET-1, CK 18 M30, and Ang-2 were higher compared to healthy or preeclamptic pregnancies, and they suggested that serum CK 18 M30 levels were being a promising test for the prediction or differential diagnosis of HELLP syndrome in PE patients [31].

There are limitations of this study. Firstly, it was a cross-sectional study with biomarkers measured only at one time point in second half of pregnancy, which could not unveil their altered levels throughout the pregnancy. Secondly, the sample size was relatively small. Well-designed prospective studies with larger sample size are needed in our future work. Other limitation that needs to be mentioned is fact that all the data were collected from a single center. However, it is novel in being the first study that investigates biomarkers of oxidative stress and pro-apoptosis in the same clinical settings in women with GDM. However, these shortcomings do not change the fact that the measured biomarkers are worthy of attention in GDM diagnosis. As summarized in a new review examining the diagnostic value of biomarkers measured from the second trimester to delivery in women with gestational diabetes, few of the more than 100 studies, most of which measure biomarkers with maternal blood analysis, biomarkers such as leptin, ficolin3/adiponectin ratio, or chemerin/fatty acid binding protein ratio, could diagnose GDM with a sensitivity and specificity of over 80%. The heterogeneity of methods used in the included articles to diagnose GDM and to assess the biomarkers is a limitation that precluded meta-analysis and allowed only narrative synthesis [32, 33].

In conclusion, women with GDM have increased levels of biomarkers of oxidized protein, lipid, and DNA and pro-apoptosis in maternal blood, and they develop abnormal oxidative stress- and pro-apoptosis-related changes that can reduce their ability to compensate for cellular stresses. This supports the role of oxidized biomolecules in the pathogenesis of GDM. With piling data related to biomarkers that changed in women with GDM, it will be possible to clarify the pathophysiology of GDM and its comorbidities. Ongoing future studies need to focus on whether it is possible to make more successful early GDM screening and diagnosis with the search of new biomarkers involved in the pathophysiology of GDM, with a promising efficacy to replace the OGTT used in current GDM screening.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by İcten Olgu Bafali, Merve Topaktas, Hatice Argun Atalmis, Sevilay Yavuz Dogu, Busra Seker Atas, Esma Ozdemir Anayurt, and Tugba Muhlise Okyay. The first draft of the manuscript was written by Filiz Yarsilikal Guleroglu and Ali Cetin, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations This hospital-based observational case-control study was conducted in at the Department of Gynecology and Obstetrics in Haseki Training and Research Hospital affiliated with the University of Health Sciences in the Sultangazi district of Istanbul, following the approval of the local ethics committee, within a 1-year period, following the appropriate clinical ethical guidelines and the valid Helsinki Declaration. Informed written consent was obtained from each pregnant woman after the purpose and nature of all procedures used were fully explained. Care was taken to include the cases in the research groups one after the other. The research did not include any aspect that may harm the participant's pregnancy care.

Conflict of interest The authors declare no competing interests.

References

1. Newman C, Kgosidialwa O, Dervan L, Bogdanet D, Egan AM, Biesty L, et al. Patient-reported outcomes (PROs) in randomised controlled trials in diabetes and pregnancy: protocol for a systematic review. BMJ Open [Internet]. 2021;11(11):e052506. Available from: http://bmjopen.bmj.com/content/11/11/e052506.abstract
2. Yuen L, Saeedi P, Riaz M, Karuranga S, Divakar H, Levitt N, et al. Projections of the prevalence of hyperglycaemia in pregnancy in 2019 and beyond: results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract [Internet]. 2019;157:107841. Available from: https://www.sciencedirect.com/science/article/pii/S0168822719312458
3. Dunne FP, Avalos G, Durkan M, Mitchell Y, Gallacher T, Keenan M, et al. ATLANTIC DIP: pregnancy outcome for women with pregestational diabetes along the Irish Atlantic Seaboard. Diabetes Care [Internet]. 2009;32(7):1205 LP – 1206. Available from: http://care.diabetesjournals.org/content/32/7/1205.abstract
4. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel–recommended criteria. Diabetes Care [Internet]. 2012;35(3):526 LP – 528. Available from: http://care.diabetesjournals.org/content/35/3/526.abstract
5. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev [Internet]. 2020;2020:8609213. Available from: https://pubmed.ncbi.nlm.nih.gov/32215179
6. Maiese K. New insights for oxidative stress and diabetes mellitus. Oxid Med Cell Longev [Internet]. 2015/05/12. 2015;2015:875961. Available from: https://pubmed.ncbi.nlm.nih.gov/26064426
7. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol [Internet]. 2019;11(3):45–63. Available from: https://pubmed.ncbi.nlm.nih.gov/31333808
8. Grindel A, Guggenberger B, Eichberger L, Pöppelmeyer C, Gschaider M, Tosevska A, Mare G, Briskey D, Brath H, Wagner KH Oxidative stress, DNA damage and DNA repair in female patients with diabetes mellitus type 2. PLoS One [Internet]. 2016;11(9):e0162082–e0162082. Available from: https://pubmed.ncbi.nlm.nih.gov/27598300
9. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: review of the underlying molecular mechanisms. J Cell Physiol. 2019;234(6):8152–61.
10. Miyasato M, Murase-Mishiba Y, Bessho M, Miyawaki M, Imbe H, Tsutsumi C, Tanimoto K, Imagawa A, Terasaki J, Hanafusa T. The cytokeratin-18 fragment level as a biomarker of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. Clin Chim Acta. 2014;433:184–9.
11. Zhao C, Lou F, Li X, Ma J, Zhu Z, Li H, Zhai Y, Chen H, Zhang Q, Liu Z, Xiao S. Correlation of CD3+/CD4+, and serum CK-18 fragment levels with glucose and lipid metabolism in elderly type 2 diabetes patients with nonalcoholic fatty liver disease. Am J Transl Res. 2021;13(4):2546–54.
12. Hussain T, Murtaza G, Metwally E, Kalhoro DH, Kalhoro MS, Rahu BA, Sahito RGA, Yin Y, Yang H, Chughtai MI, Tan B. The role of oxidative stress and antioxidant balance in pregnancy. Mediators Inflamm. 2021;2021:9962860–11.
13. Shang M, Zhao J, Yang L, Lin L. Oxidative stress and antioxidant status in women with gestational diabetes mellitus diagnosed by IADPSG criteria. Diabetes Res Clin Pract. 2015;109(2):404–10.
14. Urbaniak SK, Boguszewska K, Szewczuk M, Kaźmierczak-Barańska J, Karwowski BT. 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a potential biomarker for gestational diabetes mellitus (GDM) development. Molecules. 2020;25(1).
15. Qian L, Zhang L, Wu L, Zhang J, Fang Q, Hou X, Gao Q, Li H, Jia W. Elevated serum level of cytokeratin 18 M65ED is an independent indicator of cardiometabolic disorders. J Diabetes Res. 2020;2020:5198359–10.
16. Cave M, Falkner KC, Henry L, Costello B, Gregory B, McClain CJ. Serum cytokeratin 18 and cytokine elevations suggest a high prevalence of occupational liver disease in highly exposed elastomer/polymer workers. J Occup Environ Med. 2011;53(10):1128–33.
17. Coustan DR, Lowe LP, Metzger BE, Dyer AR. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: paving the way for new diagnostic criteria for gestational diabetes mellitus. Am J Obstet Gynecol. 2010;202(6):654.e1-6.
18. Fong A, Serra A, Herrero T, Pan D, Ogunyemi D. Pre-gestational versus gestational diabetes: a population based study on clinical and demographic differences. J Diabetes Complications. 2014;28(1):29–34.
19. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-a concise review. Saudi Pharm J SPJ Off Publ Saudi Pharm Soc. 2016;24(5):547–53.
20. Vichova T, Waldauf P, Karpisek M, Jarkovsky J, Motovska Z. Oxidative stress markers, thioredoxin 1 and 8-isoprostane, in relation to ischemic time in patients with ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention. Polish Arch Intern Med. 2021;131(7–8):755–8.
21. Kapustin R, Chepanov S, Kopteeva E, Arzhanova O. Maternal serum nitrotyrosine, 8-isoprostane and total antioxidant capacity levels in pre-gestational or gestational diabetes mellitus. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol. 2020;36(sup1):36–42.
22. Rueangdetnarong H, Sekararithi R, Jaiwongkam T, Kumfu S, Chattipakorn N, Tongsong T, et al. Comparisons of the oxidative stress biomarkers levels in gestational diabetes mellitus (GDM) and non-GDM among Thai population: cohort study. Endocr Connect [Internet]. 2018/04/17. 2018;7(5):681–7. Available from: https://pubmed.ncbi.nlm.nih.gov/29666171
23. Stanek A, Brożyna-Tkaczyk K, Myśliński W. Oxidative stress markers among obstructive sleep apnea patients. Oxid Med Cell Longev. 2021;2021:9681595–8.
24. Karacay O, Sepici-Dincel A, Karcaaltincaba D, Sahin D, Yalvaç S, Akyol M, Kandemir Ö, Altan N. A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24-36 weeks of gestation. Diabetes Res Clin Pract. 2010;89(3):231–8.
25. Li H, Yin Q, Li N, Ouyang Z, Zhong M. Plasma markers of oxidative stress in patients with gestational diabetes mellitus in the second and third trimester. Obstet Gynecol Int. 2016;2016:3865454–8.
26. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersole de Strihou C, Monnier VM, Witztum JL, Kurokawa K. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J Clin Invest. 1997;100(12):2995–3004.
27. Liang M, Wang J, Xie C, Yang Y, Tian JW, Xue YM, Hou FF. Increased plasma advanced oxidation protein products is an early marker of endothelial dysfunction in type 2 diabetes patients without albuminuria 2. J Diabetes. 2014;6(5):417–26.
28. Qiu C, Hevner K, Abetew D, Enquobahrie DA, Williams MA. Oxidative DNA damage in early pregnancy and risk of gestational diabetes mellitus: a pilot study. Clin Biochem. 2011;44(10–11):804–8.

29. Cheng Y, Qin K, Huang N, Zhou Z, Xiong H, Zhao J, Zhang Y, Yu S. Cytokeratin 18 regulates the transcription and alternative splicing of apoptotic-related genes and pathways in HeLa cells. Oncol Rep. 2019;42(1):301–12.
30. Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. J Hepatol. 2014;60(5):1063–74.
31. Karakus S, Bozoklu Akkar O, Yildiz C, Sancakdar E, Cetin M, Cetin A. Serum levels of ET-1, M30, and angiopoietins-1 and -2 in HELLP syndrome and preeclampsia compared to controls. Arch Gynecol Obstet. 2016;293(2):351–9.
32. Di Filippo D, Wanniarachchi T, Wei D, Yang JJ, Mc Sweeney A, Havard A, et al. The diagnostic indicators of gestational diabetes mellitus from second trimester to birth: a systematic review. Clin Diabetes Endocrinol [Internet]. 2021;7(1):19. Available from: https://doi.org/10.1186/s40842-021-00126-7.
33. Huhn EA, Rossi SW, Hoesli I, Göbl CS. Controversies in screening and diagnostic criteria for gestational diabetes in early and late pregnancy. Front Endocrinol (Lausanne). 2018;9:696.

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ORIGINAL ARTICLE

Total cholesterol and postprandial triglyceride levels as early markers of GDM in Asian Indian women

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Abstract

Background/objective Early detection and prompt treatment of GDM will go a long way in reducing the burden of T2DM. This study was undertaken to evaluate whether abnormal lipid profile including fasting as well as postprandial triglycerides, fasting total cholesterol, LDL cholesterol, and HDL cholesterol at 12 weeks of gestation (first trimester) may be used as simple predictive risk markers for developing GDM at a later stage.

Methods It was a prospective case-control study carried out in 45 subjects with GDM and selected equal number of age and BMI matched non-GDM subjects. A mixed meal consisting of two plain parathas and 20-g butter was given to all the subjects to evaluate 4-h triglyceride levels. Lipid levels were measured in all subjects at 11–13 weeks of gestation. At 24–28 weeks, all subjects were screened for GDM using a standardized oral glucose tolerance test.

Results The study found significantly higher levels of fasting cholesterol, LDLc, and PPTg levels in subjects in their first trimester in those with GDM compared to those without GDM. Logistic regression analysis revealed higher odds of GDM with increasing 4-h PPTg levels {OR=1.01(1.001-1.02), p=0.02} and fasting cholesterol [OR=1.02 (1.001-1.03), p=0.001]. The ROC curve generated for the combined model of total cholesterol and PPTg has a higher area under the curve as compared to other parameters.

Conclusion A combination of elevated fasting cholesterol and PPTg levels at an early stage of gestation can significantly predict future GDM.

Keywords Gestational diabetes mellitus · Total cholesterol · Postprandial triglycerides · HDL cholesterol · Biomarker

Introduction

Gestational diabetes mellitus (GDM) is growing as a major public health issue as its prevalence varies from 3.8 to 21% in different parts of the world including India [1–4]. GDM imposes adverse effects on both maternal and fetal health [5]. Subjects with GDM remain susceptible to progress to type 2 diabetes mellitus (T2DM) and hence are major contributors in the escalation of the prevalence of T2DM [3]. Early detection and prompt treatment of GDM will go a long way in reducing this burden, and hence early screening with appropriate biomarkers has been the subject of intense research. Risk factors, such as higher age [6], obesity [7], family history [8], fasting insulin [9], HOMA-IR [9], serum adiponectin [10], leptin [11], SHBG [12], and lipid levels [13] which are also established risk factors for T2DM, have been found to be associated with increased risk of GDM. Increased insulin resistance, decreased insulin secretion, and altered insulin signaling have been reported as hallmarks of pregnancy owing to the underlying altered hormonal milieu [12]. However, the exact phenomenon underlying the onset of GDM is not fully understood. Recently, few studies have shown higher lipid levels, more specifically postprandial triglycerides (PPTg) as an early phenomenon associated with the onset of insulin

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resistance and with an increased risk of T_2DM [14]. PPTg may also be associated with the risk of GDM as its pathogenesis is believed to be similar to T2DM. Although many studies have evaluated fasting lipid profile (total cholesterol, triglyceride, HDLc, LDLc, and Tg/HDLc) as an early marker for GDM, the results failed to show consistency [13, 15–21]. There are no studies, to the best of our knowledge, evaluating the role of PPTg levels as a predictor of GDM. Intolerance to the standardized high fat load in GDM subjects which sometimes stimulates nausea and vomiting could be a major limitation in using fat challenge to study postprandial lipids. This study was undertaken to evaluate whether abnormal lipid profiles including fasting as well as postprandial triglycerides, fasting total cholesterol, LDLc, and HDLc at 12 weeks of gestation (first trimester) may be used as simple predictive risk markers for developing GDM at a later stage.

Materials and methods

It was a prospective case-control study carried out in the Department of Endocrinology and Department of Obstetrics & Gynaecology at University College of Medical Sciences & GTB Hospital. Ethical clearance was obtained from postgraduate review committee of University college of Medical Sciences and written consent was obtained from all the study participants. A total of 450 antenatal females with a singleton pregnancy with 11–13 weeks of gestation were recruited in the study. Written informed consent was obtained from all the participants. Women with overt diabetes mellitus were excluded from the study. Subjects were called in the morning for a fat challenge test following the overnight fasting of 10–12 hours. All the women who underwent the fat challenge test were asked to come at 24–28 weeks of gestation for an OGTT to diagnose GDM. After OGTT, we identified 45 subjects with GDM and selected an equal number of age and BMI-matched subjects in the control group (non-GDM subjects). These groups were formed from the 450 subjects who were recruited in the study 11–13 weeks ago. In fat challenge test, a mixed meal consisting of two plain parathas and 20-g butter (Amul) was given to all the subjects. This contained a total of 396 kcal which came mainly from 22.8-g fat, 31.4-g carbohydrate, and 5.3-g protein. 2-ml blood samples were collected in the fasting state just before the meal, i.e., at 0 h, for fasting lipids including TGs and 1 ml again after 4 h of fat challenge to assess postprandial TGs response. GDM was characterized on the basis of International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria [1].

Biochemical estimation

Plasma glucose was estimated using glucose oxidase/peroxidase method using Randox kit (catalogue number GL2614), serum triglyceride by the method of Werener and Gabriesulsen using Randox Kit(catalog number TR3823), total cholesterol by Allen et al. method *(catalogue number CH8019)*, and *HDL-cholesterol by third generation direct homogeneous assay (catalogue number CH 3811). All the samples were evaluated in duplicate as per standard laboratory practices. LDL-cholesterol and VLDL-cholesterol were calculated by Friedewald's equation.*

Statistical analysis

All the data were represented as mean ± SD. p value ≤0.05 was considered statistically significant. Study parameters were compared using an independent t test. Logistic regression analysis was applied for lipid levels in the first trimester to predict the risk of GDM. *ROC curves were generated for lipid parameters. The statistical analysis was performed using SPSS 20.0.*

Results

The study included 45 subjects with GDM and equal number of age and BMI matched non-GDM subjects. The mean age of subjects in the GDM group was 25.31 ± 3.12 years and in non-GDM group was 24.16 ± 3.0 years. The mean BMI of the study subjects was 24.75±2.76 kg/m^2. Different lipid parameters were analyzed concerning the risk of GDM.

The study found significantly higher levels of fasting cholesterol, LDLc, and PPTg levels in subjects in their first trimester in those with GDM compared to those without GDM. However, there was no significant difference in any other lipid parameter between the groups (Table 1). Serum insulin was higher in the GDM group but was not significantly different as compared to the non-GDM group.

The relative risk calculation on 75th percentile value of different lipid parameters at 95% confidence intervals is given in Table 2.

Logistic regression analysis revealed higher odds of GDM with increasing 4-h PPTg levels [OR=1.01(1.001–1.02), p=0.02] and fasting cholesterol [OR=1.02(1.001–1.03), p=0.001].

Based on the ROC curve, we found that a cut-off value of 157mg/dl of fasting cholesterol at an early stage of pregnancy can predict the risk of GDM with 76% sensitivity and specificity. Similarly, a cut-off value of 107 mg/dl for LDL and 143mg/dl for PPTg can be used as early predictors for GDM as shown in Table 3 and Figure 1. The ROC curve generated for the combined model of total cholesterol and PPTg has a higher area under curve as compared to that of both the parameters independently. Also, at a 20% false positivity rate, the detection rate of total cholesterol and combined model of total cholesterol and PPTg were more than 70%. Further ROC generated for all the quartiles of the data set for cholesterol

Table 1 Comparison of lipid parameters *in the first trimester between the GDM and non-GDM groups*

Parameters	GDM mean ± SD	Non-GDM mean ±SD	*p* value
Fasting total cholesterol (mg/dl)	227.20±115.42	146±45.21	<0.001
Fasting triglycerides (mg/dl)	124.69±60.99	106.07±35.24	0.082
Fasting LDLc (mg/dl)	161.89±113.32	85.23±45.52	<0.001
Fasting VLDLc (mg/dl)	24.94±12.60	21.12±7.04	0.08
Fasting HDLc (mg/dl)	40.53±8.55	38.95±8.67	0.387
Postprandial-TG'S (4 h)	176.42±73.41	137.88±43.90	0.008
FastingTg/HDLc	3.22±2.87	2.87±1.23	0.17
PPTg/HDLc	4.57±2.57	3.70±1.57	0.06

revealed that the third quartile ROC has 0.86 AUC and a cut-off value of 173 mg/dl of cholesterol has predicted GDM with 81% sensitivity and 100% specificity (Figure 2).

Discussion

The present study found significantly higher levels of total cholesterol, LDL cholesterol, and postprandial triglyceride levels at 11–13 weeks of gestation in those who were later diagnosed with GDM at 25–28 weeks of gestation as compared to that of those who remained normal glucose tolerant. PPTg levels were significantly higher despite the observation that fasting Tg levels were similar in both groups. Fasting total cholesterol and LDL cholesterol levels were strong predictors of GDM followed by PPTg. ROC curve analysis showed fasting total cholesterol as a highly sensitive and specific test at an early stage of pregnancy to predict the risk of GDM. The results were enhanced further when we used the combined model consisting of total cholesterol, LDL cholesterol, and postprandial triglyceride as a predictor.

This is the first prospective cohort study, to the best of our knowledge, which has evaluated PPTg as a biomarker to predict GDM risk alone and in combination with other 1st-trimester lipid parameters. However, the strength of this prediction was next only to fasting total and LDL cholesterol which emerged as stronger predictors of GDM. The relatively modest effects of PPTg in predicting the risk of GDM despite several studies suggesting that they are strong predictors for T2DM [22–24] may be due to several factors. Firstly, the amount of fat used in this study was much lower, i.e., 22.8 gm as compared to 62.5 g used in standard fat tolerance tests performed in previous studies in T2DM subjects [23]. Secondly, the mixed meal consisting of Parathas and butter instead of the whipped cream used earlier could also have affected postprandial Tg responses. These changes were necessary as the pregnant patients could not tolerate the usual fat meal. It is also possible that lipid responses in pregnancy may be different than in the nonpregnant state. Despite these limitations, postprandial Tg levels when combined with total cholesterol and LDL cholesterol levels significantly increased the area under the curve in ROC prediction analysis for GDM.

We did not observe any significant difference in terms of fasting triglycerides at 11–13 weeks of gestation. This indicates that PPTg levels increase earlier than fasting triglycerides levels and may be more useful as a biomarker to predict GDM. Daniel et al. in 2004 found in their prospective study that each 20mg/dl increase in triglyceride levels was associated with a 10% increase in GDM risk [25]. In another study by Lai et al, it was demonstrated that the higher midtrimester TG levels were accompanied by higher glucose in subjects with GDM [26]. Only one study has elucidated the status of 5-h postprandial triglyceride levels in GDM subjects; however, they did not have any control group in their study to compare the difference in PPTg levels [27]. The increase in PPTg at 11–13 weeks of gestation indicates that it has a role in the development of GDM. Earlier it has been demonstrated that higher PPTGs levels were associated with a higher risk of diabetes both in human and animal models [14, 22, 28–30]. However, their role in the prediction of GDM has not been

Table 2 Risk of GDM on the basis of 75th percentile value of different 1st-trimester lipid parameters

Lipid parameters	Relative risk	Confidence interval	*p* value	Estimated value on 75th percentile (mg/dl)
Fasting total cholesterol	3.51	1.41–8.79	<0.001	>216
Fasting LDLc	3.50	1.41–8.74	<0.001	>144
Fasting HDLc	1.2	0.71–2.01	0.31	<33
Fasting triglycerides	1.3	0.78–2.39	0.16	>131
Postprandial triglycerides (4 h)	1.58	0.87–2.89	0.07	>173

Table 3 ROC curve indices for lipid parameters

Test results variable	Area under curve	*p* value	Value (mg/dl)	Sensitivity (%)	specificity (%)	Detection rate for false positive rate of 10% (95% CI)	Detection rate for false positive rate of 20% (95% CI)
Total Cholesterol	0.81	.001	157	76	76	71 (0.72–0.90)	73 (0.72–0.90)
LDL-c	0.80	.001	107	71	80	64 (0.70–0.89)	71 (0.70–0.89)
PPTG	0.64	.026	143	60	56	22 (0.52–0.75)	38 (0.52–0.75)
Combined (total cholesterol, PPTG)	0.844	.001	---	----	----	69 (0.76–0.92)	78 (0.76–0.92)

evaluated before. A few earlier studies which correlated fasting TGs and TAGs with GDM have found conflicting results. Whereas few of them have reported elevated levels of TAGs and TGs among GDM patients [31], others have failed to demonstrate significant differences from non-GDM pregnant women [32, 33]. Emet et al. did not observe positive associations between GDM and fasting TGs [32]. Similarly, Rizzo et al. did not find any differences in the concentration of fasting TGs between GDM women and controls at 24–28 weeks of gestation [33] in a smaller sample size. However, most of these studies used fasting TGs measured at 24–28 weeks and not in the 1st trimester which could have allowed GDM prediction.

Our findings report a higher level of total cholesterol and LDL cholesterol levels at an early stage of gestation that might be associated with a higher risk of GDM. Similarly, some studies have demonstrated that women who developed GDM had a higher concentration of fasting cholesterol at an early stage of gestation [15, 16, 34]. However, other studies with a design such as ours wherein early lipid markers were evaluated to predict the risk of GDM were unable to display any significant changes in terms of cholesterol and LDL cholesterol [13, 17, 19]. The differences may be attributed to the difference in ethnicity of the subjects and different time point of sample collection while some studies were cross-sectional. There are different

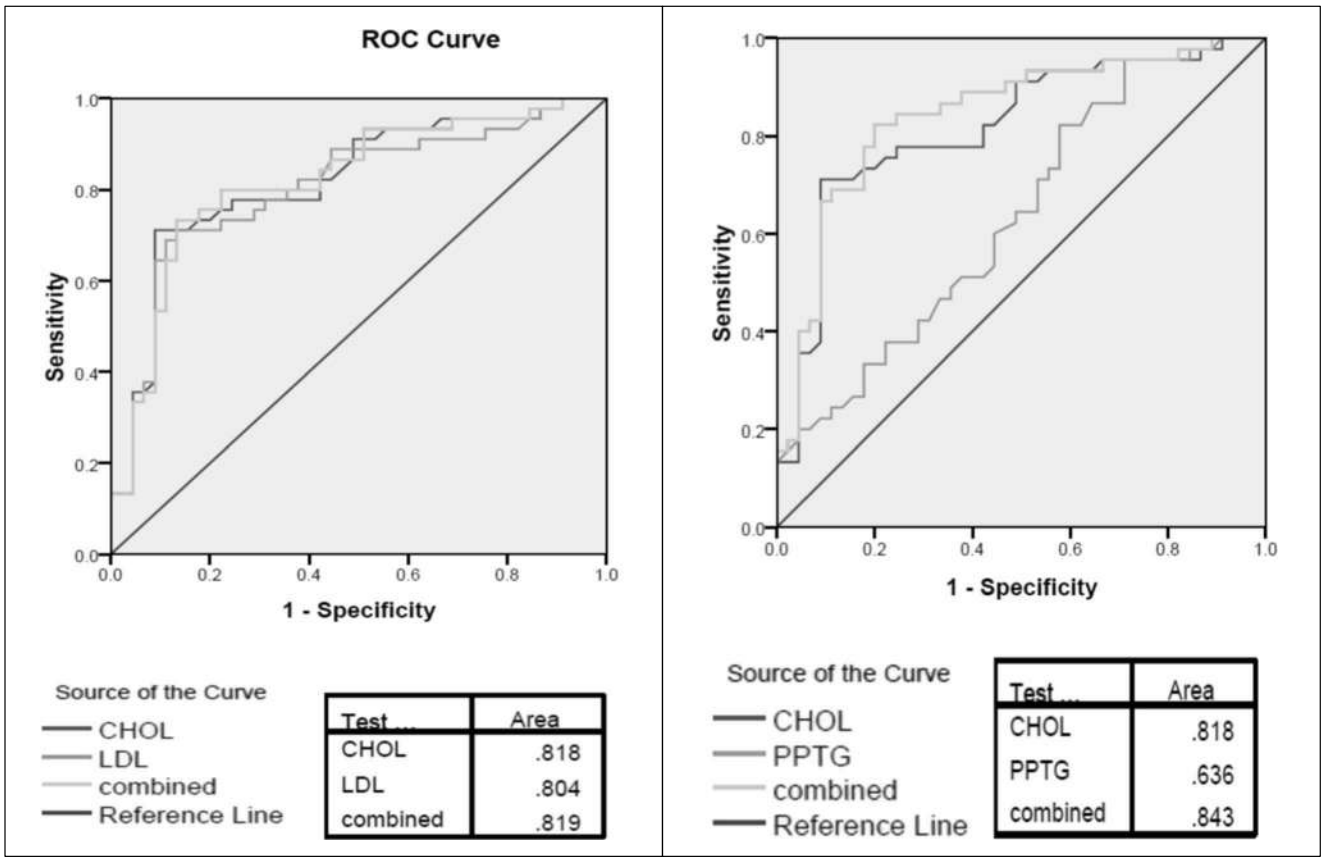

Fig. 1 ROC Curve generated for lipid parameters for detection of GDM risk

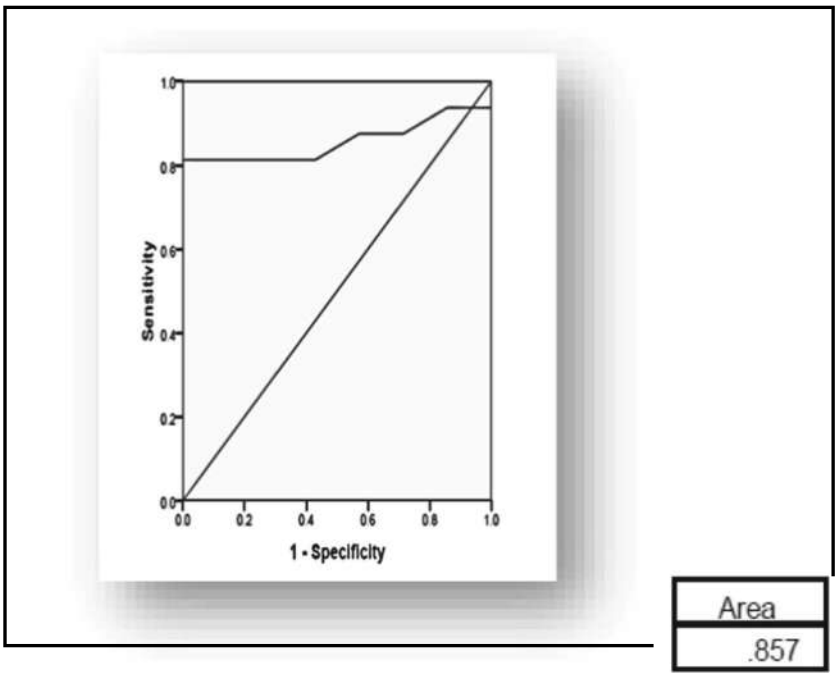

Fig. 2 ROC Curve generated for cholesterol (third quartile) for detection of GDM risk

hypotheses provided in this context as to how elevated early trimester lipids could be associated with a higher risk of GDM. One proposed mechanism behind this may be that cholesterol levels increase in pregnancy due to enhanced synthesis of cholesterol-based hormones such as oestradiol, prolactin, human somatomammotropin, cortisol, and progesterone all of which result in insulin resistance and are diabetogenic [35]. Excessive synthesis of cholesterol-based hormones might result in increased lipid synthesis resulting in fat accumulation and lipotoxicity [35]. Excessive lipid accumulation may lead to elevated oxidative stress, which further correlates with insulin resistance [36]. Also, lipotoxicity may lead to the direct destruction of the function of & e_x1D6FD; cells of the pancreas [37]. The reduction in lipoprotein lipase activity in pregnancy also increases circulating triglycerides postprandially which promotes ectopic lipid accumulation, lipotoxicity, and insulin resistance [38]. Hence, measurements of lipid levels could be an early marker of the diabetogenic potential of these pregnancy-related hormones. The particularly impressive performance of fasting 1st trimester total cholesterol levels in our study which further enhance on combining with PPTg levels suggests that at least among Asian Indians, this parameter may be a useful marker for prediction of risk of GDM.

Total cholesterol can be measured with ease, and its assay is substantially cost-effective; it can be considered as a reliable early biomarker for predicting GDM later in pregnancy. Combining total cholesterol, and PPTg level at 4 h after a modest fat challenge can significantly enhance the predictive efficacy of early trimester lipids for the prediction of GDM.

Conclusion

A combination of elevated fasting cholesterol and PPTg levels at an early stage of gestation can significantly predict future GDM.

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Data and resource sharing availability Data will be available the reasonable request.

Declarations

Ethics approval Ethical clearance was obtained from postgraduate review committee of University College of Medical Sciences and written consent was obtained from all the study participants.

Conflict of interest The authors declare no competing interests.

References

1. Metzger BE. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676–82.
2. Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Kapur A. Pregnancy and diabetes scenario around the world: India. Int J Gynecol Obstet. 2009;104(SUPPL.).
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047–53.
4. Lee KW, Ching SM, Ramachandran V, Yee A, Hoo FK, Chia YC, Wan Sulaiman WA, Suppiah S, Mohamed MH, Veettil SK.

Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2018;18(1):1–20.
5. Alia S, Pugnaloni S, Borroni F, Mazzanti L, Giannubilo SR, Ciavattini A, Vignini A. Impact of gestational diabetes mellitus in maternal and fetal health: An update. Diabetes Updates. 2019;5(3): 1–6.
6. Lao TT, Ho LF, Chan BCP, Leung WC. Maternal age and prevalence of gestational diabetes mellitus [6]. Diabetes Care. 2006;29(4):948–9.
7. Yen IW, Lee CN, Lin MW, Fan KC, Wei JN, Chen KY, Chen SC, Tai YY, Kuo CH, Lin CH, Hsu CY, Chuang LM, Lin SY, Li HY. Overweight and obesity are associated with clustering of metabolic risk factors in early pregnancy and the risk of GDM. PLoS One. 2019;14(12):1–9.
8. Chan LY-S, Wong SF, Ho LC. Diabetic family history is an isolated risk factor for gestational diabetes after 30 years of age. Acta Obstet Gynecol Scand. 2002;81(2):115–7.
9. Carnethon MR, Palaniappan LP, Burchfiel CM, Brancati FL, Fortmann SP. Incidence of Type 2 Diabetes in Black and White Adults. Diabetes Care. 2002;25(8):1358–64.
10. Williams MA, Qiu C, Muy-Rivera M, Vadachkoria S, Song T, Luthy DA. Plasma adiponectin concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. J Clin Endocrinol Metab. 2004;89(5):2306–11.
11. Soheilykhah S, Mojibian M, Rahimi-Saghand S, Rashidi M, Hadinedoushan H. Maternal serum leptin concentration in gestational diabetes. Taiwan J Obstet Gynecol. 2011;50(2):149–53.
12. Veltman-Verhulst SM, van Haeften TW, Eijkemans MJC, de Valk HW, Fauser BCJM, Goverde AJ. Sex hormone-binding globulin concentrations before conception as a predictor for gestational diabetes in women with polycystic ovary syndrome. Human Reproduction (Oxford, England). 2010;25(12):3123–8.
13. Koukkou E, Watts GF, Lowy C. Serum lipid, lipoprotein and apolipoprotein changes in gestational diabetes mellitus: a cross-sectional and prospective study. J Clin Pathol. 1996;49(8):634–7.
14. Aslam M, Aggarwal S, Sharma KK, Galav V. Postprandial hypertriglyceridemia predicts development of insulin resistance glucose intolerance and type 2 diabetes. 2016;25(11(1)):1–15.
15. M. S, S.M. N, M. M, C.-M. M, N. S, K. N. First-trimester prediction of gestational diabetes mellitus: examining the potential of combining maternal characteristics and laboratory measures. Diabetes. 2010;59(12):3017–3022.
16. Kumru P, Arisoy R, Erdogdu E, Demirci O, Kavrut M, Ardıc C, Aslaner N, Ozkoral A, Ertekin A. Prediction of gestational diabetes mellitus at first trimester in low-risk pregnancies. Taiwan J Obstet Gynecol. 2016;55(6):815–20.
17. Wang J, Li Z, Lin L. Maternal lipid profiles in women with and without gestational diabetes mellitus. Medicine (United States). 2019;98(16).
18. Hollingsworth DR, Grundy SM. Dorothy r. hollingsworth and scott m. grundy. 1982;31(December):1092–1097.
19. Khan SH. Serum triglycerides and cholesterol status in patients with. 2017;(January).
20. K. R. B, S. V, R. P. S. A study of lipid parameters among GDM and non GDM pregnant women: a hospital based study. International Journal of Reproduction, Contraception, Obstet Gynecol. 2017;6(12):5488.
21. Quinlivan JA, Danielle L. Cholesterol abnormalities are common in women with prior gestational diabetes. J Diabetes Metab. 2013;04(04):4–7.
22. Madhu SV, Kant S, Srivastava S, Kant R, Sharma SB, Bhadoria DP. Postprandial lipaemia in patients with impaired fasting glucose, impaired glucose tolerance and diabetes mellitus. Diabetes Res Clin Pract. 2008;80(3):380–5.
23. Mishra BK, Velmurugan M, Gambhir JK, Madhu SV. Postprandial lipemia and its relation to TCF7L2 gene polymorphisms in normoglycemic first-degree relatives of type 2 diabetes patients. Int J Diabetes Dev Ctries. 2019;39:268–72.
24. Mishra BK, Banerjee BD, Agrawal V, Madhu SV. Association of PPARγ gene expression with postprandial hypertriglyceridaemia and risk of type 2 diabetes mellitus. Endocrine. 2020;68:549–56.
25. Enquobahrie DA, Williams MA, Qiu C, Luthy DA. Early pregnancy lipid concentrations and the risk of gestational diabetes mellitus. Diabetes Res Clin Pract. 2005;70(2):134–42.
26. Lai M, Fang F, Ma Y, Yang J, Huang J, Li N, Kang M, Xu X, Zhang J, Wang Y, Peng Y. Elevated midtrimester triglycerides as a biomarker for postpartum hyperglycemia in gestational diabetes. J Diabetes Res. 2020;2020:1–7.
27. Hernandez TL, Van Pelt RE, Anderson MA, Daniels LJ, West NA, Donahoo WT, et al. A higher-complex carbohydrate diet in gestational diabetes mellitus achieves glucose targets and lowers postprandial lipids: a randomized crossover study. Diabetes Care. 2014;37(5):1254–62.
28. Axelsen M, Smith U, Eriksson JW, Taskinen MR, Jansson PA. Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. Ann Intern Med. 1999;131(1):27–31.
29. Kumar V, Madhu SV, Singh G, Gambhir JK. Post-prandial hypertriglyceridemia in patients with type 2 diabetes mellitus with and without macrovascular disease. J Assoc Physicians India. 2010;58:603–7.
30. Madhu SV, Mittal V, Krishna Ram B, Srivastava DK. Postprandial lipid abnormalities in type 2 diabetes mellitus. J Assoc Physicians India. 2005;53:1043–6.
31. Li G, Kong L, Zhang L, Fan L, Su Y, Rose JC, Zhang W. Early pregnancy maternal lipid profiles and the risk of gestational diabetes mellitus stratified for body mass index. Reprod Sci. 2015;22(6):712–7.
32. Emet T, Ustüner I, Güven SG, Balık G, Ural UM, Tekin YB, et al. Plasma lipids and lipoproteins during pregnancy and related pregnancy outcomes. Arch Gynecol Obstet. 2013;288(1):49–55.
33. Rizzo M, Berneis K, Altinova AE, Toruner FB, Akturk M, Ayvaz G, Rini GB, Spinas GA, Arslan M. Atherogenic lipoprotein phenotype and LDL size and subclasses in women with gestational diabetes. Diabet Med. 2008;25(12):1406–11.
34. Sánchez-Vera I, Bonet B, Viana M, Quintanar A, Martín MD, Blanco P, Donnay S, Albi M. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. Metab Clin Exp. 2007;56(11):1527–33.
35. Chatuphonprasert W, Jarukamjorn K, Ellinger I. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. Front Pharmacol. 2018;9(SEP):1–29.
36. Medina-Gomez G, Gray SL, Yetukuri L, Shimomura K, Virtue S, Campbell M, Curtis RK, Jimenez-Linan M, Blount M, Yeo GSH, Lopez M, Seppänen-Laakso T, Ashcroft FM, Orešič M, Vidal-Puig A. PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. PLoS Genet. 2007;3(4):e64.
37. Oh YS, Bae GD, Baek DJ, Park EY, Jun HS. Fatty acid-induced lipotoxicity in pancreatic beta-cells during development of type 2 diabetes. Front Endocrinol. 2018;9(JUL):1–10.
38. Herrera E, Lasunción MA, Gomez-Coronado D, Aranda P, López-Luna P, Maier I. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol. 1988;158(6 Pt 2):1575–83.

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ORIGINAL ARTICLE

Correlation analysis of triglycerides to high-density lipoprotein-cholesterol ratio associated with gestational diabetes mellitus

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Abstract

Background Gestational diabetes mellitus (GDM) initiates when a woman's pancreas could not act appropriately to bypass the diabetogenic condition during pregnancy. It is increasing across the world, including Bangladesh. Triglycerides (TG) and high-density lipoprotein-cholesterol (HDLC) are strongly connected with insulin resistance in pregnant women.

Objectives Observation of the role of lipid profiles and TG/HDL cholesterol ratio associated with fasting glucose in GDM subjects.

Methods In this experiment, a total of 232 individual subjects consisting of 132 GDM-positive and 100 GDM-negative pregnant women were examined and observed from 24 to 28 weeks of their pregnancy period. For this study, we had collected blood samples from selected women before and after breakfast and analyzed blood glucose level, triglyceride cholesterol level, HDL, low-density lipoprotein (LDL), and TG/HDL ratio.

Results TG and LDL-cholesterol were significantly higher ($p < 0.001$) in GDM individuals (220.95 ± 67.4 and 149.54 ± 32.4, respectively) than those of the non-GDM (160.98 ± 59.67 and 129.18 ± 34.18, respectively). On the contrary, HDL-cholesterol level was comparatively lower in GDM-positive women than non-GDM subjects. In this case, the optimum cut-off point was 3.8 for the TG/HDL-C ratio with 62% sensitivity and 78% specificity by oral glucose tolerance test (OGTT).

Conclusion Significantly ($p < 0.001$) higher TG/HDL ratio was found in GDM women compared with those in non-GDM. TG/HDL ratios are independently associated with the risks of GDM, which might be a good marker in predicting GDM risk.

Keywords Triglycerides · High-density lipoprotein-cholesterol · Gestational diabetes mellitus · Pregnancy · Oral glucose tolerance test

Introduction

GDM is defined as the incidence of glucose intolerance during pregnancy. This incidence has become a burning problem worldwide [1]. Type 2 diabetis and obesity have a strong correlation with GDM, and 5% of total pregnancies are related to GDM [2–4]. Women of the Indian subcontinent are more prone to GDM than white women of other countries [5]. The percentage of GDM in Bangladeshi women is around 15%, leading to type 2 diabetes in 10% of the identified cases within 10 years [6]. About 7% of total pregnancies become complicated due to GDM, leading to 200,000 plus cases annually in the USA [7]. A study on Asian women showed GDM incidence rate of 9.2% [8]. There are several reasons behind the development of GDM, among which the most prominent causes include polycystic ovary syndrome (PCOS), hypertension or pregnancy-related hypertension, strong family history of diabetes, obesity 25 + ages, persistent glucosuria, and a GDM in a previous pregnancy [9]. Even many women are living with type 2 diabetes; however, a small group is also included in the rare type, i.e.,

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monogenetic and mitochondrial diabetes which was previously misclassified as type 1 or type 2 diabetes. GDM is usually predicted as a physiological state of insulin resistance, regulated by the high percentage of diabetogenic steroid hormones. Their combined function decreases the sensitivity of insulin receptors within target tissues [10]. Various studies suggest that the patients having insulin resistance as well as type 2 diabetes have ectopic lipid and lipoprotein profile, such as lower high-density lipoproteins (HDLs), elevated triglycerides (TGs), and higher low-density lipoproteins (LDLs) [11]. In Bangladesh, a number of women do not show interest in testing lipid profile during pregnancy due to ignorance, and approximately more than 50% of pregnant women do not test their lipid profile in the first 3 months of pregnancy. However, predicting the incidence of GDM in later pregnancy is possible through the fasting glucose level test [12]. As there is a relationship between TG levels and glucose tolerance during early pregnancy [11, 13], the work designated to measure the triacylglycerol, LDLs, HDLs, and to evaluate the LDL-C/HDL-C, and TG/HDL-C ratio of GDM-positive pregnant women to correlate the incidence of higher triglyceride in GDM condition.

Methods

Study design and population

This cross-sectional research was driven in the Department of Obstetrics and Gynecology in collaboration with the Biochemistry and Cell Biology Department of the Bangladesh University of Health Sciences (BUHS) hospital. The duration of the study was from April 2018 to April 2019. A total of 232 pregnant women were included in this study. All subjects were picked from the Department of Gynecology and Obstetrics, BUHS hospital. The subjects were screened at 24–28 weeks of their pregnancy and maternal age of 20–50 years and followed for the next 4 weeks of pregnancy. Informed consent was taken from all of the interested participants who fulfilled the inclusion criteria of the study. The inclusion criteria of the study were screening of women with 24–28 weeks of pregnancy and maternal age of 20–50 years with no pre-existing diabetes mellitus. In contrast, exclusion criteria were pregnancy with known type 1 and type 2 diabetes, and any other previously recorded medical disorders including hyperlipidemia, hypertension, cardiovascular diseases (CVD), liver cirrhosis, chronic renal failure as well as anemia, and endocrine disorders.

Sociodemographic features

Information regarding basic socioeconomic and demographic features (e.g., education, occupation, and monthly income) of the participants has been collected verbally. Family income classification was made according to 2006 GNI per capita and was calculated using the WB Atlas method (The Daily Star, 2011).

Subject screening and sample collection

GDM was diagnosed following the procedure described in the latest revision of the American Diabetes Association's guidelines [14]. Briefly, the plasma glucose of the participants was measured after taking 100 g of glucose orally. The participant was suspected of GDM if fasting plasma glucose was > 95 mg/dl, blood sugar > 180 mg/dl 1 h after glucose intake, and blood sugar > 155 mg/dl 2 h later.

Venous blood (~ 6 ml) was collected from the subjects in the early morning before breakfast (8.00–9.00 a.m.). They were then provided glucose (75 g in 300 ml of water) to drink and requested not to take any other meal, and the second blood sample (~ 3.00 ml venous blood) was collected after 2 h of glucose intake in a plain tube and kept for 30 min to be clotted. Then centrifuged for 10 min at 3000 rpm, and upper transparent serum portion was separated by pipette. The samples were preserved at 40℃ and serum was frozen at − 30℃. Then blood glucose levels, triglyceride, cholesterol, HDL cholesterol, and LDL cholesterol were measured following the standard procedure.

Analytical methods

The test analysis was done using a clinical chemistry analyzer (Dimension RxL siemens, USA). Serum glucose and TG were measured by Glucose oxidase (GOD/PAP)/GLUC Flex reagent cartridge (Cat No: DF 40, UK) and GPO-PAP/TGL Flex reagent cartridge (Cat No: DF 69A), respectively. Serum cholesterol and HDL-C were measured by CHOD-PAP/CHOL Flex reagent cartridge (Cat No: DF 27, UK) and AHDL Flex reagent cartridge (Cat No: DF 488, UK). On the other hand, serum insulin was determined by an ELISA method (Cat No: EIA-2935, DRG-International, Germany). The homeostasis model assessment of the insulin resistance (HOMA-IR) method was applied to calculate insulin resistance index.

Statistical analysis

SPSS version 16.0 was used for statistical analysis. Data were expressed as mean ± SD, median (range), and/or number where appropriate. Student's unpaired *t* test was used to compare two groups. Correlation analysis between normally distributed variables was examined by the Pearson's correlation test. Sensitivity and specificity were calculated by the McNemar Chi-square test for early detecting the disease group where p value of < 0.05 was taken as a level of

significance [15]. The result was expressed as parametric test, mean ± SD, and number (%). For detecting the risk of GDM, the ROC curve (receiver operating characteristic curve) was built to examine sensitivity and specificity.

Results

Sociodemographic status of the study subjects

Among the examined 232 subjects, 132 subjects were GDM and 100 subjects were non-GDM. The sociodemographic variables including education, employment, and monthly income of the participants were analyzed for this study and summarized in Table 1. Regarding education level, it was observed that the illiteracy rate was higher for the non-GDM participants (47%) than the GDM participants (21.2%). In addition, both the GDM and non-GDM participants showed that the majority of them were housewives (92.4% and 93%, respectively). In the case of monthly family income, it was found that more than half of the respondents' income level was below 6000 BDT both in GDM and non-GDM groups (57.6% and 61%, respectively).

Clinical characteristics of the study subjects

Clinical characteristics of GDM and non-GDM subjects are illustrated in the Table 2. It was observed, among all the variables, that only BMI significantly ($p < 0.001$) differed in GDM subjects (25.13 ± 3.72) than that of non-GDM subjects (23.54 ± 2.06). But, in the case of age, gestational age, parity, systolic blood pressure, diastolic blood pressure, and mean blood pressure, there were no significant ($p > 0.05$) differences between GDM and non-GDM subjects.

Table 1 Sociodemographic status of the study subjects ($n = 232$)

Variable	Mean ± SD		*p*-Value
Education	GDM ($n = 132$)	Non-GDM ($n = 100$)	< 0.001
Illiterate	28 (21.2%)	47 (47%)	
SSC	13 (9.8%)	13 (13%)	
Graduate	91 (69%)	40 (40%)	
Occupation			
Housewife	122 (92.4%)	93 (93%)	< 0.002
Service	10 (7.6%)	7 (7%)	
Income (BDT)			
(< 6000)	76 (57.6%)	61 (61%)	< 0.001
(> 6000)	56 (42.4%)	30 (30%)	

The result was expressed as a parametric test, mean ± SD, and number (%), whereas appropriate, $p = 0.05$, was considered as statistically significant. Significant test was calculated by the Student's *t* test. *GDM*, gestational diabetes mellitus, *Non GDM*, non-gestational diabetes mellitus. Family income classification was made according to 2006 GNI per capita and was calculated using the WB Atlas method (The Daily Star, 2011). *Income*, monthly family income in Bangladeshi Taka (BDT)

Table 2 Clinical characteristic of the study subjects ($n = 232$)

Variable	Mean ± SD		*p* value
	GDM	Non-GDM	
Age (years)	29.84 ± 3.75	29.78 ± 4.62	0.901
BMI (Kg/m^2)	25.13 ± 3.72	23.54 ± 2.06	0.001
Gestational age (years)	1.76 ± 0.42	1.79 ± 0.40	0.655
Parity	1.40 ± 0.49	1.36 ± 0.48	0.522
SBP (mm of Hg)	113.29 ± 11.7	113.55 ± 13.16	0.877
DBP (mm of Hg)	74.43 ± 7.32	74.60 ± 7.23	0.862
MBP (mm of Hg)	88.3 ± 7.94	87.58 ± 8.50	0.461

Results are expressed as mean ± SD. Significant level calculated by Student's *t* test. *p* value < 0.05 as significant level. *BM*, body mass index; *SB*, systolic blood pressure; *DBP*, diastolic blood pressure; *MBP*, mean blood pressure

Glycemic and lipidemic status of the study subjects (n = 232)

Table 3 showed the glycemic and lipidemic status of the study subjects, where both the serum fasting blood glucose (SFBG) and serum 2 h after blood glucose (SFBG 2 h) were significantly ($p = < 0.001$) higher in GDM subjects compared to those in the non-GDM. On the other hand, HDL-cholesterol was significantly ($p = < 0.001$) higher in non-GDM subjects, and total cholesterol and LDL-cholesterol significantly ($p = < 0.001$) increased in the GDM subjects. Moreover, TG/HDL-cholesterol ratio and TG/LDL-cholesterol ratio also significantly ($p = < 0.05$) differed between GDM and non-GDM subjects.

Table 3 Glycemic and lipidemic status of the study subjects ($n = 232$)

Variable	Mean ± SD		*p* value
	GDM	Non-GDM	
SFBG (mmol/dl)	7.88 ± 7.94	5.22 ± 0.81	0.001
SFBG 2 h (mmol/dl)	10.45 ± 2.78	6.27 ± 0.78	0.001
Triglycerides (mg/dl)	220.95 ± 67.4	160.98 ± 59.67	0.001
Total cholesterol (mg/dl)	211.12 ± 40.42	193.08 ± 59.67	0.001
HDL-cholesterol (mg/dl)	39.44 ± 7.39	43.83 ± 6.73	0.001
LDL-cholesterol (mg/dl)	149.54 ± 32.4	129.18 ± 34.18	0.001
TG/HDL-cholesterol ratio	5.75 ± 1.88	3.68 ± 1.63	0.001
TG/LDL-cholesterol ratio	1.60 ± .68	1.37 ± 1.63	0.022

Results are expressed as mean ± SD. Significant level calculated by Student's *t* test. *p* value < 0.05 as significant level, serum fasting blood glucose level, serum 2 h after glucose level. *S TG*, serum triglyceride; *S T Chol*, total cholesterol; *HD*, high-density lipoprotein, *LDL*, low density lipoprotein; *TG/HDL*, triglyceride/high-density lipoprotein–cholesterol ratio; *TG/LDL*, triglyceride/ low-density lipoprotein–cholesterol ratio)

The coefficient of correlation of TG/HDL ratio with other confounding variables

Table 4 showed the coefficient correlation of TG/HDL cholesterol ratio with other confounding variables (age, education, gestational age, parity, BMI, MBP, and SFBG 2 h). There was significant positive relation with BMI (0.980/0.002), SFBG (0.569/0.050), and SFBG 2 h level (0.651/0.001) in GDM subjects. On the other hand, the non-GDM subjects had no significant relation with them.

Coefficient of correlation of TG/LDL ratio with other confounding variables

Coefficient correlations of TG/LDL cholesterol ratio with other confounding variables (age, education, gestational age, parity, BMI, MBP, fasting blood sugar, 2 h after glucose) are presented in Table 5. There was significant positive relation with age, BMI, SFBG, and SFBG 2 h in GDM subjects. On the other hand, the non-GDM subjects showed a negative correlation with mean blood pressure (0.057).

Sensitivity and specificity of TG/HDL and TG/LDL ratio to predict the risk of GDM

Sensitivity and specificity were examined to detect the risk of GDM. The optimal cut-off value analyzed for TG/HDL-C with GDM was 3.8 in the 75th percentile with the sensitivity 62%, specificity 82%, PPV (positive predictive value) 67%, and NPV (negative prediction value) 66%, respectively. Another predictor, TG/LDL ratio, found 75th percentile, and optimum cut-off value was 1.5 and sensitivity 60%, specificity 72%, PPV 70%, and NPV 60%, respectively (Table 6).

Table 4 Coefficient of correlation of TG/HDL ratio with other confounding variables

Variable	GDM (r/p)	Non-GDM (r/p)
Age (years)	0.072/0.409	0.093/0.357
Education	0.009/0.914	0.074/0.621
Gestational age (years)	0.084/0.341	0.080/0.426
Parity	0.063/0.473	0.154/0.127
BMI (Kg/m^2)	0.980/0.002	0.054/0.596
MBP (mm of Hg)	0.584/0.048	0.078/0.438
SFBG (mmol/dl)	0.569/0.050	0.009/0.928
SFBG 2 h (mmol/dl)	0.651/0.001	0.015/0.882

Results are expressed as coefficient of correlation calculated by parametric Pearson's correlation analysis" p value < 0.05 as significant level. *BMI*, body mass index; *MBP*, mean blood pressure, *S*, serum

Table 5 Coefficient of correlation of TG/LDL ratio with other confounding variables

Variable	GDM (*r*/*p*)	Non-GDM (*r*/*p*)
Age (years)	0.972/0.003	0.005/0.960
Education	0.059/0.502	0.064/0.524
Gestational age (years)	0.100/0.253	0.038/0.708
Parity	0.122/0.165	0.144/0.153
BMI (Kg/m^2)	0.569/0.050	0.129/0.200
MBP (mm of Hg)	0.076/0.387	−0.191/0.057
SFBG (mmol/dl)	0.667/0.038	0.184/0.067
SFBG 2 h (mmol/dl)	0.014/0.018	0.014/0.889

Results are expressed as coefficient of correlation calculated '*r*' by parametric Pearson's correlation analysis" p value < 0.05 as significant level. *BMI*, body mass index; *MBP*, mean blood pressure; *S*, serum

Discussion

GDM can have severe consequences for both mother and fetus during pregnancy and perinatal period, and throughout the future life span [8]. There is an increased chance of babies from GDM mothers suffering from DM or cardiovascular diseases (CVD) in adult life (known as fetal programming of adult disease) [16, 17]. On the other hand, latent DM or CVD can be triggered during pregnancy and can be chronic or recurrent even after the postpartum period. It is now known that the incidence and risk factors for diseases of the fetus or later life born from GDM mother vary significantly from population to population [17]. Thus, it is important to investigate these factors in different ethnic, social, and cultural backgrounds. This study sought to address the important risk factors for GDM among diverse populations.

The results of this study showed significant differences between the GDM and non-GDM individuals regarding TG, HDL-C, LDL-C levels, and TG/LDL-C ratio, and TG/HDL ratio. Various studies have shown similar results and confirmed the occurrence of hyperlipidemia in complex pregnancies with gestational diabetes [18–20]. In our experiment, TG level was significantly higher in women with GDM compared to those without GDM ($p = < 0.001$). Again, GDM subjects showed significantly ($p = < 0.001$) more elevated total cholesterol and LDL cholesterol values but lower HDL cholesterol values comparing with non-GDM subjects.

In the current study, the correlation analysis has shown the significant positive correlation of GDM subjects with age (p-0.003), BMI ($p = < 0.050$), fasting blood glucose level ($p = 0.038$), and after 2 h blood glucose level (0.028). The findings and parameters of Barat et al. are entirely similar to our result [21]. Maryam et al. [22] found the TG and HDL levels in GDM, and non-GDM individuals were statistically different. Here, TG levels were higher with lower HDL serum levels

Table 6 The sensitivity and specificity of TG/HDL and TG/LDL ratio to predict the risk of GDM

Variable	Optimum cut-off value	Sensitivity	Specificity	PPV	NPV
TG/HDL ratio	75th per 3.8	62%	82%	67%	66%
TG/LDL ratio	75th per 1.5	60%	72%	70%	60%

The results were expressed as sensitivity, specificity, *PPV*, positive predictive value, and *NPV*, negative value, analyzed by the *Mc Namara* chi-square test

($p = < 0.0001$). Furthermore, cholesterol levels in women with GDM were non-significantly lower than non-GDM ($p = 0.239$), and LDL serum levels were significantly lower in subjects with GDM. Wang et al. [23] found similar levels of total cholesterol and LDL-C between most of the women with GDM or without GDM. However, in this research, LDL-C levels were significantly different ($p = < 0.0001$). Inversely, Russell et al. [22] found that total cholesterol levels in the GDM group were lower than in the control group, but this difference was not statistically significant ($p = 0.239$).

Many studies, including Donovan et al. [24], have suggested that women with GDM have increased levels of LDL-C and total cholesterol, which are partially similar to the present study. This variation may occur due to rational differences or environmental effects on the selected individuals. Furthermore, obesity can be an essential factor, as the percentage of many hormones, including insulin, can alter cholesterol and LDL levels [25–27]. It was found that obesity in women can lead to low HDL and high TG levels [28]. On the other hand, smoking has a significant effect on increasing cholesterol and LDL levels [29, 30], which was observed in European countries where smoking practice is very common [31, 32].

Some important findings related to this work have confirmed a significant increase in serum lipid profile, including the concentration of TG/LDL-C and TG/HDL ratios in GDM-infected mothers compared to healthy pregnancies [33, 34]. Our study results showed that TG/HDL-cholesterol was significantly ($p = < 0.001$) higher (5.75) in GDM subjects as compared to non-GDM subjects (3.68). Another marker showed TG/LDL-cholesterol also had significant difference ($p = < 0.022$) between GDM subjects (1.60) and non-GDM subjects. The area under the ROC curve of TG/HDL-C to detect GDM was 0.786 (95% CI, 0.590–0.865). The optimal cut-off point proposed by the ROC analysis for TG/HDLC and GDM was 3.8, with sensitivity and specificity of 62% and 82%, respectively. Another one was the TG/LDL ratio which had an optimum cut-off value of 1.5 with sensitivity and specificity of 60% and 72%, respectively. The positive and negative predictive values of the TG/HDL-C were 4.4% and 79.1%, respectively. An almost similar result was observed by Jameshorani [35]. Finally, we suggest that lipid profiling might be a good tool for risk assessment of gestational diabetes in individuals, especially the TG/HDL ratio can work as a marker to identify gestational diabetes.

The limitation of our study is that a small number of participants were selected for the study, and they were confined to a particular region of our country, and they may not be representative for the general population of the whole country. Moreover, we lacked some potential information required for the intensive conclusion. Therefore, to elucidate the pinpoint conclusion, further investigation is needed using a large number of samples from diverse sources, including different races, ethnic groups, locations, and age groups.

Conclusion

Among all the complications during pregnancy, GDM is a commonly occurring disease in recent times. TG to HDL-C ratio is an indicator of GDM. As the TG/HDL-C ratio is associated with insulin resistance, different lipid ratios have been used to predict the risk of different GDMs. In this cross-sectional case–control study, the TG/HDL ratios (3.8) were significantly higher in women with GDM compared to those with non-GDM. In conclusion, we can say that there is a relationship of gestational diabetes with the HDL-C and the TG ratio. Therefore, lipid profiling during pregnancy is strongly recommended as it can help in immediate management strategies to prevent the harmful effects of hyperlipidemia related to GDM pregnancy.

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Data availability The dataset will be available upon request unless there are legal or ethical reasons for not doing so.

Code availability Not applicable.

Declarations

Ethics approval Ethical issue of this study was approved by the ethical review board of the Institute of Biological Sciences (IBSc) of the University of Rajshahi, Rajshahi-6205, Bangladesh, under the certificate

number 255(14)/320/IAMEBBC/IBSc. Informed written consent was taken from all patients enrolled in this study.

Consent to participate The written consent from the participants was taken, describing that the survey report will be published in the journal.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Bharathi KR, Vijayalakshmi S, Shrunga RP. A study of lipid parameters among GDM and non GDM pregnant women: a hospital based study. Int J Reprod Contraception Obstet Gynecol. 2017;6:5488.
2. Van Assche FA, Aerts L, Holemans K. Metabolic alterations in adulthood after intrauterine development in mothers with mild diabetes. Diabetes. 1991;40:106–8.
3. Van Assche FA, Aerts L, Holemans K. Maternal diabetes and the effect for the offspring. Verh K Acad Geneeskd Belg. 1992;54.
4. Rosenn B, Tsang RC. The effects of maternal diabetes on the fetus and neonate. Ann Clin Lab Sci. 1991;21:153–70.
5. American College of Obstetricians and Gynecologists. Assessment of risk factors for preterm birth. Clinical management guidelines for obstetrician-gynecologists. ACOG Practice Bulletin. 2001; 31.
6. Mohiuddin AK. Diabetes fact: Bangladesh perspective. Int J Diabetes Res. 2019;2:14–20.
7. American Diabetes Association. Gestational diabetes mellitus (Position Statement). Diabetes Care. 2004;27(Suppl. 1):S88-90.
8. Cheung NW, Wasmer G, Al-Ali J. Risk factors for gestational diabetes among Asian women. Diabetes Care. 2001;24:955–6.
9. Wang Y, Zhao X, Zhao H, Ding H, Tan J, Chen J, et al. Risks for gestational diabetes mellitus and pregnancy-induced hypertension are increased in polycystic ovary syndrome. Biomed Res Int. 2013;2013.
10. Monir N, Zeba Z, Rahman A. Comparison of knowledge of women with gestational diabetes mellitus and healthy pregnant women attending at hospital in Bangladesh. J Sci Found. 2018;16:20–6.
11. Li G, Kong L, Zhang L, Fan L, Su Y, Rose JC, et al. Early pregnancy maternal lipid profiles and the risk of gestational diabetes mellitus stratified for body mass index. Reprod Sci. 2015;22:712–7.
12. Zhu WW, Yang HX, Wei YM, Yan J, Wang ZL, Li XL, Wu HR, Li N, Zhang MH, Liu XH, Zhang H. Evaluation of the value of fasting plasma glucose in the first prenatal visit to diagnose gestational diabetes mellitus in China. Diabetes Care. 2013;36:586–90.
13. Nolan CJ, Riley SF, Sheedy MT, Walstab JE, Beischer NA. Maternal serum triglyceride, glucose tolerance, and neonatal birth weight ratio in pregnancy: a study within a racially heterogeneous population. Diabetes Care. 1995;18:1550–6.
14. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol. 1982;144(7):768–73.
15. Trajman A, Luiz RR. McNemar. $\chi 2$ test revisited: comparing sensitivity and specificity of diagnostic examinations. Scand J Clin Lab Invest. 2008;68:77–80. https://doi.org/10.1080/00365510701666031.
16. Monteiro LJ, Norman JE, Rice GE, Illanes SE. Fetal programming and gestational diabetes mellitus. Placenta. 2016;48:S54-60.
17. Dorner G, Plagemann A, Reinagel H. Familial diabetes aggregation in type I 532 diabetics: gestational diabetes an apparent risk factor for increased diabetes 533 susceptibility in the offspring. Exp Clin Endocrinol. 1987;89:84–90.
18. Bartha JL, Comino-Delgado R, Martinez-Del-Fresno P, Fernandez-Barrios M, Bethencourt I, Moreno-Corral L. Insulin-sensitivity index and carbohydrate and lipid metabolism in gestational diabetes. J Reprod Med Obstet Gynecol. 2000;45:185–9.
19. Clark CM, Qiu C, Amerman B, Porter B, Fineberg N, Aldasouqi S, Golichowski A. Gestational diabetes: should it be added to the syndrome of insulin resistance? Diabetes Care. 1997;20:867–71.
20. Couch SC, Philipson EH, Bendel RB, Wijendran V, Lammi-Keefe CJ. Maternal and cord plasma lipid and lipoprotein concentrations in women with and without gestational diabetes mellitus: predictors of birth weight? J Reprod Med Obstet Gynecol. 1998;43:816–22.
21. Barat S, Ghanbarpour A, Bouzari Z, Batebi Z. Triglyceride to HDL cholesterol ratio and risk for gestational diabetes and birth of a large-for-gestational-age newborn. Casp J Intern Med. 2018;9:368–75.
22. Russell MA, Carpenter MW, Coustan DR. Screening and diagnosis of gestational diabetes mellitus. Clin Obstet Gynecol. 2007;50:949–58.
23. Wang J, Li Z, Lin L. Maternal lipid profiles in women with and without gestational diabetes mellitus. Medicine (Baltimore). 2019;98:e15320.
24. Donovan BM, Spracklen CN, Schweizer ML, Ryckman KK, Saftlas AF. Intimate partner violence during pregnancy and the risk for adverse infant outcomes: a systematic review and meta-analysis. BJOG An Int J Obstet Gynaecol. 2016;123:1289–99.
25. Meshkini M, Alaei-Shahmiri F, Mamotte C, Earnest J. Ethnic variation in lipid profile and its associations with body composition and diet: differences between Iranians, Indians and Caucasians living in Australia. J Immigr Minor Heal. 2017;19:67–73.
26. Feingold K, Brinton EA, Grunfeld C. The effect of endocrine disorders on lipids and lipoproteins. Endotext. 2020.
27. Sikaris KA. The clinical biochemistry of obesity. Clin Biochem Rev. 2004;25:165–81.
28. Bahrami H, Sadatsafavi M, Pourshams A, Kamangar F, Nouraei M, Semnani S, et al. Obesity and hypertension in an Iranian cohort study; Iranian women experience higher rates of obesity and hypertension than American women. BMC Public Health. 2006;6.
29. Lakshmi AS, Lakshmanan A, Ganesh Kumar P, Saravanan A. Effect of intensity of cigarette smoking on haematological and lipid parameters. J Clin Diagnostic Res. 2014;8.
30. Rashan MA, Dawood OT, Razzaq HAA, Hassali MA. The impact of cigarette smoking on lipid profile among Iraqi smokers. Int J Collab Res Intern Med Public Heal. 2016;8.
31. Halimi L, Haghdoost AA, Alizadeh SM. Prevalence of cigarette smoking among Iranian women: a systematic review and meta-analysis. Med J Islam Repub Iran. 2013;27:132–40.
32. Mansouri M, Sadeghi O, Roshanfekr P, Sharifi F, Varmaghani M, Yaghubi H, et al. Prevalence of smoking and its association with health-related behaviours among Iranian university students: a large-scale study. East Mediterr Heal J. 2020;
33. Bao W, Dar S, Zhu Y, Wu J, Rawal S, Li S, et al. Plasma concentrations of lipids during pregnancy and the risk of gestational diabetes mellitus: a longitudinal study. J Diabetes. 2018;10:487–95.
34. Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. BJOG An Int J Obstet Gynaecol. 2015;122:643–51.
35. Jameshorani M, Arefzadeh A, Khalighinejad P, Ranjbar S, Mazloomzadeh S. Evaluation of triglyceride to high-density lipoprotein cholesterol ratio and atherogenic indices in gestational diabetes mellitus. J Pharm Res Int. 2018;22:1–9.

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ORIGINAL ARTICLE

Comparison of serum cytokines between gestational diabetes mellitus and normal pregnancy women: a pilot study

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Abstract

Aim Gestational diabetes mellitus (GDM) is associated with a higher risk of postpartum type 2 diabetes mellitus and a series of complicates, such as adverse obstetric and perinatal outcome. We aimed to evaluate cytokines profile in women with GDM.

Materials and methods Based on a 75g oral glucose tolerance testing (OGTT), the participants were divided into two groups: GDM (n=28) and normal glucose tolerance (NGT) (control group, n=20). Flow cytometry was used to assess serum cytokines. The levels of glycosylated hemoglobin (HbA1c), glucose, and high-sensitive C-reactive protein (hsCRP) were measured.

Results HsCRP, interleukin (IL)-2, and IL-6 were higher in GDM group as compared with the NGT group. HsCRP and IL-6 concentrations positively correlated to Hb1Ac and OGTT. A number of cytokines (such as IL-10, IL-8, IL-12p70, INF-γ, IL-4, IL-17, and IL-10) might exhibited beneficial effects against GDM.

Conclusions Our results showed that the GDM patients developed a low-grade inflammatory state and presented a disorder of cytokines . These observations suggest that dysregulation of concentrations of pro- and anti-inflammatory cytokines in pregnancy is associated with GDM development.

Keywords Gestational diabetes mellitus · High-sensitive C-reactive protein · Cytokines · IL-2 · IL-6

Introduction

Gestational diabetes mellitus (GDM) is a condition of disordered glucose metabolism, which is first occurring or recognized during pregnancy. Worldwide, GDM affects up to 25% of all pregnancies [1], and is usually associated with obesity, oxidative stress, endothelial function, and β-cell dysfunction [2–4]. Moreover, metabolic adaptations such as nutrient-stimulated insulin responses during pregnancy do not fully compensate in GDM, suggesting a predisposition to type 2 diabetes or an extreme manifestation of normally metabolic alterations [5]. Besides perinatal complications, GDM is always associated with a higher risk to develop cardiovascular disease and even cancer in the future [6].

Obesity is a basic risk factor for GDM, and is linked to a chronic low-grade inflammatory state [7]. White adipose tissue (WAT) is considered an endocrine organ, which excess accumulated in individuals could secret a serious of active molecules and further disrupts immune homeostasis [8]. Several studies have identified cytokines, act as immune mediators and regulators, could favor or compromise the normal pregnancy outcomes [9]. Furthermore, C-reactive protein (CRP) is an acute phase reactant produced by hepatocytes in response to injury and inflammation, Even CRP within healthy reference range, it is strongly associated to BMI, metabolic syndrome, and coronary heart disease [10, 11]. Thus, CRP could act as a marker of systemic low-grade inflammatory state. Recently, several studies support that the unbalanced production of anti- and pro-inflammatory cytokines is involved in the initiation and progression of GDM [12–14]. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-6, IL-2, and IL-1β have been found increased

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in some cases with GDM [15–17], whereas anti-inflammatory properties such as IL-4 and IL-10 were also closely associated with the pathophysiology of diabetes mellitus [18, 19]. The role of cytokines in the etiology of GDM, however, has yet to be investigated due to a plethora of controversial reports [20].

In this regard, our study has been designed to characterize the cytokines profile in patients with GDM compared to normal pregnancy women. Besides, we also aimed to investigate the relationships among glucose levels and cytokine expression in serum, attempt to better understand the mechanisms of GDM and provide a potential way for monitoring pregnancy participant's immune state.

Materials and methods

Study population and clinical samples

The study involving 48 consecutive pregnant women referred to the obstetric clinic in the Third Affiliated Hospital of Guangzhou Medical University for 24 to 28 weeks' gestation screening, including 28 women with GDM and 20 healthy pregnant women with NGT (control group). Morning fasting blood samples were obtained from all the participants in two tubes. Serum from the tube without anticoagulant was separated by centrifugation. Fasting glucose (OGTT-0h), Hb1Ac, and hsCRP were measured within 2h. Additional samples collected in tube with the anticoagulant ethylenediamine tetra acetic acid (EDTA) were centrifuged immediately and stored at −20°C for cytokines detection. Then, all the participants screened for GDM using a 75-gram, 2-h oral glucose tolerance test (OGTT). Serum samples were collected and measured at 1-h (OGTT-1h) and 2-h (OGTT-2h) post-OGTT.

According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) standard [21], a diagnosis of GDM was made if met one or more of the following criteria: fasting glucose level ≥ 5.1 mmol/L, 1-h glucose level ≥ 10.0 mmol/L, and 2-h glucose level ≥ 8.5 mmol/L. The normal control group included healthy pregnant women, with normal 75g OGTT range. Exclusion criteria were as follows: existing chronic medical conditions that have the potential to affect acute phase markers (injury, infectious diseases, autoimmune diseases). The study was approved by the Ethics Committee and all subjects gave written informed consent.

Measurement of serum glucose, high-sensitive C-reactive protein (hsCRP), and HbA1c

Serum samples for glucose tests, hsCRP, and HbA1c were prepared by centrifugation at 380*g* for 10 min at room temperature after coagulation. Serum glucose and high-sensitivity CRP (hsCRP) were measured using Roche Biochemicals (Roche, Germany). HbA1c was analyzed using high-performance liquid chromatography (HPLC) automated hemoglobin analyzer (Tosoh G7, Japan).

Cytokines analyses

The plasma levels of cytokines were quantitatively determined within a week. A total of 12 cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, IFN-γ, IFN-α) were detected on a flow cytometer (BD, USA) by using a multiple microsphere flow immunofluorescence cytokine detection kit (Raisecare Biotechnology Co., Ltd., Qingdao, China) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (version 8.0) and Statistical Package of Social Science (SPSS version 23.0). Data were expressed as a mean ± standard deviation (SD) when the variables distributed normally (Shapiro-Wilk test) or a median with interquartile range. Data were analyzed using either Student *t*-test or non-parametric Mann-Whitney *U* tests for comparison. Spearman's correlation coefficient (*r*) was used to evaluate the bivariate correlation between cytokines and metabolic parameters. p-value < 0.05 was considered to be statistically significant.

Results

Clinical characteristics of the groups studied

The basic clinical characteristics of the groups studied are summarized in Table 1. A total of 48 pregnant women were enrolled in the study. Based on the OGTT results, the patients were divided into 2 groups: GDM (n=28) and NGT (n=20). Age of GDM group was significantly higher than in NGT group [33.50 (31.00–36.80) versus 29.50 (27.00–32.00) years, p = 0.004), as expected. The GDM group had significantly higher glucose levels at OGTT-0h (p = 0.000), OGTT-1h (p = 0.000), and OGTT-2h (p = 0.000) compared to the NGT group. The HbA1c of GDM group was also significantly higher than that of NGT group (p =0.000).

The serum levels of cytokines and inflammatory maker in patients with and without GDM

The comparisons of cytokines and inflammatory maker (hsCRP) between GDM group and NGT group are presented in Table 2. Results showed that pregnant women with GDM had significant higher hsCRP (p=0.002), IL-2 (p = 0.026), and IL-6 (p = 0.004) levels. Comparisons for other cytokines demonstrated no significant difference.

Table 1 Clinical characteristics according to glycemic tolerance status

	NGT (*n*=20)	GDM (*n*=28)	*p*-value
Age (years)	29.5 (27.0–32.0)	33.50 (31.00–36.80)	0.004
Fasting glucose (mmol/L)	4.35±0.32	4.75±0.36	0.000
OGTT-1h (mmol/L)	6.44±1.50	9.89±1.17	0.000
OGTT-2h (mmol/L)	5.89±0.93	8.93±1.01	0.000
HbA1c (%)	5.00 (4.83-5.00)	5.65 (5.50-6.00)	0.000

NGT, normal glucose tolerance; *GDM*, gestational diabetes mellitus; *OGTT*, oral glucose tolerance test; *HbA1c*, glycosylated hemoglobin

Continuous variables were expressed as mean ± SD with normal distribution and median (interquartile range) without normal distribution. Parametric data were analyzed by using the two-sided Students *t*-test and non-parametric data were analyzed by using the Mann–Whitney *U* test

Statistically significant with p-value < 0.05

Correlations between serum levels of cytokines and metabolic indices

Significant correlations between cytokines and metabolic metrics were obtained in the participants and presented in Table 3. HsCRP levels were positively to correlated to HbA1c (r = 0.494, p = 0.000), OGTT-0h (r = 0.327, p = 0.023), OGTT-1h (r = 0.379, p = 0.008), and negatively with IFN-γ (r = −0.361, p = 0.012), IL-4 (r = −0.373, p =0.009), and IL-17 (r = −0.303, p = 0.036). IL-10, IFN-γ, IL-8, and IL-12P0 levels correlated negatively to OGTT-0h (r = −0.379, p = 0.008, r = -0.310, p = 0.032, r = −0.314, p = 0.03, and r = −.301, p = 0.038, respectively). IL-6 levels positively correlated to Hb1Ac (r = 0.303, p =0.036), OGTT-1h (r = 0.407, P =0.004), as well as OGTT-2h (r = 0.292, p = 0.044).

Table 2 Serum levels of cytokines and hsCRP studied in patients with and without GDM

Cytokines	NGT (*n*=20)	GDM (*n*=28)	*p*-value
hsCRP (mg/L)	2.67 (1.18–4.20)	5.32 (2.95–10.88)	0.002
IL-5 (pg/ml)	24.44 (15.82–28.83)	21.86 (14.59–30.58)	0.892
IFN-α (pg/ml)	3.15 (1.81–11.82)	2.69 (1.12–10.05)	0.170
IL-2 (pg/ml)	2.01 (1.44–3.23)	3.01 (2.17–5.24)	0.026
IL-6 (pg/ml)	5.68 (3.22–15.51)	18.18 (5.61–31.26)	0.004
IL-1β (pg/ml)	0.73 (0.46–11.36)	0.99 (0.43–22.24)	0.867
IL-10 (pg/ml)	0.78 (0.49–1.17)	0.80 (0.47–1.32)	0.942
IFN-γ (pg/ml)	7.50(1.42–17.21)	3.80 (0.03–19.74)	0.550
IL-8 (pg/ml)	10.18 (6.70–13.50)	10.58 (5.61–14.70)	0.925
IL-17 (pg/ml)	0.99 (0.43–1.88)	0.88 (0.36–1.93)	0.810
IL-4 (pg/ml)	1.18 (0.76–1.70)	0.98 (0.71–1.30)	0.517
IL-12P70 (pg/ml)	1.94 (1.32–2.82)	1.81 (1.17–2.53)	0.594
TNF-α (pg/ml)	7.60 (3.76–9.85)	5.60 (0.027–13.78)	0.482

NGT, normal glucose tolerance; *GDM*, gestational diabetes mellitus; *IL*, interleukin; *hsCRP*, high-sensitivity C-reactive protein; *IFN*, interferon; *TNF*, tumor necrosis factor

All the results were presented as median (interquartile range) and the Mann-Whitney *U* test was used for comparisons because of non-normal distribution

Statistically significant with p-value < 0.05

Discussion

Hyperglycemia induces oxidative stress in individuals, leading to a range of illnesses. The offspring of maternal patient exposed to hyperglycemia tended to have more allergies and even suffer neurological damage. Previous studies have indicated that an increase of the expression of pro-inflammatory factors caused by GDM, which in

Table 3 Speaman rank correlations between serum cytokines and metabolic indices in all subjects analyzed together

	r_s	*p*-value
hsCRP&HbA1c	0.494	0.000
hsCRP&OGTT-0h	0.327	0.023
hsCRP&OGTT-1h	0.379	0.008
hsCRP&IFN-γ	−0.361	0.012
hsCRP&IL-4	−0.373	0.009
hsCRP&IL-17	−0.303	0.036
OGTT-0h and IL-10	−0.379	0.008
OGTT-0h and IFN-γ	−0.310	0.032
OGTT-0h and IL-8	−0.314	0.030
OGTT-0h and IL-12P70	−0.301	0.038
IL-6 and Hb1Ac	0.303	0.036
IL-6 and OGTT-1h	0.407	0.004
IL-6 and OGTT-2h	0.292	0.044

IL, interleukin; *hsCRP*, high-sensitivity C-reactive protein; *IL*, interleukin; *IFN*, interferon; *OGTT*, oral glucose tolerance test; *HbA1c*, glycosylated hemoglobin

Spearman's correlation analysis was applied to determine the correlation of two non-normally distributed variables

Statistically significant with p-value < 0.05

turn leads to substantially irreversible islet damage. Thus, women with GDM carry a higher risk for development of type 2 diabetes mellitus in future [22].

In this study, the patients with GDM recognized between the 24th and 28th week of gestation had significantly higher hsCRP levels than normal pregnancy women. CRP is the most commonly used marker for evaluation of bacterial infection and some inflammatory conditions. Numerous studies have related elevated CRP level, but remained within the suggested reference range (0–10.0 mg/L), to higher BMI, obesity, lipids, and cardiovascular events. In the present study, the hsCRP level demonstrated the similar results that most GDM subjects (n=22) were under the suggested reference range but markedly higher than the NGT patients, suggesting a systemic low-grade inflammatory state. HsCRP in this study was also positively associated with OGTT-0h, OGTT-1h, and Hb1Ac, in good agreement with previous reports [23]. In addition, the range of hsCRP suggested a low-grade systemic inflammation among GDM patients.

Several mechanisms may link CRP with metabolic syndrome. Since IL-1, IL-6, and TNF-α are the main modulators of CRP [24], serum levels of these pro-inflammatory, which promote the production of CRP, were reported raised in obese individuals. In our previous work, the prepregnancy BMI of GDM was markedly higher than normal pregnancy women as many studies showed. Obesity-induced chronic low-grade inflammation is strongly associated with the development of insulin resistance and other severe complications. These findings are in agreement with the data observed in our GDM cohort, in which IL-2 and IL-6 are higher in GDM group compared with NTG group. Although IL-2 and IL-6 were significantly increased in GDM group, there was no significant correlation between them and circulating hsCRP. However, the CRP was observed negatively correlated to anti-inflammatory cytokine (such as IL-4), IL-17, and IFN-γ. IL-17 has been reported to have pro-inflammatory and anti-inflammatory effects, may exert protective effects in the development of autoimmune disease [25]. Nevertheless, the role of IL-17 in GDM still remains unclear and further investigation is needed.

On the other hand, anti-inflammatory cytokines such as IL-4, IL-5, TGF-α, and IFN-γ showed a downward trend in GDM subjects, but the difference between groups did not reach statistical significance, possibly due to small sample size and large standard deviation. Furthermore, we obtained the significant negative associations of IL-10, IFN-γ, IL-8, and IL-12p70 with fasting glucose level, but not with 1h and nor 2h serum glucose. The reason for this may need further investigation. On the basis of these findings, it can be hypothesized that these cytokines might have protective effect against insulin resistance.

A limitation of our study was the small size of patients, and larger sample size is needed to further confirm these findings. Second, we cannot exclude some maternal factors risk of GDM, such as no data on the OGTT before pregnancy. Finally, the current study only focused on women during 24 to 28 weeks' gestation. Therefore, the profile of cytokines in early pregnancy and postpartum requires further investigation to elucidate. Although the potential relationship of cytokines and underlying mechanisms with disordered glucose metabolism were not investigated in this study, the results of our study provide an alternative metabolic related cytokines or insulin-sensitive monitoring markers. However, up to the moment, this is one of the few studies that analyzed such a large variety of immunological factors in the peripheral blood of women with and without GDM.

Conclusions

Evidence suggested of that chronic inflammatory state associated with several metabolic disturbances. In this regard, it is worth characterizing the dysregulated cytokines in the women with GDM. Therefore, this acritical provides information regarding a series of cytokines changes to help understand the circling immune response to GDM. We screened the most differentially expressed cytokines between the GDM group and non-GDM group, and hsCRP, IL-2 and IL-6 are significantly increased in patients with GDM during 24 to 28 weeks' gestation. And IL-10, IFN-γ, IL-8, and IL-12p70 might act as protective factors against GDM development. Due to the limitations of this study, more studies are needed to verify the relationship between cytokines levels and prenatal and postnatal glucose metabolism in GDM patients.

Availability of data and material Not applicable

Code availability Not applicable

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by YYY and ZHJ. WZ was contributed to the manuscript drafting and revision. YP contributed to revising the manuscript. All authors read and approved the final manuscript.

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Declarations The authors declare no conflict of interest.

Conflict of interest The authors declare no competing interests.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of The Third Affiliated Hospital of Guangzhou Medical University.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Written informed consent for publication of clinical details was obtained from the patients.

References

1. Swierzewska P, Kosiński M, Wójcik M, Dworacka M, Cypryk K. Family, anthropometric and biochemical factors affecting birth weight of infants born to GDM women. Ginekol Pol. 2015;86(7): 499–503.
2. Metzger BE, et al. Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care. 2007;2(Supplement 2):251–60.
3. Boule NG, et al. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA. 2002;12(1):1218–27.
4. Nicholas, et al. Alteration of endothelial function markers in women with gestational diabetes and their fetuses. J Matern Fetal Neonatal Med. 2012.
5. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr. (5): 1256S.
6. Powe CE, et al. History of gestational diabetes mellitus and risk of incident invasive breast cancer among parous women in the Nurses' Health Study II prospective cohort. other. 2017;26(3).
7. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11(2):85–97.
8. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2014;132(6):2169–80.
9. Murthy KAS, et al. Evaluation of oxidative stress and proinflammatory cytokines in gestational diabetes mellitus and their correlation with pregnancy outcome. Indian J Endocrinol Metab. 2018;22(1):79–84.
10. Retterstol L, Eikvar L, Berg K. A twin study of C-reactive protein compared to other risk factors for coronary heart disease. Atherosclerosis. 2003;169:279–82.
11. Noordam, et al. High-sensitivity C-reactive protein, low-grade systemic inflammation and type 2 diabetes mellitus: a two-sample Mendelian randomization study. Nutr Metab Cardiovasc Dis. 2018;28(8):795–802.
12. Ferrante AW Jr. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. J Intern Med. 2007;262(4):408–14.
13. Wolf M, Sandler L, Hsu K, Vossen-Smirnakis K, Ecker JL, Thadhani R. First-trimester C-reactive protein and subsequent gestational diabetes. Diabetes Care. 2003;26(3):819–24.
14. Pantham P, Aye ILMH, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta. 2015;36(7):709–15.
15. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochem Biophys Res Commun. 2005;334(4):1092–101.
16. Jiang Y. The relationship between insulin resistance and leptin, INF-α in patients with gestational diabetes mellituus. Chinese Journal of Practical Gynecology and Obstetrics. 2005.
17. Daneshmandi, S., et al., Evaluation of Relation between IL-4 and IFN- Polymorphisms and Type 2 Diabetes. 2009.
18. Matthew, et al. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. J Biol Chem. 2004.
19. Forte GI, Pilato G, Vaccarino L, Sanacore M, Candore G, Romano GC, Testa R, Franceschi C, Capri M, Marra M, Bonfigli AR, Caruso C, Scola L, Lio D. Risk profiles in type 2 diabetes (metabolic syndrome): integration of IL-10 polymorphisms and laboratory parameters to identify vascular damages related complications. Curr Pharm Des. 2010;16(07).
20. Korkmazer E, et al. Correlation between inflammatory markers and insulin resistance in pregnancy. J Obstet Gynaecol. 2015.
21. International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. Diabetes Care. 2010;33(3):676–82.
22. Lappas M, Mundra PA, Wong G, Huynh K, Jinks D, Georgiou HM, Permezel M, Meikle PJ. The prediction of type 2 diabetes in women with previous gestational diabetes mellitus using lipidomics. Diabetologia. 2015;58:1436–42.
23. Tan KCB, Wat NMS, Tam SCF, Janus ED, Lam TH, Lam KSL. C-Reactive protein predicts the deterioration of glycemia in chinese subjects With Impaired Glucose Tolerance. Diabetes Care. 2003;26(8):2323–8.
24. Tamakoshi K. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. Int J Obes Relat Metab Disord. 2003;27:443–9.
25. Ke Y, Liu K, Huang GQ, Cui Y, Kaplan HJ, Shao H, Sun D. Anti-inflammatory role of IL-17 in experimental autoimmune uveitis. J Immunol. 2009;182(5):3183–90.

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ORIGINAL ARTICLE

Role of third-trimester OGTT in the detection of late-onset gestational diabetes in the Indian population

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Abstract

Background There are numerous guidelines defining the diagnostic strategy of gestational diabetes mellitus, but majority of them do not suggest follow-up beyond 28 weeks. Hence, this study was done to find the prevalence of abnormal glucose tolerance in the third trimester of pregnancy and to reinforce the importance of repeat screening in the third trimester of pregnancy.

Methods Four hundred sixty-eight antenatal women with normal first- and second-trimester screening tests were subjected to a repeat screening for gestational diabetes with 75 g oral glucose tolerance test (OGTT), at 32 to 34 weeks of gestation. Maternal and fetal outcomes were analyzed.

Results Of the total 468 antenatal women, 16.8% had decreased gestational glucose tolerance (DGGT) and 7.69% had gestational diabetes mellitus (GDM) diagnosed for the first time in the third trimester. Family history of diabetes was the significant risk factor for the development of GDM (p value = 0.006). There was no statistically significant difference in the maternal outcomes. Among neonatal outcomes, incidence of low APGAR score at 1 min and NICU admission for management of various complications were significantly high in women with abnormal glucose tolerance (p value = 0.0015).

Conclusion Since the incidence of detection of glucose intolerance was significantly high, our study implies the importance of screening in the third trimester of pregnancy, although there was not much difference in the adverse obstetric and neonatal outcomes.

Keywords Abnormal glucose tolerance · Gestational diabetes mellitus · Third trimester screening

Introduction

Gestational diabetes mellitus (GDM) is defined as impaired glucose tolerance with onset or first recognition during pregnancy associated with various maternal, fetal, and neonatal complications [1]. Screening and diagnosis of such an important disease have evolved over the years with different global bodies suggesting different approaches. There is a wide variation in the prevalence of GDM worldwide because of the demographic characteristics such as race, ethnicity, and the difference in screening and diagnostic methodology used. The global prevalence on average is 17% but varies between < 5% in some European countries to a staggering 27% in Southeast Asia [2]. Hence, screening is universal in most Southeast Asian countries.

India is one of the epicenters for the global burden of GDM. The prevalence of GDM in India is 19.19% according to IADPSG criteria, and by WHO criteria, 10% [3]. Although the prevalence of GDM kept on increasing in India, there was no uniform consensus on best practices for screening and diagnosis until a few years back.

In February 2018, the Ministry of Health and Family Welfare (MoHFW) released guidelines for the diagnosis of GDM in India based on the Diabetes in Pregnancy Study Group of India (DIPSI) recommendations. As per the guidelines, universal screening of all pregnant women with non-fasting 75 g of oral glucose tolerance test (OGTT) at first antenatal contact is recommended. If negative, a second test should be repeated at 24–28 weeks of gestation [4].

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If negative, no further testing is recommended thereafter although DIPSI suggested a repeat testing at 32–34 weeks of gestation [5].

This is in line with most guidelines which do not recommend testing in the third trimester when the diabetogenic maternal hormones such as progesterone, human placental growth factor, and human placental lactogen are found in their highest levels [6–10]. It could be proposed that undiagnosed late-onset glucose intolerance might be associated with adverse perinatal outcomes as seen in two cases of late stillbirth in our institution, which were retrospectively attributed to late-onset hyperglycemia.

Hence, the current study on third-trimester screening for gestational diabetes was initiated to find out the prevalence of late-onset glucose intolerance and to compare their obstetric and neonatal outcomes with women with normal glucose tolerance (NGT).

Materials and methods

This was a prospective analytical study carried out at PSG Institute of Medical Sciences and Research, a tertiary healthcare referral institution between March 1, 2018, and February 28, 2019. The sample size was calculated based on the incidence of GDM diagnosed in our institution, which was approximately 12%.

Four hundred sixty-eight antenatal women with a singleton pregnancy who presented to our outpatient department between 32 and 34 weeks of gestation with normal test results for diabetes screening in the first and second trimester were randomly selected and included in the study. Women with pre-gestational diabetes, women on steroid therapy, and those with other medical disorders were excluded from the study.

Blood samples collected after 2 h of OGTT were centrifuged and analyzed by the hexokinase method using commercial Randox kits run on an automated Randox daytonal clinical chemistry analyzer. A 2-h plasma glucose level $\geq$ 140 mg/dL was taken as GDM; values between 120 and 139 mg/dL were taken as decreased gestational glucose tolerance (DGGT). Collectively, they were grouped under abnormal glucose tolerance (AGT). A 2-h plasma glucose level < 120 mg/dL was taken as normal glucose tolerance (NGT).

Women diagnosed with AGT were initially offered medical nutrition therapy (MNT) if the 2-h plasma glucose level was < 200 mg/dL for a period of 2 weeks. Women with plasma glucose levels $\geq$ 200 mg/dL were directly started on insulin therapy with or without oral hypoglycemic agents (OHA) as per MoHFW guidelines [4]. Fasting and 2-h postprandial plasma glucose levels were monitored every 2 weeks and the treatment was continued or modified as per plasma glucose levels until delivery.

The sociodemographic characteristics, risk factors, obstetric outcomes such as gestational age at delivery, preterm labor, and mode of delivery were studied in both groups, i.e., AGT vs. NGT. The neonatal outcomes analyzed in the two groups include birth weight, complications such as birth asphyxia (APGAR < 7 at 1 and 5 min), hypoglycemia, hyperbilirubinemia, hypernatremia, intracranial hemorrhage, respiratory distress syndrome, sepsis, NICU admission, and perinatal death.

All the statistical analyses were carried out in R package version 4.0.2. Chi-square test was used for categorical variables and Student's *t* test was used for continuous variables. Logistic regression analysis was done to determine association of risk factors with late-onset GDM.

Results

Of the 468 women who underwent non-fasting 75 g OGTT between 32 and 34 weeks of gestation in our study, 75.4% ($n = 353$) had normal glucose tolerance, 16.9% ($n = 79$) had decreased gestational glucose tolerance (DGGT), and only 7.7% ($n = 36$) had GDM.

Table 1 shows maternal demographic characteristics. The mean maternal age at delivery was comparable in both groups. The mean body mass index (BMI) was higher in the AGT group and was statistically significant (24.44 vs 23.31, p value = 0.012). Of the women in AGT, 41.7% were overweight or obese (BMI $\geq$ 25) compared to 33.7% in the NGT group.

Table 2 represents the distribution of various risk factors in our study population. The majority of women in both groups were primigravida. There was no difference in the occurrence of any of these risk factors except for a family history of GDM which was found to be significant for the development of AGT in the third trimester after adjusting for confounders like age and BMI (p value = 0.0155*).

Tables 3 and 4 refer to the various obstetric and neonatal outcomes. The mean gestational age at delivery was similar in both groups which was 38 weeks. Although statistically not significant, the percentage of women having preterm births was higher in the AGT group (7.9% vs. 5.4%). There was no difference in the mode of delivery with approximately 60% of women having normal vaginal delivery in both groups.

The mean birth weight at delivery was similar in both groups. LGA (birth weight > 90th centile) occurred more frequently in the AGT group (6.1% vs. 4.5%) whereas SGA (birth weight < 10th centile) was seen frequently in NGT group (14.5% vs. 9.6%).But they were not statistically significant.

Table 1 Maternal demographic characteristics

Maternal characteristics	Category	NGT			AGT			*p* value
		n (%)	Mean	SD	*n* (%)	Mean	SD	
Age	Total	353 (100)	25.89	3.83	115 (100)	26.38	4.16	0.2682
	≤29 years	293 (83)	24.67	2.86	90 (78.3)	24.7	2.8	0.9433
	≥30 years	60 (17)	31.85	1.93	25 (21.7)	32.44	2.08	0.231
BMI	Total	353 (100)	23.31	4.09	115 (100)	24.44	4.17	0.012*
	≥25 kg/m^2	119 (33.7)	27.73	3.15	48 (41.7)	28.37	3.02	0.2236
	<25 kg/m^2	234 (66.3)	21.06	2.28	67 (58.3)	21.62	2.06	0.0572*

NGT, normal glucose tolerance; *AGT*, abnormal glucose tolerance; *SD*, standard deviation; *BMI*, body mass index.

**p* value < 0.05.

Table 2 Maternal risk factors

Risk factor	Category	NGT (*n* = 353)		AGT (*n* = 115)		OR (95% CI)	*p* value
		N	%	*n*	%		
Parity	Primigravida	236	67	80	70	0.71 (0.42–1.2)	0.1782
	Multigravida	117	33	35	30		
Family H/O DM	Yes	59	17	33	29	0.54 (0.33–0.89)	0.0155*
	No	294	83	82	71		
Hypothyroidism	Yes	93	26	31	27	0.95 (0.59–1.54)	0.8455
	No	260	74	84	73		
PCOS	Yes	8	2	6	5	0.46 (0.15–1.38)	0.167
	No	345	98	109	95		
Previous H/O GDM	Yes	8	2	3	3	0.95 (0.25–3.67)	0.9402
	No	345	98	112	97		

NGT, normal glucose tolerance; *AGT*, abnormal glucose tolerance; *GDM*, gestational diabetes mellitus; *PCOS*, polycystic ovary syndrome.

**p* value < 0.05.

Table 3 Obstetric and neonatal outcomes

Outcomes	NGT (*n* = 353)			AGT (*n* = 115)			OR (95% CI)	*p* value
	n (%)	Mean	SD	*n* (%)	Mean	SD		
Gestational age at delivery (weeks)	353 (100)	38.3	1.38	115 (100)	38.1	1.14	1.09 (0.93–1.27)	0.3034
Preterm deliveries (weeks)	19 (5.4)	34.52	1.72	9 (7.9)	35.77	0.45	0.25 (0.04–1.65)	0.1506
Birthweight (kg)	353 (100)	2.92	0.39	115 (100)	2.95	0.39	0.84 (0.49–1.44)	0.5128
LGA (>90th centile) (kg)	16 (4.5)	3.83	0.2	7 (6.1)	3.78	0.2	0.98 (0.03–2.92)	0.4271
SGA (<10th centile) (kg)	51 (14.5)	2.28	0.2	11 (9.6)	2.24	0.3	2.65 (0.2–35.02)	0.4597
APGAR < 7 at 1 min	36 (10.2)	6.63	0.93	12 (10.4)	5.75	1.95	1.31 (1.02–1.68)	0.0359*
APGAR < 7 at 5 min	7 (1.9)	6.57	0.79	4 (3.5)	5	2.16	2.65 (0.63–11.27)	0.1858

NGT, normal glucose tolerance; *AGT*, abnormal glucose tolerance; *OR*, odds ratio.

**p* value < 0.05.

The rate of admission to NICU for management of various neonatal complications was significantly high in the AGT group (*p* value = 0.0023). The most common indication for NICU admission in this group was hyperbilirubinemia. The incidence of birth asphyxia, i.e., APGAR score < 7 at 1 min, was higher in AGT group, which was statistically significant (*p* value = 0.0359). But there was no difference in the APGAR score at 5 min. The other neonatal complications such ashypoglycemia, electrolyte imbalances, and neonatal trauma did not differ in both groups. There were no cases of respiratory distress syndrome, sepsis, and neonatal mortality in the AGT group in our study.

Table 4 Obstetric and neonatal outcomes

Outcomes	Category	NGT ($n=353$) n (%)	AGT ($n=115$) n (%)	OR (95% CI)	p value
Mode of delivery	Normal vaginal delivery	214 (61)	68 (59)	-	-
	Operative vaginal delivery	34 (10)	10 (9)	1.10 (0.52–2.36)	0.799
	Emergency LSCS	68 (19)	25 (22)	0.87 (0.51–1.49)	0.6171
	Elective LSCS	37 (10)	12 (10)	1.03 (0.51–2.1)	0.9324
	NICU admission	15 (4)	15 (13)	0.31 (0.14–0.65)	0.0023*
Neonatal complications	Hyperbilirubinemia	17 (5)	12 (10)	0.19 (0.02–1.81)	0.1501
	Hyperbilirubinemia and hypernatremia	1 (0)	2 (2)	0.09 (0.003–2.18)	0.1383
	Hypernatremia	4 (1)	0 (0)	0 (0–4.86)	0.9903
	Intracranial hemorrhage	1 (0)	0 (0)	0 (0–123.7)	0.9951
	Parietal cephalhematoma	2 (1)	0 (0)	0 (0–17.02)	0.9931
	Respiratory distress syndrome	3 (1)	0 (0)	0 (0–7.77)	0.9918
	Sepsis	1 (0)	0 (0)	0 (0–123.78)	0.9952

NGT, normal glucose tolerance; *AGT*, abnormal glucose tolerance; *OR*, odds ratio; *LSCS*, lower segment caesarean section; *NICU*, neonatal intensive care unit.

*p value < 0.05.

Discussion

The incidence of GDM in the third trimester in the present study is 7.7%, which is in addition to the cases diagnosed until 28 weeks of gestation. This is less than the 13.5% rate reported by Fonseca et al. in a similar study done between 32 and 36 weeks of gestation using the IADPSG criteria [11]. A comparison between DIPSI and IADPSG criteria to diagnose GDM done in the Indian population noted that the diagnosis in almost 22.3% of women might be missed when using DIPSI criteria [12]. This could explain the vast difference in the incidence.

The systematic review and meta-analysis performed by Lee et al. identified age ≥ 25, BMI ≥ 25 kg/m^2, multiparty ≥ 2, previous history of GDM, macrosomia, family history of diabetes, PCOS, and pregnancy-induced hypertension as significant risk factors for the development of GDM in the Asian population [13]. In our study, majority of women were primigravida (approximately 70%) and although women diagnosed with AGT weighed higher than the women with NGT at the time of diagnosis, there was no difference in the distribution of overweight/obese women (BMI ≥ 25 kg/m^2) between these groups. A family history of diabetes mellitus was present in 29% of women with AGT which was the only statistically significant risk factor. Interestingly, none of the women was diagnosed with hypertensive disorders of pregnancy during pregnancy and postpartum until discharge. Although the previous history of GDM is a high-risk factor for the diagnosis of GDM, it is not reflected in our study possibly due to screening and diagnosis at early gestations [14].

Various adverse obstetric and neonatal outcomes are associated with GDM diagnosed before 28 weeks of gestation [1]. This study attempted to investigate the rate of these complications in women diagnosed with new-onset abnormal glucose tolerance in the third trimester and whether they significantly differed from the women with normal glucose tolerance. In the study by Fonseca et al., the rate of cesarean section was found to be significantly higher in women with AGT diagnosed in the third trimester [11]. The higher rate of cesarean section (CS) reported in a similar study done by Shindo et al. was explained by the performance of scheduled cesarean section for macrosomia to prevent shoulder dystocia [15]. This is in contrast to our study where there was no difference in the mode of delivery between the two groups. The mean birth weight (2.9 kg) did not differ between both groups. Only 6.1% of babies born to women with AGT were LGA with the mean birth weight among the LGA babies being 3.78 kg. The mean GA at delivery in the AGT group was also 38 weeks. This could be explained by the late onset of hyperglycemia leaving less time to adversely impact these outcomes. The strict management of GDM instituted upon diagnosis, our department policy of offering induction of labor at 38 weeks for women on medical management of GDM and at 40 weeks for women on medical nutrition therapy, could be the other possible explanations for the low incidence of macrosomia, therefore the low CS rate.

Arbib et al. analyzed the adverse perinatal outcomes associated with third-trimester abnormal OGTT through a retrospective cohort study [16]. Apart from the increased incidence of LBW in neonates of mothers with GDM, no other neonatal outcome was reported to have statistical significance. Our study shows that neonates born to mothers

with AGT have low APGAR scores at 1 min and frequently require NICU admission for the management of various complications, the most common being hyperbilirubinemia.

To our knowledge, there are no studies on the adverse outcomes associated with late-onset hyperglycemia diagnosed using the DIPSI criteria. But extrapolating the results of this study to the general population worldwide will be limited as the DIPSI criteria have been unique to the Indian population. Although there was not much difference in the adverse outcomes in our study groups (AGT vs NGT), it is worthwhile to mention that the outcome might be different when the comparison is restricted to women with GDM alone. Also, the impact of late-onset hyperglycemia on long-term metabolic outcomes is still unclear and will require further investigation.

Conclusion

There are not many studies that investigated the adverse outcomes associated with third-trimester abnormal OGTT using the DIPSI criteria. The incidence of new-onset GDM in the third trimester in this study (7.7%) did not differ much from the nation's incidence of GDM diagnosed before 28 weeks (10–14%) [4]. Although there was not much difference in the adverse obstetric and neonatal outcomes, we tend to miss identifying an important disease with long-term implications on both maternal and child health in a significant number of women when skipping screening in the third trimester [17, 18]. Hence, it is worthwhile to investigate the same with a larger sample size.

Declarations

Ethical approval The study was initiated after obtaining approval from the Institutional Human Ethics Committee, PSG IMSR.

Ethical consent After obtaining oral and written consent, detailed history taking and risk factor assessment were done following which they were subjected to the non-fasting 75 g OGTT (DIPSI criteria).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. HAPO study Cooperative research group, Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse pregnancy outcomes. NEngl J Med. 2008;358:1991–2002
2. International Diabetes Federation. IDF diabetes Atlas, 9th edn. Brussels, Belgium: 2019. Available at: https://www.diabetesatlas.org. Accessed Nov 2020
3. Li KT, Naik S, Alexander M, Mathad JS. Screening and diagnosis of gestational diabetes in India: a systematic review and meta-analysis. Acta Diabetol. 2018;55(6):613–25.
4. Government of India. Maternal and health division, diagnosis and management of gestational diabetes mellitus: technical and operational guidelines. New Delhi, India: Ministry of Health& Family Welfare, New Concept Information Systems; 2018.
5. Gestational Diabetes Mellitus Guidelines [Internet]. [Cited 2020 Dec 20]. Available from: https://www.japi.org/r2f4d464/gestational-diabetes-mellitus-guidelines. Accessed Nov 2020
6. Sonagra AD, Biradar SM, Dattatreya K, Murthy DSJ. Normal pregnancy-a state of insulin resistance. J Clin DiagnRes. 2014;8:CC01–3.
7. Committee on practice bulletins—obstetrics. ACOG Practice Bulletin No. 190: Gestational diabetes mellitus. Obstet Gynecol.2018; 131:e49–e64.
8. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes. Diabetes Care.2018;41(S1): S13–S27.
9. Hod M, Kapur A, Sacks DA, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes Mellitus: a pragmatic guide for diagnosis, management, and care. Int J Gynecol Obstet. 2015;131(S3):S173–211.
10. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33:676–682
11. Fonseca A, Lopes J, Clode N. Glucose intolerance in the third trimester is not predictive of adverse outcomes. Int J Gynecol Obstet. 2019;147:108–14.
12. Vij P, Jha S, Gupta S, Aneja A, Mathur R, Waghdhare S, Panda M. (2015). Comparison of DIPSI and IADPSG criteria for diagnosis of GDM: a study in a north Indian tertiary care center. Int J Diabetes Dev Ctries. 2015;35. https://doi.org/10.1007/s13410-014-0244-5.
13. Lee KW, Ching SM, Ramachandran V, et al. Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2018;18:494. https://doi.org/10.1186/s12884-018-2131-4.
14. Diabetes in pregnancy: management of diabetes and its complications from pre-conception to the postnatal period. [(Accessed on 21 May 2021)]; Available online: https://www.nice.org.uk/guidance/cg63.
15. Shindo R, Aoki S, Nakanishi S, Misumi T, Miyagi E. Impact of gestational diabetes mellitus diagnosed during the third trimester on pregnancy outcomes: a case-control study. BMC Pregnancy Childbirth. 2021;21(1):246.
16. Arbib N, Gabbay-Benziv R, Aviram A, Sneh-Arbib O, Wiznitzer A, Hod M, Chen R, Hadar E. Third trimester abnormal oral glucose tolerance test and adverse perinatal outcome. J Matern Fetal Neonatal Med. 2017;30(8):917–21.
17. Shou, Chong1; Wei, Yu-Mei2; Wang, Chen2; Yang, Hui-Xia2, Updates in long-term maternal and fetal adverse effects of gestational diabetes mellitus, maternal-fetal medicine: October 2019 - Volume 1 - Issue 2 - p 91–94
18. Scholtens DM, Kuang A, Lowe LP, Hamilton J, Lawrence JM, Lebenthal Y, Brickman WJ, Clayton P, Ma RC, McCance D, Tam WH, Catalano PM, Linder B, Dyer AR, Lowe WL Jr, Metzger BE; HAPO Follow-up Study Cooperative Research Group; HAPO follow-up study cooperative research group. hyperglycemia and adverse pregnancy outcome follow-up study (HAPO FUS): maternal glycemia and childhood glucose metabolism. Diabetes Care. 2019 Mar;42(3):381–392

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ORIGINAL ARTICLE

Outcome of glucose tolerance condition in patients with normal glucose tolerance with either persistently high or low 1-h postchallenge glucose levels in 75 g oral glucose tolerance test

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Abstract

Purpose of the study This study aimed to investigate whether persistently high 1-h postchallenge glucose (PG) levels in a 75 g oral glucose tolerance test (75 g OGTT), as well as persistently low 1-h PG levels, are a risk factor for reclassification from normal glucose tolerance (NGT) into impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) among participants continually observed for 11 years.

Methods This single-center retrospective study used the electronic records of all participants undergoing Ningen Dock (health checkup) at Kiryu Kosei General Hospital between 2008 and 2018. In 2008, 361 of 523 participants who received Ningen Dock had NGT. Of the 361 participants, 109 received 75 g OGTT yearly for 11 years (2008–2018), and 72 of these 109 participants showed either persistently high 1-h PG (> 155 mg/dL) or persistently low 1-h PG (< 155 mg/dL) levels. These 72 participants with NGT were analyzed to observe the 1-h PG effect on glucose tolerance alteration for 11 years. They were divided into persistently low 1-h PG ($N = 50$) and persistently high 1-h PG ($N = 22$) groups.

Results In the low 1-h PG group, 49 participants remained to have NGT, and the remaining 1 was reclassified as having IGT. In the high 1-h PG group, 8 remained to have NGT, whereas 10 and 4 were reclassified as having IGT and T2DM, respectively.

Conclusion High 1-h PG levels may be a risk factor for reclassification from NGT into IGT or T2DM, but not the low 1-h PG levels.

Keywords 75 g oral glucose tolerance · 1-h postchallenge glucose level in 75 g oral glucose tolerance · Type 2 diabetes mellitus · Impaired glucose tolerance · Normal glucose tolerance

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Introduction

The 1-h postchallenge glucose (PG) level in a 75 g oral glucose tolerance test (75 g OGTT) is reportedly important in predicting the reclassification of patients from normal glucose tolerance (NGT) into type 2 diabetes mellitus (T2DM) [1]. Patients who initially have NGT may also be reclassified to impaired glucose tolerance (IGT), and predicting this reclassification facilitates clinicians in providing the appropriate treatment or prevention. In this study, we aimed to investigate whether persistently elevated 1-h PG levels, as well as persistently low 1-h PG levels, are a risk factor for reclassification from NGT into IGT and T2DM among participants who were continually observed for 11 years.

Materials and methods

In this single-center retrospective study, we used the electronic records of all participants who underwent 75 g OGTT and glycated hemoglobin (HbA1c) measurement during their Ningen Dock (health checkup) at Kiryu Kosei General Hospital between 2008 and 2018.

On day 1 of hospitalization, study participants were requested to fast overnight (for 12 h). On day 2, blood samples were drawn at 8:00 AM and 2 h after the oral administration of 75 g of glucose as the 75 g OGTT. Within < 1 h after venous blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) and fluoride, plasma was separated from the cells. Then, plasma glucose concentration was determined by a hexokinase method using the Synchro CX4/CX5 glucose analyzer (Beckman Instruments, Fullerton, CA). Both intra- and inter-assay coefficients of variation were < 2% at < 7 mmol/L. HbA1c in blood samples collected in EDTA was assessed by high-performance liquid chromatography (Bio-Rad DIAMAT, Ivry-sur-Seine, France), with a normal value range of 4.5–6.5%.

In this study, NGT, IGT, and T2DM were defined as < 110, < 110, and < 126 mg/dL for the fasting plasma glucose (FPG), < 140, 140–200, and ≤ 200 mg/dL for the 2-h PG, and < 6.2%, 6.2–6.5%, and < 6.5% for the HbA1c (normal range as NGT: 4.6–6.2%), respectively.

In 2008, 523 participants received Ningen Dock, of which 361 had NGT according to the 75 g OGTT and HbA1c level results. Using the data of these participants, we evaluated the factors affecting the 1-h PG levels through the analysis of variance. Of the 361 participants, 109 received 75 g OGTT yearly for 11 years (2008–2018), and 72 of these 109 participants had either persistently high 1-h PG (> 155 mg/dL) or persistently low 1-h PG (< 155 mg/dL) levels. A patient was considered to have a high 1-h PG level when the upper range in 1-h PG for the transition from NGT to T2DM was 8.6 mmol/L or 154.8 mg/dL [1].

We then evaluated the 72 participants with NGT to observe the effect of 1-h PG on glucose tolerance alteration for 11 years. These participants were further divided into persistently low 1-h PG ($N = 50$) and persistently high 1-h PG ($N = 22$) groups.

The ethics committee at Kiryu Kosei General Hospital approved our study, which conformed to the Declaration of Helsinki (as 2–K015). Every Ningen Dock, the participants were asked whether they agree to use their Ningen Dock's data for future clinical study and presentation. Thus, all of the eligible participants provided written informed consent prior to participation.

After Ningen Dock, the physician explained to the participants their medical results. These participants also received suggestions and advice regarding necessary diet and lifestyle modifications, as well as abnormal results, by a registered nurse, a registered dietician, and their physician.

Statistical analysis

All statistical data were analyzed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA). All numerical values are expressed as means ± SD. Multiple comparisons of the variables were conducted using Dunnett's test. In addition, we used the Wilcoxon rank-sum test (for non-normally distributed data) and the analysis of variance for comparing continuous variables according to group, and the chi-square test for categorical variables. All tests for significance and the resulting *p* values were two-sided, with a level of significance at 5%.

Results

Characteristics of participants in 2008

Table 1 summarizes the characteristics of the 361 NGT participants in 2008. Age, height (HT), body weight (BW), body mass index (BMI), abdominal circumference, serum creatinine (SCr), triglyceride (TG), low-density

Table 1 Multivariate analysis of factors that affect 1- and 2-h PG levels

	1-h PG < 155	155 < 1-h PG	*p*
Sex (M/F)	35/15	17/5	
Age	53.7 ± 7.7	56.2 ± 7.9	*N.S*
BL	165.7 ± 8.6	166.1 ± 9.6	*N.S*
BW	63.8 ± 11.2	64.8 ± 12.5	*N.S*
BMI	23.1 ± 3.1	23.3 ± 3.0	*N.S*
SBP	117.5 ± 14.3	127.5 ± 10.9	< 0.01
DBP	73.9 ± 9.3	78.7 ± 7.4	< 0.01
Waist circumference	85.1 ± 8.6	86.2 ± 8.1	*N.S*
SCr	0.8 ± 0.2	0.8 ± 0.2	*N.S*
TG	131.2 ± 115.4	130.4 ± 64.6	*N.S*
HDL-c	68.5 ± 19.4	57.4 ± 14.8	< 0.01
LDL-c	132.0 ± 30.0	123.7 ± 25.8	*N.S*
UA	5.6 ± 1.4	6.0 ± 1.4	*N.S*
FPG	92.3 ± 8.1	95.7 ± 7.1	< 0.05
2-h PG	107.1 ± 21.6	115.9 ± 19.5	*N.S*
Hb	14.4 ± 1.3	14.6 ± 1.3	*N.S*
HbA1c	5.1 ± 0.3	5.2 ± 0.3	< 0.05

Summarized results of the multivariate analysis of the factors affecting 1- and 2-h PG levels. *1-h PG* 1-h post challenge glucose level in 75 g oral glucose tolerance test, *2-h PG* 2-h postchallenge glucose level in 75 g oral glucose tolerance test

lipoprotein cholesterol (LDL-c), uric acid (UA), 2-h PG level (in a 75 g OGTT), and hemoglobin (Hb) levels were not significantly different between the persistently low and persistently high 1-h PG groups. Conversely, the levels of systolic blood pressure (SBP; $p < 0.01$), diastolic blood pressure (DBP; $p < 0.01$), high-density lipoprotein cholesterol (HDL-c; $p < 0.01$), FPG ($p < 0.05$), and HbA1c ($p < 0.05$) significantly differed between such groups.

Table 2 Characteristics of participants in 2008

	1-h PG	2-h PG
Age	< 0.0001	< 0.0001
SBP	0.0002	< 0.0001
TG	0.0056	0.0003
FPG	< 0.0001	< 0.0001
HbA1c (NGSP)	< 0.0001	< 0.0001
Sex	0.4451	0.0118
Total cholesterol	0.01944	0.08522
BMI	0.6145	0.55158
DBP	0.49654	0.75783
Abdominal circumference	0.98182	0.3317
HDL-c	0.1424	0.28321
LDL-c	0.06843	0.17571
UA	0.40548	0.33127

The characteristics of the participants were compared between participants with persistently high (> 155 mg/dL) and persistently low (< 155 mg/dL) 1-h PG levels. *HT* height (cm), *BM* body weight (kg), *BMI* body mass index (kg/m^2), *SBP* systolic blood pressure (mmHg), *DBP* diastolic blood pressure (mmHg), *SCr* serum creatinine (mg/dL), *TG* triglyceride (mg/dL), *HDL-c* high-density lipoprotein cholesterol (mg/dL), *LDL-c*, low-density lipoprotein cholesterol (mg/dL), *UA* uric acid (mg/dL), *FPG* fasting plasma glucose (mg/dL), *1-h PG* 1-h postchallenge glucose level in 75 g oral glucose tolerance test (mg/dL), *2-h PG* 2-h postchallenge glucose level in 75 g oral glucose tolerance test (mg/dL), *Hb* hemoglobin (g/dL), *HbA1c* glycated hemoglobin (%)

Analysis of multiple comparisons for factors affecting the 1-h PG level

Multiple comparisons for factors affecting the 1-h PG level were analyzed in 361 participants diagnosed with NGT according to the results of 75 g OGTT and HbA1c levels in 2008 (Table 1). Age ($p < 0.0001$, $p < 0.0001$) and the levels of SBP ($p = 0.0002$, $p < 0.0001$), TG ($p = 0.0056$, $p = 0.0003$), FPG ($p < 0.0001$, $p < 0.0001$), and HbA1c ($p < 0.0001$, $p < 0.0001$) independently affected both the 1- and 2-h PG levels, respectively (Table 2). Sex ($p = 0.4451$, $p = 0.0118$) and the total cholesterol levels ($p = 0.01944$, $p = 0.08522$) weakly influenced 1- and 2-h PG levels, respectively. However, BMI, DBP, abdominal circumference, HDL-c level, LDL-c level, and UA level did not influence both PG levels.

Change in 1-h PG levels between 2008 and 2018

As described earlier, 72 participants showed continuously either high (> 155 mg/dL) or low (< 155 mg/dL) 1-h PG levels yearly for 11 years. As shown in Fig. 1, the 1-h PG level remained > 155 mg/dL in the high 1-h PG group ($N = 22$) and < 155 mg/dL in the low 1-h PG group ($N = 50$) throughout the entire observation period. Thus, the 1-h PG values in the high group were consistently higher than those in the low group during the observation period ($p < 0.01$).

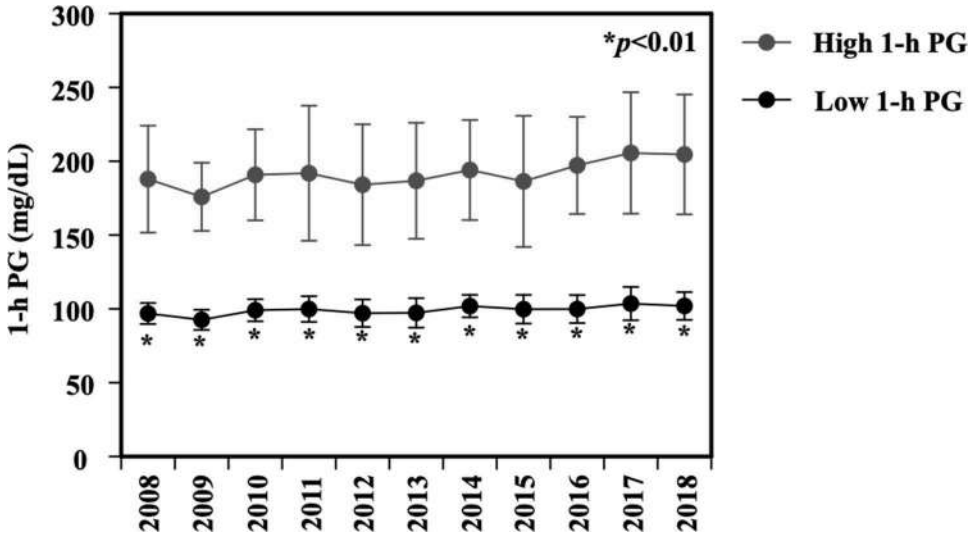

Fig. 1 Changes in 1-h PG levels. A total of 72 participants showed either persistently high 1-h PG (> 155 mg/dL) or persistently low 1-h PG (< 155 mg/dL) levels yearly for 11 years. The high 1-h PG group remained to have > 155 mg/dL (red circle and line, $N = 22$), and the low 1-hPG group remained to have < 155 mg/dL (black circle and line, $N = 50$). *$p < 0.01$ -h PG, 1-h postchallenge glucose level in 75 g oral glucose tolerance test (mg/dL)

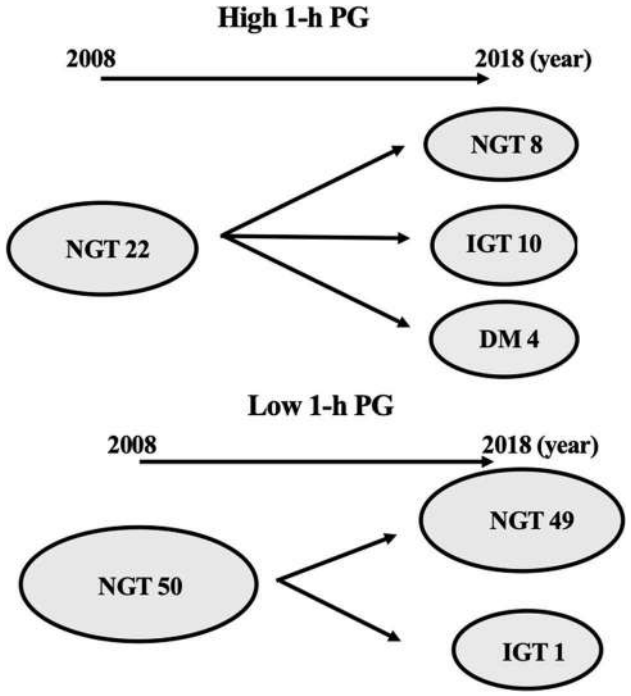

Fig. 2 Reclassification of glucose tolerance from NGT over an 11-year period in participants with either low or high 1-h PG levels. The upper image (labeled as "High 1-h PG") represents the reclassification from NGT into IGT and T2DM in participants with high. 1-h PG levels (> 155 mg/dL). The lower image (labeled as "Low 1-h PG") represents the reclassification from NGT into IGT in participants with low 1-h PG levels (< 155 mg/dL). Each number represents the number of participants 1-h PG, 1-h postchallenge glucose level in 75 g oral glucose tolerance test; NGT, normal glucose tolerance; IGT, impaired glucose; T2DM, type 2 diabetes mellitus

Transition of glucose tolerance condition from NGT after 11 years

In the low 1-h PG group, 49 participants remained to have NGT, while only 1 was reclassified as having IGT after 11 years (Fig. 2). In the high 1-h PG group, 8 participants remained to have NGT, whereas 10 and 4 were reclassified as having IGT and T2DM, respectively, after 11 years.

Discussion

In this clinical study, risk factors such as BMI, DBP, abdominal circumference, and the levels of total cholesterol, HDL-c, LDL-c, and UA, which are linked to both atherosclerosis and metabolic syndrome, were not independent risk factors for 1-h PG levels. Conversely, age, SBP, FPG, and HbA1c were independent risk factors affecting both the 1- and 2-h PG levels. Meanwhile, sex was an independent risk factor for 2-h PG alone, and total cholesterol for 1-h PG alone. Thus, unexpectedly, some factors affected both the 1- and 2-h PG levels independently, and some of them behaved differently as risk factors for 1- and 2-h PG levels. Therefore, 1- and 2-h PG cannot always be controlled together.

A 1-h PG level of > 155 mg/dL might predict the reclassification from NGT into T2DM [1]. Although early-phase insulin secretion is reportedly impaired in 1-h PG [2], our study did not evaluate insulin levels at 75 g OGTT. Therefore, we cannot confirm whether different insulin responses or insulin sensitivities can lead to the different outcomes of glucose tolerance condition in NGT participants with either persistently high or low 1-h PG levels. Nevertheless, considering that age, HT, BW, BMI, and abdominal circumference were not significantly different between the high and low 1-h PG groups, these groups might not have marked differences in terms of peripheral insulin action.

Large-scale epidemiological studies conducted in other populations, including Koreans [3], Israelis [4], Japanese [5], Chinese [6], Native Americans [7], Asian Indians [8], Peruvians [9], and Thais [10], confirmed that 1-h PG is strongly and independently with incident diabetes. The huge difference between our study and the previous studies is that we evaluated participants with either persistently high 1-h PG (> 155 mg/dL) or persistently low 1-h PG (< 155 mg/dL) level, which was confirmed by 75 g OGTT results obtained yearly for 11 years (2008–2018).

Such results revealed that persistently high 1-h PG levels might be a risk factor for reclassification into IGT and T2DM from NGT. Meanwhile, persistently low 1-h PG levels may not be a substantial risk factor for the same reclassification. Considering that SBP, FPG, and HbA1c levels were significantly different between the high and low 1-h PG groups, this difference might partially explain the difference in the transition of glucose tolerance condition from NGT after a period of 11 years.

As described above, every after Ningen Dock, all of the participants were explained on their medical results by their physician. They also received suggestions and advice about necessary measures for diet and lifestyle modifications, as well as abnormal data, by a registered nurse, a registered dietician, and their physician annually. Although occupation and economic status differed between participants, all of the participants were recommended to consistently practice healthy lifestyle measures, such as appropriate diet, smoking cessation, and BW reduction, as emphasized every year during Ningen Dock throughout the observation period.

As shown in Table 1, BW, BMI, and waist circumference were statistically not different between the high and low 1-h PG groups. Thus, as a baseline, interests in health seemed

to be similar between the two study groups. However, given that Ningen Dock is costly, this clinical study mainly investigated patients who could afford Ningen Dock, suggesting a certain grade of bias.

Meanwhile, this clinical study has limitations that warrant discussion. Although this clinical study contains a strong point such that the participants consistently underwent 75 g OGTT yearly for 11 years (2008–2018), our sample size was small ($N=78$), with a limited range on ethnicities, age, sex, and weight. Thus, our results are not fully generalizable. A study involving a larger cohort with a wider range of demographics and a multicenter study design is required to verify the current results.

In conclusion, persistently high 1-h PG levels may be a risk factor for reclassification into IGT and T2DM from NGT, but not the persistently low 1-h PG levels.

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Author contribution KK (Kashima) collected the data. EY, TS, AO, KO (Okada), TW, KK (Kikkawa), KO (Ohshima), and SO analyzed the data. JO, MY, and SO prepared the manuscript.

Data availability The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The ethics committee at Kiryu Kosei General Hospital approved our study, which conformed to the Declaration of Helsinki (as 2-K015).

Consent to participate Whenever the participants received Ningen Dock, they were asked whether they agree to use their Ningen Dock's data for future clinical study and presentation.

Consent for publication All of the authors have also agreed to submit our manuscript to your journal.

Conflict of interest The authors declare no competing interests.

References

1. Jagannthan R, Neves JS, Dorcely B, Chung ST, Tamura K, Rhee M, Bergman M. The oral glucose tolerance test: 100 years later. Diabetes Metab Syndr Obes. 2020;13:3787–805.
2. Sato M, Tamura Y, Someya Y, Takeno K, Kaga H, Kadowaki S, Sugimoto D, Kakehi S, Funayama T, Furukawa Y, Suzuki R, Nakagata T, Nishitani-Yokoyama M, Shimada K, Daida H, Aoki S, Sato H, Kawamori R, Watada H. Characteristics associated with elevated 1-hour plasma glucose levels during a 75-g oral glucose tolerance test in non-obese Japanese men. J Diabetes Investig. 2020;11:1520–3.
3. Oh TJ, Lim S, Kim KM, Jang H, Cho NH. One-hour postload plasma glucose concentration in people with normal glucose homeostasis predicts future diabetes mellitus: a 12-year community-based cohort study. Clin Endocrinol (Oxf). 2017;86:513–9.
4. Bergman M, Chetrit A, Roth J, Jagannathan R, Sevick M, Dankner R. One-hour post-load plasma glucose level during the OGTT predicts dysglycemia: observations from the 24 year follow-up of the Israel Study of Glucose Intolerance, Obesity and Hypertension. Diabetes Res Clin Pract. 2016;120:221–8.
5. Oka R, Aizawa T, Miyamoto S, Yoneda T, Yamagishi M. One-hour plasma glucose as a predictor of the development of type 2 diabetes in Japanese adults. Diabet Med. 2016;33:1399–405.
6. Kuang L, Huang Z, Hong Z, Chen A, Li Y. Predictability of 1-h postload plasma glucose concentration: a 10-year retrospective cohort study. J Diabetes Investig. 2015;6:647–54.
7. Paddock E, Hohenadel MG, Piaggi P, Vijayakumar P, Hanson RL, Knowler WC, Krakoff J, Chang DC. One-hour and two-hour postload plasma glucose concentrations are comparable predictors of type 2 diabetes mellitus in Southwestern Native Americans. Diabetologia. 2017;60:1704–11.
8. Sai Prasanna N, Amutha A, Pramodkumar TA, Anjana RM, Venkatesan U, Priya M, Pradeepa R, Mohan V. The 1 h post glucose value best predicts future dysglycemia among normal glucose tolerance subjects. J Diabetes Complications. 2017;31:1592–6.
9. Lizarzaburu-Robles JC, Torres-Aparcana L, Mansilla R, Valera J, Vargas G, Vento F, Laca J, Cornetero V, Herman WH. A cross-sectional study of the association between the 1-hr oral glucose tolerance test and the metabolic syndrome in a high-risk sample with impaired fasting glucose. Endocr Pract. 2020;26:529–34.
10. Thewjitcharoen Y, Elizabeth Jones A, Butadej S, Nakasatien S, Chotwanvirat P, Wanothayaroj E, Krittiyawong S, Himathongkam T, Himathongkam T. Elevated 1-h post-load plasma glucose levels in Thai people with normal glucose tolerance associated with high diabetes and cardiovascular risk. Diabet Med. 2019;1:1548.

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ORIGINAL ARTICLE

Post-usual meal C-peptide as a reliable and practical alternative to C-peptide following glucagon or standardized mixed-meal for β-cell reserve: a comparative study between three stimulatory methods in different types of diabetes

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Abstract

Introduction Formal stimulation tests for β-cell secretory reserve, like glucagon stimulation test (GST) or mixed meal tolerance test, are cumbersome, hence, difficult to use in clinical practice. C-peptide response at 2-h following usual meal, measured simultaneously with plasma glucose, may be used as an alternative.

Methods We compared C-peptide at 2-h post usual meal (uCP) with C-peptide at 6 min in GST (gCP), and C–peptide at 90 min following standardized mixed meal (Sustacal®) (sCP) in 112 participants, of which 64.3% had T2DM, 12.5% had T1DM, and the remaining 23.2% had other forms of diabetes (LADA, FCPD, MODY, PNDM).

Results Correlations between uCP & gCP and uCP & sCP were assessed in different groups and subgroups. We found either very strong or moderately strong correlation in each categories except in T2DM treated with OAD, where gCP showed fair correlation with uCP. In the receiver operating characteristic (ROC) curves of uCP to define insulin dependence (gCP and sCP thresholds of < 1.8 ng/mL) and to differentiate insulin-requiring from non-insulin-requiring diabetes using equivalent threshold, AUC 0.967 (95% CI, 0.934–1.000) for gCP < 1.8 ng/ml; and 0.993 (95% CI, 0.983–1.002) for sCP < 1.8 ng/ml was found. uCP cut-off of < 1.8 ng/ml had a sensitivity of 94–100% and specificity of 93% for insulin dependency, with 93–95% of patients being identified correctly.

Conclusions uCP is a valid, cost-effective, and practical alternative to gCP or sCP in resource restricted settings, where glucagon and standardized mixed meal are not easily available, and adds to the cost of the diagnostic test.

Keywords Glucagon stimulation test · Standardized mixed meal · Post-usual meal C-peptide · Stimulated C-peptide · β-cell reserve

Introduction

C-peptide estimation, a reliable method of evaluating β-cell secretory reserve, is widely used in management of patients with diabetes mellitus (DM). Assessment of endogenous insulin secretion is often required in clinical practice to characterize the type of DM, particularly in children and adolescents, and to identify responsiveness to oral antidiabetic agents (OADs), in patients reluctant to take insulin. C-peptide may be measured in fasting state or following formal stimulation tests, like glucagon stimulation test (GST) or after standardized mixed-meal (SMM); stimulated C-peptide, however, is preferred over fasting value [1].

The prototypical mixed-meal (MM) tolerance test (MMTT), often considered the "gold-standard," is invasive, and intensive due to frequent venous sampling, required to measure area under the curve (AUC) C-peptide, in addition to measurement of peak C-peptide immunoreactivity. Single 90-min SMM stimulated C-peptide value has subsequently been proposed as an effective alternative to MMTT

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[2]. The SMM preparations, widely used in clinical trials are Sustacal® and Boost®, which are not available in many countries, and the fixed-calorie administered through these test meals, irrespective of body habitus, may not provide adequate stimulus for every patient. The drawbacks of GST are the need for intravenous (IV) injection, and cost of glucagon that adds to the total expenditure of the test. C-peptide assessment in non-fasting or "random" samples, following oral or IV glucose, after sulfonylurea (SU), or after administering "custom-made" MM with broadly similar nutritional content to that of SMM, has been used as substitutes [3]. Assessment of "stimulated" C-peptide following usual meal, a person consumes seems clinically more prudent outside major research trials.

Estimation of venous plasma glucose (VPG) at 2-h post habitual meal is done routinely in day to day practice; simultaneous estimation of C-peptide provides "stimulated" C-peptide value in addition to the post-prandial plasma glucose (PPPG) concentrations. The number of venipuncture thus is reduced, and the PPPG also helps to interpret the C-peptide value, making this protocol practical and patient-friendly.

We compared C-peptide concentrations following usual meal (uCP) with glucagon stimulated C-peptide (gCP), and post-SMM C-peptide (sCP) values in patients with different types of diabetes, aiming to establish uCP as a valid, cost-effective, and practical alternative to gCP or sCP in resource restricted settings. To the best of our knowledge, none of the studies performed so far, compared uCP both with gCP and sCP in a single cohort of different types of diabetes.

Materials and methods

This cross sectional study was performed between January 2019 and December 2019, and was approved by the ethical committee of the institute [Memo No. MMC/IEC-2019/193/1(12) dated 28/01/2019]. Patients with established DM, willing to volunteer for the study, and not having any one of the exclusion criteria, seen during this period, were included irrespective of the type of DM. Informed written consent was obtained from each of the participants.

Patients with primary OAD failure, positive anti-glutamic acid decarboxylase (GAD) antibody, and/or positive anti-islet antigen 2 (IA-2) antibody, with/without past history of documented diabetic ketoacidosis (DKA) was considered as Type 1 DM (T1DM), those with positive anti-GAD and/or anti-IA2 antibody with secondary OAD failure (having documented normoglycemia for at least 6 months with OADs) was considered as latent autoimmune diabetes in adults (LADA), and those with dilated main pancreatic duct (MPD) with multiple ductal calculi, without history of alcohol intake was considered as fibrocalculous pancreatic diabetes (FCPD). We included patients with T1DM early in the course of the disease (newly diagnosed or less than 3 years duration), patients with T1DM beyond their proposed "honeymoon period" (more than 5 years duration), type 2 DM (T2DM) with relatively shorter duration (newly diagnosed or diagnosed within 5 years), long standing T2DM (more than 10 years duration), patients of LADA, FCPD, maturity-onset diabetes of the young (MODY), and permanent neonatal diabetes mellitus (PNDM). The patient with PNDM had mutation in the insulin gene with primary OAD failure without DKA. Two patients with MODY had agenesis of the body and tail of pancreas, bilateral multiple renal cysts, hypomagnesemia, and hyperuricemia, suggestive of MODY type-5, and one had mutation of hepatocyte nuclear factor (HNF)-1α gene (MODY type-3). The total cohort was divided into three groups, T1DM, T2DM, and others, the latter included patients with LADA, FCPD, MODY, and PNDM. Patients with severe co-morbidities (stage 3, 4, 5 chronic kidney disease, chronic liver disease, chronic heart failure, chronic obstructive pulmonary disease), severely ill patients [systemic inflammatory response syndrome (SIRS), sepsis, patients with DKA or idiopathic hyperosmolar hyperglycemic state (IHHS), malignancy, acquired immunodeficiency syndrome (AIDS), active tuberculosis], and pregnant females were excluded.

Detailed history was obtained and all patients underwent thorough clinical examination. Following baseline biochemical investigations including glycated hemoglobin (HbA1c), stimulated C-peptide measurements were performed subsequently on 3 consecutive days, following usual meal (uCP) on day 1, with SMM (sCP) on day 2, and post-glucagon (gCP) on day 3. C-peptide was estimated only if simultaneous VPG was more than 90 mg/dl, in samples drawn after stimulation tests.

Ongoing anti-hyperglycemic agents were continued till previous night in all the three occasions (usual meal, glucagon, Sustacal). uCP and sCP were measured following scheduled morning dose of anti-hyperglycemic agent(s), if any, eliminating any possible confounding effect. It helped us to quantify maximum C-peptide response with insulin secretagogues in type 2 DM, FCPD, or MODY, which in turn could identify people who were likely/unlikely to respond to OADs. Such an effort was made for two reasons. First, C-peptide response following secretagogues helped us to counsel those needle-phobic patients whether they were going to achieve satisfactory glycemic control with oral agents or not. Second, in clinical practice, post-meal glucose is measured after usual meal on usual medications, to adjust OAD dosage. We applied the same logic to quantify C-peptide response following insulin secretagogues, and thus, to identify OAD responders. Stimulated C-peptide response (irrespective of the method use) without OAD could have misidentified OAD responders as OAD non-responders.

Stimulation tests

With usual meal

Patients consumed their habitual meals at scheduled time of the day. Ongoing anti-hyperglycemic agents were administered on usual times. Samples were withdrawn 2-h after starting of the largest meal (lunch).

With SMM

We used Sustacal® (Nestle Health Sciences, Colombo, Sri Lanka), that contains 456 kcal/100 g of powder (47% carbohydrate, 35% fat, 16% protein, and dietary fiber 2%). A total of 1.4 g/kg (6 ml/kg) of the formula was administered up to a maximum of 86 g (360 ml) after an overnight fast. The patients were instructed to continue their scheduled dose of OAD and insulin till the night before, and also the morning dose of OAD and insulin, if any. Samples were withdrawn at 90 min.

With glucagon

The GSTs were carried out in the morning after an overnight fast skipping the scheduled morning dose of anti-diabetic agents. A bolus dose of 1 mg of biosynthetic glucagon hydrochloride (GlucaGen®, Novo Nordisk, Denmark) was given IV. Samples were withdrawn 6 min after injection.

VPG was measured using the glucose oxidase method, HbA1c by high-performance liquid chromatography (HPLC) in Biorad D-10 system, and serum C-peptide by electrochemiluminescence immunoassay in cobas e 411 analyzer (Roche, Basel, Switzerland) within 6 h of venipuncture. The average inter- and intra-assay coefficient of variations (CV) was less than 5% in this platform. The concentration of C-peptide was reported in ng/ml (3 ng/ml = 1 nmol/L).

Statistical analysis

Data was entered in Microsoft spreadsheet and then analyzed. All statistical analyses were conducted with the IBM® SPSS® Statistics ver. 26.0 software (SPSS, Chicago, IL). Values of C-peptides were not normally distributed, so data was expressed as median and interquartile range (IQR). We assessed the correlation between uCP & gCP and uCP & sCP using Spearman's rank correlation coefficient (r_s). Values of 0.8 or higher suggested very strong correlation, between 0.6 and 0.8 indicated moderate correlation, while that between 0.3 and 0.6 indicated fair correlation. We also performed Bland–Altman analysis to evaluate the agreement among these three stimulatory methods. The standard deviation (SD) of all the individual differences was calculated as a measure of variability from which the limits of agreement (LOA) were determined. LOA represent the range of values in which agreement between methods fell within ± 2 SD for 95% of the sample. Bias and precision estimates of ± 0.6 ng/ml (this value is accepted as a cut-off of absolute insulin deficiency, hence, any change up to 0.6 ng/ml may be considered clinically insignificant) and ± 3.0 ng/ml (this value is considered as the cut-off of "normal" cell-response), respectively, were established a priori as the maximum parameters that would indicate acceptable agreement between methods and precision of the difference. This study aimed to establish a stimulatory protocol for assessing β-cell reserve that is not only easy to perform but also to look for insulin dependence, hence deciding on management strategy. Accuracy, thus, was of utmost importance, and we therefore chose a stringent criterion for agreement.

Receiver operating characteristic (ROC) curves were constructed to compare the ability of uCP with that of gCP and sCP, for the already established stimulated C-peptide cut-off value of 1.8 ng/ml to discriminate insulin dependency from OAD responsivity. The areas under the ROC curves were calculated. An AUC of 0.5 indicated no discriminating value of the test. An AUC exceeding 0.7 carried a discriminating strength of statistical significance, while value exceeding 0.8 indicated excellent discrimination. We then assessed the utility of uCP in correctly identifying patients with insulin dependency in relation to established threshold of gCP and sCP (< 1.8 ng/ml). The maximum sensitivity and specificity of uCP were obtained from the ROC curves and using the Youden index.

We also assessed the influence of concomitant VPG on stimulated C-peptide. Fisher's exact test was used for frequencies. $p < 0.05$ was considered significant.

Results

The entire cohort of 112 participants (40 males and 72 females) was divided into 3 groups: T1DM ($n = 14$, 12.5%), T2DM ($n = 72$, 64.3%), and others ($n = 26$, 23.2%), which consisted of 16 patients with LADA, 6 patients with FCPD, 3 patients with MODY, and 1 patient with PNDM. Total of 30 patients of the entire cohort had autoimmune diabetes. Mean age of the study population was 38.61 (± 15.16) years (range, 3–73 years). Among patients with T1DM, 2 had disease duration of more than 5 years. Forty patients with T2DM had been diagnosed with diabetes within 5 years, while remaining 32 patients had disease duration of 10 years or more. All patients with T1DM and "other" forms of diabetes were on insulin, while 16 patients with T2DM were on insulin therapy. The mean HbA1c was 8.35 (± 2.11) % overall, while mean HbA1c for T1DM, T2DM, and others groups were 9.53 (± 2.84) %, 7.90 (± 1.51) %, and 9.02 (± 2.97) % respectively.

The median values of stimulated C-peptide by three different methods among the different groups and subgroups have been summarized in Table 1 and Fig. 1. The numerical values of stimulated C-peptide were highest with SMM in all groups and subgroups.

The correlation between uCP, gCP, and sCP have been shown in Table 2. For T1DM group, we found a very strong correlation between uCP & gCP (r_s, 0.845) and uCP & sCP (r_s, 0.831). For T2DM group, the correlation was moderately strong between uCP & gCP (r_s, 0.631), and very strong between uCP & sCP (r_s, 0.817). The correlations were also very strong in patients allocated in the others group (r_s, 0.885 for uCP & gCP; 0.934 for uCP & sCP). The findings were not much different in the various subgroups we looked at. We found moderately strong or very strong correlation between these tests in all the subgroups except in T2DM patients on OAD; in that subgroup, fair correlation was obtained between uCP & gCP (r_s, 0.408), and also between gCP & sCP (r_s, 0.540). All the correlations were statistically significant.

The Bland–Altman analysis has been summarized in Table 3, and the Bland–Altman plots are shown in Fig. 2.

Table 1 C-peptide response (ng/ml) by the three different stimulatory methods in different groups and subgroups ($n=112$). Data is expressed in median (IQR)

Subgroups	uCP	gCP	sCP
T1DM ($n=14$)	0.34 (0.09–0.72)	0.26 (0.17–0.95)	0.42 (0.12–1.11)
T2DM ($n=72$)	6.00 (3.37–8.56)	5.82 (3.56–8.18)	7.20 (4.87–8.56)
T2DM ≤ 5 years ($n=40$)	6.74 (5.17–8.56)	6.69 (4.26–7.98)	7.32 (5.58–9.08)
T2DM ≥ 10 years ($n=32$)	4.88 (2.95–9.08)	5.29 (3.25–8.66)	6.01 (3.61–8.23)
T2DM with BMI < 23 kg/m^2 ($n=42$)	6.71 (3.17–8.88)	5.64 (3.95–8.47)	7.19 (4.89–9.41)
T2DM with BMI ≥ 23 kg/m^2 ($n=30$)	5.84 (5.10–7.15)	6.56 (3.41–8.04)	7.21 (4.74–8.10)
T2DM on insulin ($n=16$)	2.07 (1.15–3.12)	3.57(1.9–5.21)	4.42 (1.96–5.04)
T2DM on OAD ($n=56$)	6.74 (5.31–8.91)	6.92 (4.91–8.33)	7.44 (6.33–9.08)
Others ($n=26$)	1.37 (0.17–2.42)	1.63 (0.21–2.15)	1.73 (0.43–2.61)
Autoimmune diabetes ($n=30$)	0.20 (0.10–1.17)	0.25 (0.17–1.73)	0.59 (0.19–1.86)
Non-autoimmune diabetes ($n=82$)	5.60 (3.09–7.62)	5.60 (3.34–7.86)	7.01 (4.02–8.40)

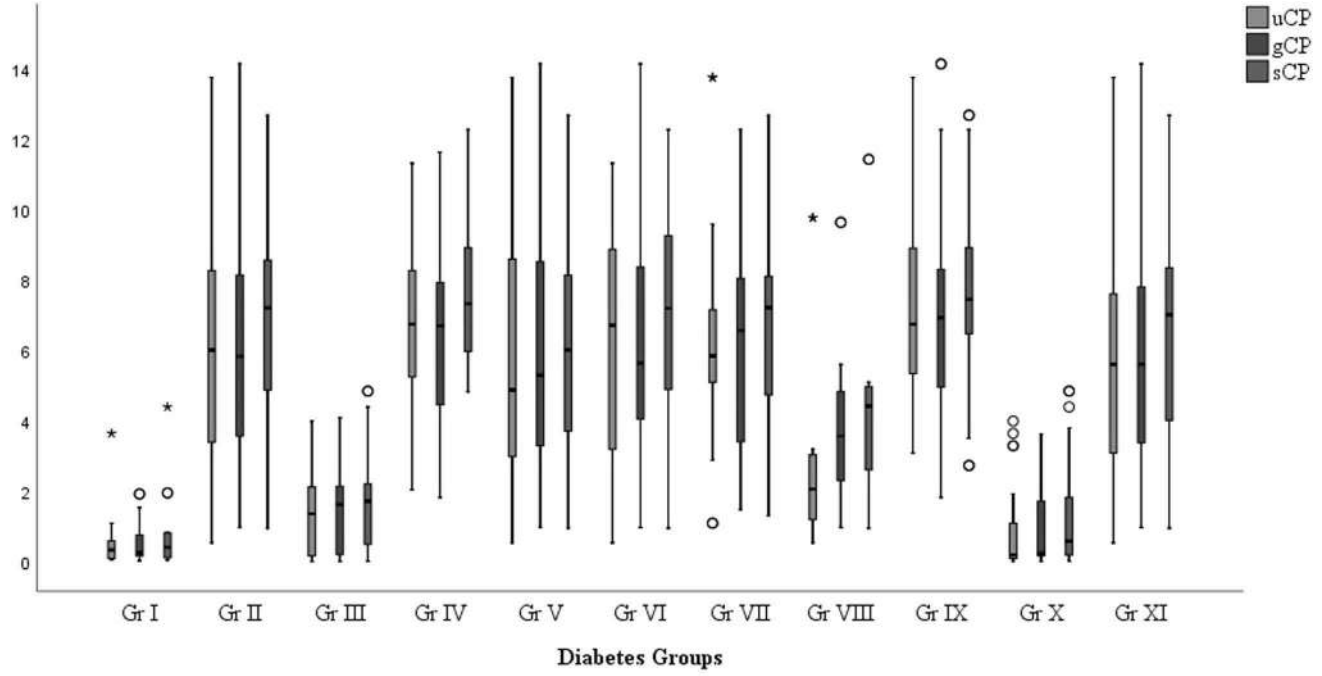

Fig. 1 Clustered boxplot showing median stimulated C-peptide (with IQR) values among different groups and subgroups of the study population ($n=112$). Small circles denote "outliers" and stars denote "far outliers"

Table 2 Correlation between C-peptide values by three different stimulatory methods in different groups and subgroups of the study population ($n=112$)

Subgroups	gCP and sCP		gCP and uCP		sCP and uCP	
T1DM ($n=14$)	r_s	0.969	r_s	0.845	r_s	0.831
	p	<0.000	p	<0.000	p	<0.000
T2DM ($n=72$)	r_s	0.715	r_s	0.631	r_s	0.817
	p	<0.000	p	<0.000	p	<0.000
T2DM ≤ 5 years ($n=40$)	r_s	0.654	r_s	0.701	r_s	0.678
	p	<0.000	p	<0.000	p	<0.000
T2DM ≥ 10 years ($n=32$)	r_s	0.768	r_s	0.650	r_s	0.879
	p	<0.000	p	<0.000	p	<0.000
T2DM with BMI < 23 kg/m^2 ($n=42$)	r_s	0.655	r_s	0.606	r_s	0.853
	p	<0.000	p	<0.000	p	<0.000
T2DM with BMI ≥ 23 kg/m^2 ($n=30$)	r_s	0.857	r_s	0.693	r_s	0.761
	p	<0.000	p	<0.000	p	<0.000
T2DM on insulin ($n=16$)	r_s	0.976	r_s	0.833	r_s	0.762
	p	<0.000	p	<0.000	p	0.001
T2DM on OAD ($n=56$)	r_s	0.540	r_s	0.408	r_s	0.665
	p	<0.000	p	0.002	p	<0.000
Others ($n=26$)	r_s	0.863	r_s	0.885	r_s	0.934
	p	<0.000	p	<0.000	p	<0.000
Autoimmune diabetes ($n=30$)	r_s	0.848	r_s	0.847	r_s	0.864
	p	<0.000	p	<0.000	p	<0.000
Non-autoimmune diabetes ($n=82$)	r_s	0.786	r_s	0.722	r_s	0.858
	p	<0.000	p	<0.000	p	<0.000

$p<0.05$ is significant (2-tailed)

Table 3 Bland–Altman analysis for agreement between uCP & gCP and uCP & sCP in three groups

Groups	Analysis method	SD[a]	Bias[b] (±2SD)	95% C.I. for the bias	Lower LOA[c] (95% CI)	Upper LOA[c] (95% CI)
T1DM	uCP relative to gCP	0.687	0.003 (±1.374)	−0.357–0.363	−1.994- −0.748	0.754–2.000
	uCP relative to sCP	0.335	0.265 (±0.670)	0.089–0.441	−0.710- −0.101	0.631–1.240
T2DM	uCP relative to gCP	2.708	0.216 (±5.416)	−0.409–0.842	−6.284- −4.117	4.550–6.717
	uCP relative to sCP	1.610	0.918 (±3.220)	0.546–1.290	−2.950- −1.658	3.494–4.782
Others	uCP relative to gCP	0.755	−0.010 (±1.510)	−0.300–0.280	−2.021- −1.016	0.997–2.002
	uCP relative to sCP	0.465	0.299 (±0.930)	0.120–0.477	−0.942- −0.322	0.919–1.539

[a]*SD* standard deviation (random variation from the two different methods)

[b]*Bias* mean difference in values obtained with the two different methods (systematic variation between methods)

[c]*LOA* limits of agreement around the bias

Both in T1DM and others group, the LOAs were small enough to suggest that uCP can be used with confidence in place of either gCP or sCP. However, in the T2DM group, the LOAs were large to indicate that uCP cannot be used as an alternative to either the gCP or to the sCP for clinical purposes. This was likely due to significant differences in uCP, gCP, and sCP values in 16 patients in the T2DM group. All these patients were on OAD, and though the C-peptide values, obtained by three methods were widely separated, the lowest values were much higher than the established cut-off of insulin dependency (1.8 ng/ml), making this particular findings in Bland–Altman analysis clinically irrelevant.

We took uCP cut-off of less than 1.8 ng/ml to denote insulin dependency and found AUC of 0.967 (95% CI, 0.934–1.000) for gCP < 1.8 ng/mL, and 0.993 (95% CI, 0.983–1.002) for sCP < 1.8 ng/mL (Fig. 3). Taking GST as reference, we found uCP cut-off of < 1.8 ng/mL having a sensitivity of 94% and specificity of 93% to denote insulin dependence. Ninety-three percent of patients were correctly classified to have insulin dependence. On the other hand, considering sCP of < 1.8 ng/ml as reference, uCP of < 1.8 ng/ml had a sensitivity of 100% and specificity of 93% for insulin dependency with 95% of patients being correctly classified. No statistically significant association

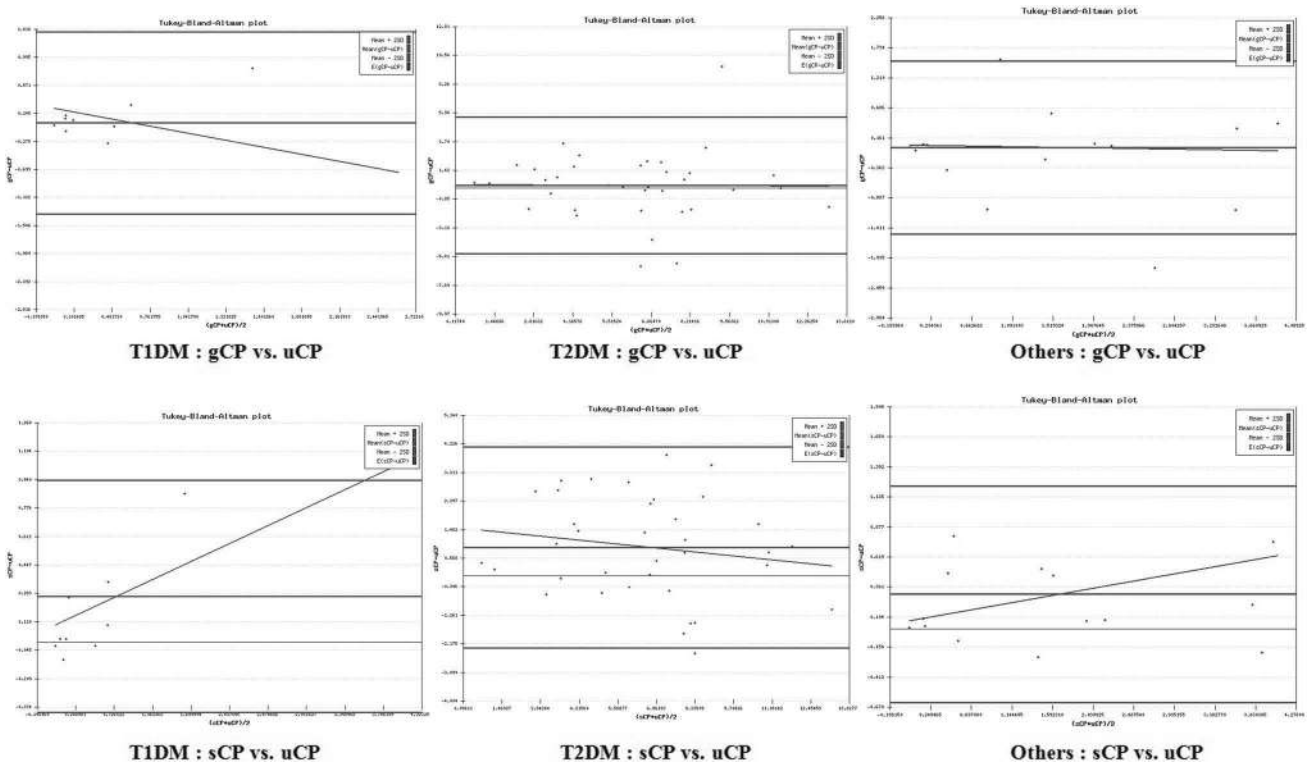

Fig. 2 Bland–Altman plots in three groups. Red lines denote mean, maroon lines denote LOA (mean ± 2SD), and green lines denote line of equality. Dots denote scattered points

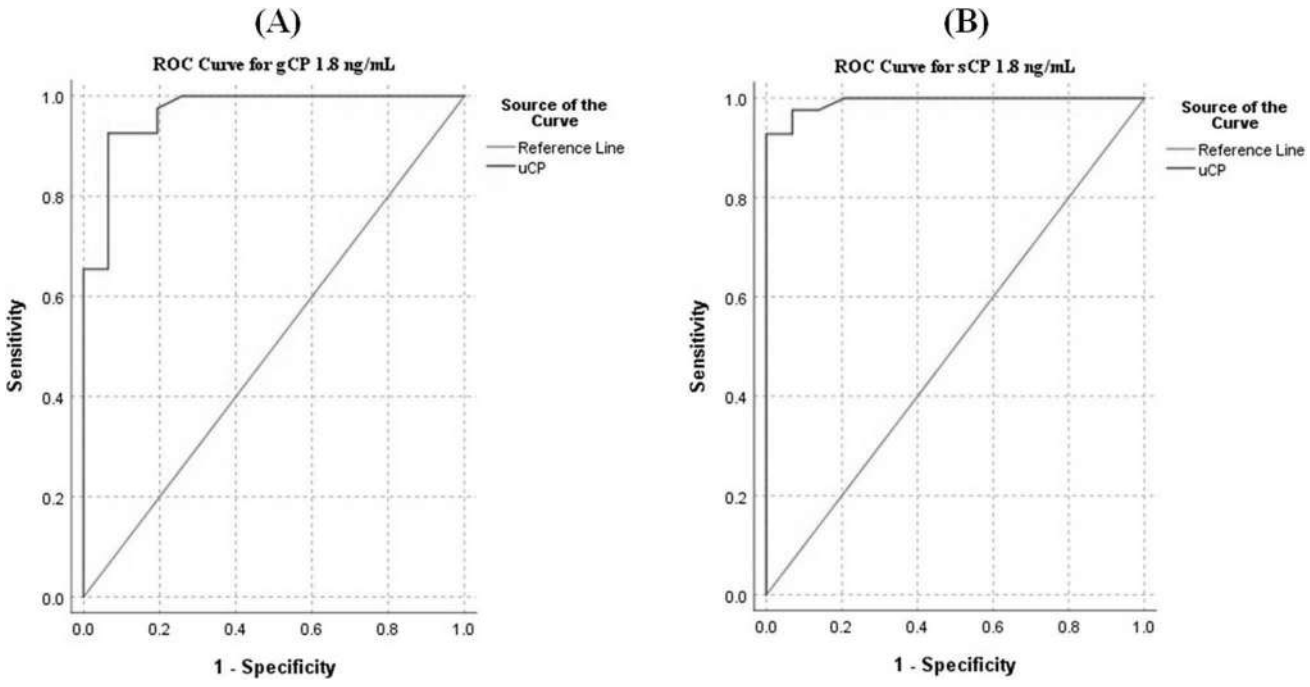

Fig. 3 Receiver operating characteristic (ROC) curves showing ability of uCP to define insulin dependence using equivalent thresholds (< 1.8 ng/mL). **a** for gCP (**b**) for sCP

between stimulated C-peptide responses in any of the methods with concomitant VPG was found.

Discussion

Though different stimulatory methods are being used to assess β-cell secretory reserve, each of these tests has its own limitations. The two formal stimulation tests, GST and SMM have been compared earlier, and SMM was found to be more reproducible and better tolerated than GST for the assessment of β-cell function in patients with T1DM [4]. Similarly, in non-insulin dependent DM, C-peptide response with MM was comparatively higher than glucagon, and the responses demonstrated modest correlation [5]. The higher C-peptide following MM in comparison to glucagon stimulation likely represents the β-cell stimulatory effects of gut-derived peptides and micronutrients present in the meal.

However, the recommended SMM formulations, used in large trials are not freely available in most of the countries, and a number of custom-made preparations, differing widely in their energy and macronutrient contents have been used as alternatives. For example, one study from Denmark used a meal that consisted of one soft boiled egg, half a slice of rye bread with butter, two slices of white bread with butter, 15 g cheese, 20 g jam, 200 ml skimmed milk and tea or coffee [5]. MM used in Nigeria to evaluate β-cell response in T2DM contained a sandwich made from whole wheat bread, fried tomatoes, lettuce, onions, and 1% fat milk added to a cup of tea [6]. A Brazilian study administered a stimulus consisting of 200 ml of 2% cow milk and seven crackers to assess endogenous insulin secretion in a cohort of T1DM [7]. A Dutch study compared two different meals with comparable total energy content and macronutrient composition and found no difference in estimates of β-cell function; the MM consisted of 150 g Egg McMuffin, 50 g croissant, 10 g butter, 15 g jam, 200 ml full fat milk, 20 ml cream, and 11 ml lemon flavored concentrated syrup and the liquid meal contained 1 sachet Calshake vanilla, 240 ml full-fat milk, 20 g sunflower oil, 5 g Fantomalt powder, and 27 g Protifar Plus [8]. Commercially available formulations like 160 ml of Fortisip Compact have also been used [9].

As different formulations have been found to be effective in stimulating β-cells, and preparing such meals are lot more cumbersome than using usual meals, we compared habitual meals during formal stimulation tests. In our study, the correlations between uCP and sCP in all groups and subgroups were statistically significant and clinically relevant. Similar observation was also established in an earlier study, comparing 90 min C-peptide response following SMM with random non-fasting C-peptide (sample was taken within 5-h of a meal between 9 a.m. and 5 p.m.) in insulin-treated patients [9]. This is the only study we came across that compared SMM with usual meal, though the authors relied on random C-peptide.

We also established statistically significant and clinically meaningful correlation between uCP and gCP in all groups and subgroups. This finding was concordant with previously published studies that came across strong correlation between post meal C-peptide and post glucagon C-peptide, both in insulin-treated and non-insulin treated diabetes [10–13]. However, none of these studies, except one (Koskinen et al.) looked at agreement between these stimulatory methods, and relied only on correlation coefficients [12]. We performed Bland–Altman analysis and found agreements in T1DM and others group, and not in T2DM group. However, the finding in the latter was not clinically relevant; in addition, we found the difference between uCP and gCP statistically insignificant when we performed Wilcoxon's signed-rank test in that particular group to assess the significance of the differences between paired measurements. Post-meal C-peptide could effectively discriminate between insulin-requiring and non-insulin-requiring patients in all these studies including ours. We noted that the overall sensitivity of uCP of less than 1.8 ng/ml to denote insulin dependency was 94–100% and specificity of 93% irrespective of VPG, consistent with findings obtained from earlier studies [9].

Chronic hyperglycemia might impair endogenous insulin secretion after a meal load, but has little effect on GST; hence, post-meal C-peptide might get underestimated, and GST supposedly is the best stimulatory test when HbA1c is 9.0% or more [14]. However, we could not find such an association in T1DM and others group with mean HbA1c of 9.53% and 9.02% respectively. Glucotoxicity and lipotoxicity are known to interfere with β-cell secretory reserve. The stimulation tests in our study were performed in consecutive day; hence, we can confidently rule out confounding effects of chronic hyperglycemia in interpretation of correlation between these tests. The mean HbA1c (8.35%) in this particular cohort was consistent with the overall glycemic control of the diabetes population in India, ranging from 7.7 to 8.9% in the recently performed population based studies [15, 16]. Majority of our patients are to be evaluated with this degree of background hyperglycemia and our findings shall give confidence to the treating physicians.

C-peptide values are considered "stimulated," when concurrently measured plasma glucose is more than 144 mg/dl, and this "random" C-peptide, irrespective of last meal was found to be superior to gCP in differentiating T1DM from T2DM [17, 18]. Acute normalization of plasma glucose (90–100 mg/dl) was found to reduce endogenous insulin secretion, following GST in non-insulin treated patients with long standing hyperglycemia (HbA1c:10.2%) [17]. However, we could not find statistically significant association between C-peptide response

and concomitant VPG in any of the three stimulatory tests, suggesting adequate β-cell stimulation depends on factors other than glucose. Similar observation has also been noted in earlier studies [19]. The insulinotropic effect of glucagon is independent of ambient plasma glucose, and the rise in insulin concentration occurs earlier than rise in plasma glucose following intravenous glucagon [20]. Similarly, rise in gut-derived hormones including incretin hormones or other micronutrients (leucine, arginine) present in the meal may themselves act as insulin secretagogues, in addition to potentiating the response to glucose [21]. Though we did not find any association between C-peptide concentrations and simultaneous VPG, C-peptide estimation should not be performed if VPG is less than 90 mg/dl, as endogenous insulin secretion ceases at a glycemic threshold of 80–85 mg/dl [22].

Our study had certain limitations. First, we evaluated 112 participants with different types of diabetes, which may not be sufficient to draw a firm conclusion. The largest study so far which compared random C-peptide with gCP, for classification of diabetes based on C-peptide cut-off, enrolled 1093 patients [18]. However, strength of the cohorts in all the earlier studies that looked into the correlation between different stimulatory methods was less than 76 except one with 142 [9–13]. This particular study may be considered as a pilot study. We are very much interested to replicate this study involving larger population in future. Second, we continued OADs, like SU and gliptins, during the stimulation tests, which might have affected C-peptide responses. Third, we measured uCP at 120 min and sCP at 90 min. C-peptide following oral stimulation peaks at variable time points and might have interfered with results.

To conclude, 120-min C-peptide response following usual meal is an effective and valid alternative to formal stimulation tests. The SMM preparations are not available in India, and making a standardized custom-made preparation is cumbersome in routine practice. Glucagon injection is difficult to procure, costly, and requires IV administration. PPPG at 2 h after usual meal is a standard of care in patients with diabetes; simultaneous C-peptide measurement at the same time point makes uCP a practical way of evaluating β-cell reserve.

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Data availability Available.

Code availability Not applicable.

Declarations

Ethics approval The study was approved by the Intuitional Ethical Committee of Midnapore Medical College and Hospital. This has been mentioned in the "Materials and methods" section. The authors are prepared to provide all necessary documentations related to compliance with ethical standards if required during peer review or after publication.

Consent to participate All the participants provided written consent and this has been mentioned in the "Materials and methods" section.

Research involving human participants and/or animals The study involved human participants.

Informed consent Informed consents were obtained from all participants, and this has already been mentioned in the "Materials and methods" section.

Consent for publication All the authors provided consent for publication of the study findings and agreed to the authors' list mentioned above.

Conflict of interest/competing interests None of the authors have any conflicts of interest.

References

1. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30(7):803–17.
2. Besser RE, Shields BM, Casas R, Hattersley AT, Ludvigsson J. Lessons from the mixed-meal tolerance test: use of 90-minute and fasting C-peptide in pediatric diabetes. Diabetes Care. 2013;36(2):195–201.
3. Leighton E, Sainsbury CA, Jones GC. A practical review of C-peptide testing in diabetes. Diabetes Ther. 2017;8(3):475–87.
4. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H, Type 1 Diabetes Trial Net Research Group; European C-Peptide Trial Study Group. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care. 2008;31(10):1966–71.
5. Gjessing HJ, Damsgaard EM, Matzen LE, Faber OK, Frøland A. The beta-cell response to glucagon and mixed meal stimulation in non-insulin dependent diabetes. Scand J Clin Lab Invest. 1988;48(8):771–7.
6. Young EE, Chinenye S, Unachukwu CN. Beta cell response to a mixed meal in Nigerian patients with type 2 diabetes. BMC Endocr Disord. 2012;12:11.

7. Pozzan R, Dimetz T, Gazzola HM, Gomes MB. The C-peptide response to a standard mixed meal in a group of Brazilian type 1 diabetic patients. Braz J Med Biol Res. 1997;30(10):1169–74.
8. Brodovicz KG, Girman CJ, Simonis-Bik AM, Rijkelijkhuizen JM, Zelis M, Bunck MC, Mari A, Nijpels G, Eekhoff EM, Dekker JM. Postprandial metabolic responses to mixed versus liquid meal tests in healthy men and men with type 2 diabetes. Diabetes Res Clin Pract. 2011;94(3):449–55.
9. Hope SV, Knight BA, Shields BM, Hattersley AT, McDonald TJ, Jones AG. Random non-fasting C-peptide: bringing robust assessment of endogenous insulin secretion to the clinic. Diabet Med. 2016;33(11):1554–8.
10 Faber OK, Binder C. C-peptide response to glucagon. A test for the residual beta-cell function in diabetes mellitus. Diabetes. 1977;26(7):605–10.
11. Fujioka Y, Okura T, Sumi K, Matsumoto K, Shoji K, Nakamura R, Matsuzawa K, Izawa S, Kato M, Taniguchi S, Yamamoto K. Normal meal tolerance test is preferable to the glucagon stimulation test in patients with type 2 diabetes that are not in a hyperglycemic state: comparison with the change of C-peptide immunoreactivity. J Diabetes Investig. 2018;9(2):274–8.
12. Koskinen PJ, Viikari JS, Irjala KM. Glucagon-stimulated and postprandial plasma C-peptide values as measures of insulin secretory capacity. Diabetes Care. 1988;11(4):318–22.
13 Escobar-Jiménez F, Herrera Pombo JL, Gómez-Villalba R, Nuñez del Carril J, Aguilar M, Rovira A. Standard breakfast test: an alternative to glucagon testing for C-peptide reserve? Horm Metab Res. 1990;22(6):339–41.
14. Funakoshi S, Fujimoto S, Hamasaki A, Fujiwara H, Fujita Y, Ikeda K, Takahara S, Seino Y, Inagaki N. Analysis of factors influencing postprandial C-peptide levels in Japanese patients with type 2 diabetes: comparison with C-peptide levels after glucagon load. J Diabetes Investig. 2011;2(6):429–34.
15. Mohan V, Shah SN, Joshi SR, Seshiah V, Sahay BK, Banerjee S, Wangnoo SK, Kumar A, Kalra S, Unnikrishnan AG, Sharma SK, Rao PV, Akhtar S, Shetty RV, Das AK, DiabCare India 2011 Study Group. Current status of management, control, complications and psychosocial aspects of patients with diabetes in India: results from the DiabCare India 2011 Study. Indian J Endocrinol Metab. 2014;18(3):370–8.
16. Borgharkar SS, Das SS. Real-world evidence of glycemic control among patients with type 2 diabetes mellitus in India: the TIGHT study. BMJ Open Diabetes Res Care. 2019;7(1):e000654.
17. Nosari I, Lepore G, Maglio ML, Cortinovis F, Pagani G. The effect of various blood glucose levels on post-glucagon C-peptide secretion in type 2 (non insulin-dependent) diabetes. J Endocrinol Invest. 1992;15(2):143–6.
18. Berger B, Stenström G, Sundkvist G. Random C-peptide in the classification of diabetes. Scand J Clin Lab Invest. 2000;60(8):687–93.
19. Koskinen P, Viikari J, Irjala K, Kaihola HL, Seppälä P. C-peptide determination in the choice of treatment in diabetes mellitus. Scand J Clin Lab Invest. 1985;45(7):589–97.
20. Samols E, Marri G, Marks V. Promotion of insulin secretion by glucagon. Lancet. 1965;2(7409):415–6.
21. Hannon TS, Kahn SE, Utzschneider KM, Buchanan TA, Nadeau KJ, Zeitler PS, Ehrmann DA, Arslanian SA, Caprio S, Edelstein SL, Savage PJ, Mather KJ, RISE Consortium. Review of methods for measuring β-cell function: design considerations from the Restoring Insulin Secretion (RISE) Consortium. Diabetes Obes Metab. 2018;20(1):14–24.
22. Cryer PE, Arbelaez AM. Hypoglycaemia. In: Melmed S, Auchus RJ, Goldfine AB, Koenig RJ, Rosen CJ, editors. Williams textbook of endocrinology. 14th ed. Philadelphia: Elsevier; 2020. p. 1525–51.

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ORIGINAL ARTICLE

Anthropometric indices and their predictive ability on metabolic syndrome in west China

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Abstract

Aims To evaluate the relationship between anthropometric indices, including abdominal volume index (AVI), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), conicity index (C index), body mass index (BMI), body roundness index (BRI), body adiposity index (BAI), A body shape index (ABSI) and cardiovascular risk factors, and their abilities to predict metabolic syndrome (MetS) in adults.

Methods A cross-sectional study of 76,915 participants (30,912 females and 46,003 males) aged between 14 and 100 years was conducted. AVI, WHR, WHtR, BMI, conicity index (C index), BRI, BAI, and ABSI were measured and calculated. Pearson correlation analysis and linear regression analysis were used to examine the relationship between anthropometric indicators and the components of MetS, while binary logistic regression analysis was used to assess the relationship between anthropometric indicators and overall MetS. The receiver operating characteristic curve (ROC) was used to analyze and calculate the area under the curve (AUC). Then, a 95% confidence interval (95% CI) was calculated to evaluate the ability of anthropometric indicators to predict MetS and determine the optimal cutoff value of each anthropometric indicator. The optimal cutoff value was determined by the Youden index.

Results MetS prevalence was 21.71% in males and 9.5% in females. Participants with MetS were older and had higher values of glucose, triglyceride, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), systolic blood pressure (SBP), diastolic blood pressure (DBP) than those without MetS. The high-density lipoprotein cholesterol (HDL-C) values of males and females with MetS were lower than the values found in those without MetS. Mean values of all anthropometric indicators in men and women with MetS were significantly higher than the mean values in those without MetS. After adjusting for age, alcohol consumption, and smoking, anthropometric indexes AVI, WHR, WHtR, C index, BMI, and BRI were all associated with cardiovascular risk factors ($p < 0.001$). Among men over the age 60 years old, an AVI cutoff of 16.0 predicted MetS with a sensitivity of 74.70% and a specificity of 84.90%. The area under the ROC curve was 0.84 ($p < 0.001$). Among women over the age of 60 years, an AVI cutoff of 12.8 predicted MetS with a sensitivity of 90.13% and a specificity of 63.72%. The area under the ROC curve was 0.80 ($p < 0.001$). Among men aged 30–60 years, an AVI cutoff of 16 predicted MetS with a sensitivity of 80.44% and a specificity of 82.36%. The area under the ROC curve was 0.85 ($p < 0.001$). Among women aged 30–60 years, an AVI cutoff of 12.82 predicted MetS with a sensitivity of 87.72% and a specificity of 83.47%. The area under the ROC curve was 0.90 ($p < 0.001$). Among men under the age of 30 years, an AVI cutoff of 16.22 predicted MetS with a sensitivity of 87.97% and a specificity of 88.65%. The area under the ROC curve was 0.92 ($p < 0.001$). Among women under the age of 30 years, an AVI cutoff of 12.79 predicted MetS with a sensitivity of 95.92% and a specificity of 93.79%. The area under the ROC curve was 0.97 ($p < 0.001$). AVI showed the strongest ability to distinguish MetS across genders and different age groups.

Conclusion AVI, WHR, WHtR, BMI, C index, and BRI were all associated with cardiovascular risk factors. The anthropometric index is a useful screening tool for MS, its components, and cardiovascular risk factors. Among all the indices examined, AVI can best distinguish MetS.

Keywords Abdominal volume index · Anthropometric index · Cardiovascular risk factors · Metabolic syndrome

Extended author information available on the last page of the article

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the world, and its prevalence varies with territory and culture. The prevalence, morbidity, and mortality of CVD are on the rise in China, and CVD is the number one leading cause of death, with percentages as 44.60% and 42.51% of total deaths in rural and urban areas [1]. The mortality rate from CVD in rural areas has exceeded and continued to exceed the urban level since 2009. The increasing burden of CVD has become a major public health concern. In this sense, the prevention of CVD has become a public health priority, especially for those considered at high cardiovascular risk. Clinically, effective strategies must be developed to fully identify these patients.

Metabolic syndrome (MetS) describes a cluster of conditions that pose as risk factors for CVD and other illnesses such as type 2 diabetes and stroke. It has become a major health problem in both developing and developed countries, with prevalence rising globally. According to the National Cholesterol Education Program [2], MetS is made up of the presence of any three or more of the following conditions: (a) blood glucose greater than 5.6 mmol/L (100 mg/dL) or drug treatment for elevated blood glucose 2; (b) high-density lipoprotein (HDL) cholesterol < 1.0 mmol/L (40 mg/dL) in men, < 1.3 mmol/L (50 mg/dL) in women or drug treatment for low HDL-C; (c) blood triglycerides > 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides; (d) waist circumference > 102 cm in men, or > 88 cm in women; (e) blood pressure > 130/85 mmHg or drug treatment for hypertension. Individuals meeting at least three of the five components of MetS are considered as having the condition. People with MetS have a higher risk of insulin resistance, low levels of chronic inflammation, and oxidative stress [3]. MetS can also contribute to the early development of coronary heart disease (CHD) [4]. Therefore, it is crucial to identify MetS early and determine which anthropometric indicators have the highest predictive ability for MetS.

The use of methods to determine body composition began in the 1940s and has been extended to various methods as indicators of health status, treatment progress, and functional status in medicine and research. The measurements and indices make it easy for researchers to use anthropometry as a method of assessing cardiovascular risk because it is simple to measure, low cost, and clinically applicable. Additionally, they provide invaluable information on risk factors that can help prevent and treat diseases. Perissinotto et al. [5] found that non-pathological factors, such as age, sex, and geographical area, may influence anthropometric measurement and should be considered. Anthropometric indices have been the focus of much research on obesity, cardiovascular health, and diseases. However, certain issues such as the possible redistribution of fat [6], the selection of the most appropriate statistical method, and the optimal measurement technique are all important factors to consider for meaningful measurement in any given population.

The assessment of obesity was extrapolated from anthropometric measurements. With the increasing prevalence of obesity, the development of a reliable anthropometric obesity index has become an important public health issue. Obesity not only closely reflects the risk of glucose metabolism disorders but identifying it also helps clinicians advise patients on health promotion and awareness. Body mass index (BMI), an anthropometric parameter, is the most commonly used index for assessing overweight and obesity. It has long been used to assess obesity because of its simplicity, ease of calculation, and clear association with mortality and obesity-related complications. However, BMI alone does not reflect the amount or the distribution of body fat that comes from the variation in any given population under study, including factors such as age, sex, ethnicity, and type of obesity. While overall obesity is measured by BMI, measurements of waist circumference (WC) and hip circumference (HC) and their associated ratios have been identified as better indicators of body size and obesity-related risks. A common form of abdominal obesity is the waist-to-hip ratio (WHR), a ratio calculated by dividing WC over HC, or WC, and overall obesity is measured by body mass index (BMI). WHR [7] is determined by dividing waist circumference by hip circumference. It is used to describe how body fat is distributed, and if it is concentrated centrally or in the limbs. Both overall obesity and poor body fat distribution are independently associated with the development of type 2 diabetes mellitus (DM). Central obesity as assessed by WHR or WC is a better indicator of type 2 diabetes risk than BMI is. Sometimes weight gain or loss causes similar changes in WC and HC without changing the final ratio. For the reason, WHR is not very useful in assessing changes in weight change. Also, WC does not account for variations in height. Therefore, it may underestimate the risks in short people, while overestimating the risks of tall people. While both WHR and WC are based on longitudinal measurements, the reliability of the estimation of central obesity by WC and WHR might also be affected in individuals whose umbilical line falls below the buttocks and the pendulum abdomen.

To address these issues, the abdominal volume index (AVI) [8] is developed as an anthropometric obesity assessment. As the name implies, it measures total volume. It is a theoretical estimate of total abdominal volume, including abdominal fat and adipose tissue volume as measured between the pubic symphysis and the xiphoid process, and

is calculated by using WC and HC according to the formula [2 cm (WC) ^{2} + 0.7 cm (WC-HC) ^{2}]/1000. The formula is derived from the formula for calculating the volume of a cylinder and the volume formula of a cone. It is easier to estimate volume with the AVI formula than to estimate the volume with a truncated cone. Women's fat distribution is mainly concentrated in the lower body, while men are mainly distributed in the upper body. The geometric figures of the obesity pattern in women and men are upright and inverted cones, respectively. The obesity pattern based on which AVI is calculated is similar to calculating the volume of a truncated cone. The advantage of using AVI to estimate abdominal volume is that it predicts the possible occurrence of both obesity patterns. Overseas studies [9] have found it to be a reliable measurement, closely linked to impaired glucose tolerance (IGT) and type 2 diabetes (DM). However, studies on AVI and its ability to predict other diseases including cardiovascular risk factors are still rare.

Conicity index (C index) is an indicator of abdominal obesity proposed by Ruperto et al. [10], based on geometric reasoning models. It is built upon the fact that the less fat individuals have in the center, the closer they resemble a cylinder. However, if individuals accumulate fat around the waist, their body shape seems to shift from the cylinder to the tip cone. The C index has no units and ranges theoretically from 1.00 (perfect cylinder) to 1.73 (double cone, namely two cones, where the base is the same). It is obtained by using WC, height, and weight of any given individual according to the following formula: "WC (m) / $(0.109 \times \sqrt{\text{weight [kg]/height [m]}})$." Compared with WHR, the C index adjusts for the height as well as the weight of the individual, and can directly compare abdominal fat across individuals and even across different populations without requiring hip measurement [11].

There is growing evidence suggesting that abdominal fat plays a role in the development of MetS. Developing new anthropometric indicators to evaluate abdominal fat is thus necessary. To address this issue, several new measurements have been developed including the body adiposity index (BAI) by Bergman and colleagues [11], the A body shape index (ABSI) by Krakauer and Krakauer [12], and the body roundness index (BRI) by Thomas and colleagues [13]. These indices aim to estimate body fat distribution. BMI is known to have limited accuracy, as its value may differ for men and women with similar percentages of body fat. To make up for this deficiency, Bergman et al. proposed that BAI as an indicator for evaluating body fat composition. It is calculated based on height and HC and shows a high correlation with adult body fat percentage (BF%) [11]. BAI can be used to reflect the percentage of body fat of adult men and women of different races without numerical correction. ABSI [14] is an index that standardizes WC by BMI and height, while ABSI assesses visceral, abdominal, and general obesity at the same time, and is a better predictor of premature death than WC and BMI [15]. BRI is based on a new geometric model that quantifies a person's body shape independently of height. It can predict body fat percentage and visceral adipose tissue and can be used as a visual tool for health assessment.

Finally, waist-to-height ratio (WHtR) is also an indicator of abdominal obesity and has been used to assess metabolic disorders in studies. WHtR shows that individuals' WC should not exceed half their height, and show better sensitivity in assessing health risks than measurements of isolated WC in different populations.

There is no complete agreement on the best anthropometric indicators for assessing MetS status and risk. The best measure of obesity as a predictor of cardiometabolic risk remains elusive. Due to differences in race, genetic makeup, diet and lifestyle, culture, and economic conditions between foreign and domestic populations, more research is needed in the Chinese population. Additionally, the diversity of anthropometric methods, particularly the newly proposed AVI and other anthropometric indicators, and their ability to predict MetS remain controversial and have not been widely analyzed or studied. The purpose of this study was to assess the relationship between AVI and cardiovascular risk factors and to compare the ability of AVI and other anthropometric indicators in predicting MetS in adults.

Methods

Data collection

A cross-sectional study of 76,915 participants (30,912 females and 46,003 males) aged between 14 and 100 years was conducted between December 2013 and December 2014 in the metropolis of Chengdu city in west China, the details of which had been documented elsewhere [16]. Inclusion criteria were that the participant was a resident of metropolitan Chengdu and willing to participate in the study. Exclusion criteria were the following: unwilling participants, patients with severe heart, liver and kidney diseases, malignant tumors, mental disorders, persons unable to take care of themselves, persons diagnosed with eating difficulties, pregnant women, and persons with physical disabilities. Demographic information including sex, age, medical history (hypertension or diabetes mellitus), family history, smoking status (current smoking defined as ≥ 20 cigarettes/month for ≥ 6 months), drinking status (current alcohol use defined as at least once/week for ≥ 6 months), and other relevant information were collected.

Anthropometric assessment

Body weight, height, blood pressure, WC, and HC were measured and recorded by skilled nurses. All participants were requested to stand upright with arms hanging freely in light clothing without shoes. All measurements were taken using inelastic tape on the skin. WC was measured straight at the midpoint between the last rib and the iliac crest to the nearest 0.1 cm at the end of a normal breath [16]. The average of three measurements was recorded. HC was measured at the widest portion of the buttock. Height was measured by a nonelastic ruler to the nearest 0.5 cm. Weight was measured to the nearest 0.1 kg in light clothing and without shoes using a Weight scale (Wujin, RGT-120, Wujin Medical Equipment Co. Ltd., Jiangsu, China). Blood pressure was measured seated, by a sphygmomanometer (Yuyue, GB3053-93, Yuyue Medical Equipment Co., Ltd., Jiangsu, China) on the right arm after a 5-min rest. The mean of two measurements with at least a 1-min interval was taken. Anthropogenic indices were calculated by the following formulas:

$$\mathrm{BMI} = \mathrm{weight}/\mathrm{height2(kg/m2)}.$$

$$\mathrm{WHR} = \mathrm{WC(cm)}/\mathrm{HC(cm)}$$

$$\mathrm{CI} = \mathrm{WC(m)}/\left(0.109 \times \sqrt{\mathrm{weight}[\mathrm{kg}]/\mathrm{height}[\mathrm{m}]}\right)$$

$$\mathrm{AVI} = \left[2 \times \mathrm{x(WC)}^2(\mathrm{cm}^2) + 0.7(\mathrm{WC–HC})^2(\mathrm{cm}^2)\right]/1000$$

$$\mathrm{BAI} = \left[\mathrm{HC(cm)}/\mathrm{height}^{3/2}(\mathrm{m})\right]–18$$

$$\mathrm{WHtR} = \mathrm{WC(cm)}/\mathrm{height(cm)}$$

$$\mathrm{BRI} = 364.2–365.5 \times \sqrt{1 - \left[\frac{[\mathrm{WC(m)}/(2\times\pi)]}{[0.5\times\mathrm{height(m)}]}^2\right]^2}$$

$$\mathrm{ABSI}\left(\mathrm{m}^{11/6}\,\mathrm{kg}^{-2/3}\right) = \mathrm{WC(m)}/\left(\mathrm{BMI}[\mathrm{kg/m}^2]^{2/3} \times \mathrm{height}[\mathrm{m}]^{1/2}\right)$$

Laboratory measurements

Blood samples were drawn from the antecubital vein after fasting for more than 12 h. Without being placed in the freezer, the blood samples were centrifuged at 3000 rpm for 10 min and then tested. Fasting plasma glucose (FPG), total cholesterol(TC), high-density lipoproteins cholesterol (HDL-C), low-density lipoproteins cholesterol (LDL-C), triglycerides (TG), Cystatin C (Cys-c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GGT), total bilirubin (TB), direct bilirubin (DB), serum uric acid (UA), serum creatinine (Scr), and blood urea nitrogen (BUN) were analyzed by a biochemical autoanalyzer (ROCHE Cobas 8000, ROCHE Diagnostics, Basel, Switzerland) at the Department of Clinical Laboratory Diagnostics, West China Hospital Sichuan University [16]. TB and DB were assayed by the Vanadate oxidase method. FPG concentrations were assayed by the hexokinase method. TGs in plasma were analyzed by glycerol oxidation. TC was assayed by means of the enzymic method. HDL-C and LDL-C were analyzed by homogeneous enzyme colorimetry. ALT, AST, and γ-GGT were measured through the enzyme rate method. UA and BUN were measured by means of the enzyme coupling method. Cystatin C (Cys-c) was assayed by emulsion enhanced immune projection turbidimetry. Scr was measured by means of the picric acid method.

Diagnostic criteria for MetS and its components

MetS was diagnosed when the participant shows the presence of three or more following factors according to the National Cholesterol Education Program-Adult Treatment Panel III(NCEP-ATPIII)criteria [2]: abdominal obesity: WC ≥ 90 cm in men or WC ≥ 80 cm in women; Increased TG ≥ 150 mg/dL (1.7 mmol/L); Decreased HDL-C, defined as HDL-C < 40 mg/dL (1.03 mmol/L) in male or < 50 mg/dL (1.29 mmol/L) in female; blood pressure ≥ 130/85 or receiving treatment; fast blood glucose (FBG) ≥ 100 mg/dL (5.6 mmol/L), or type 2 diabetes mellitus or receiving treatment.

Statistical analysis

The normality of the distribution was evaluated using the Kolmogorov–Smirnov test. Results were expressed as a number (percentages) or mean ± standard deviations. IBM SPSS v24.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Student's *t*-test was used to assess mean differences between males and females. Comparisons of means were assessed by analysis of variance (ANOVA), followed by Tukey's test. Pearson correlation analysis and linear regression analysis were used to examine the relationship between anthropometric indicators and the components of MetS, while binary logistic regression analysis was used to assess the relationship between anthropometric indicators and MetS. The receiver operating characteristic curve (ROC) and the area under the Curve (AUC) of the ROC were designed to assess the abilities of the anthropometric indexes to predict MetS, and determine the cutoff points for each indicator. Cutoff points were got after calculating the Youden's index (sensitivity + specificity-1). The AUC was compared using MedCalc by DeLong et al.'s [17] (version 13.0; MedCalc Software, Mariakerke, Belgium). Statistical significance was considered at $p < 0.05$.

Table 1 Baseline characteristics of participants and their cardiovascular risk factors and anthropometric indicators

Characteristics	Total ($n=76,915$)		Male ($n=46,003$)		Female ($n=30,912$)	
	No MetS	MetS	No MetS	MetS	No MetS	MetS
	($n=63,988$)	($n=12,927$)	($n=36,014$)	($n=9989$)	($n=27,974$)	($n=2938$)
Age (years)	43.16 ± 12.24	50.33 ± 12.13	44.24 ± 12.59	48.62 ± 11.63	41.77 ± 11.61	56.15 ± 11.99
FBG (mmol/L)	5.13 ± 0.82	6.32 ± 1.91	5.20 ± 0.95	6.37 ± 2.00	5.04 ± 0.60	6.15 ± 1.56
TG (mmol/L)	1.41 ± 1.07	2.74 ± 1.94	1.65 ± 1.24	2.89 ± 2.04	1.10 ± 0.7	2.22 ± 1.45
LDL-C (mmol/L)	2.79 ± 0.76	3.05 ± 0.84	2.91 ± 0.76	3.02 ± 0.83	2.65 ± 0.74	3.13 ± 0.87
HDL-C (mmol/L)	1.54 ± 0.40	1.22 ± 0.31	1.39 ± 0.35	1.17 ± 0.28	1.73 ± 0.38	1.38 ± 0.33
TC (mmol/L)	4.77 ± 0.88	5.16 ± 1.00	4.83 ± 0.88	5.14 ± 0.98	4.70 ± 0.87	5.22 ± 1.04
SBP (mmHg)	110.95 ± 14.33	127.25 ± 15.03	113.98 ± 13.94	126.84 ± 14.63	107.06 ± 13.87	128.65 ± 16.22
DBP (mmHg)	72.57 ± 9.20	82.93 ± 9.58	74.72 ± 9.23	83.84 ± 9.42	69.80 ± 8.39	79.83 ± 9.48
PP (mmHg)	38.38 ± 10.16	44.32 ± 13.1	39.25 ± 10.33	43 ± 12.44	37.26 ± 9.84	48.83 ± 14.24
Heart rate (beat/min)	71.88 ± 8.89	73.12 ± 8.74	70.96 ± 8.93	72.74 ± 8.5	73.05 ± 8.69	74.42 ± 9.39
cystatin C (mmol/L)	0.84 ± 0.14	0.92 ± 0.17	0.90 ± 0.14	0.94 ± 0.16	0.77 ± 0.12	0.88 ± 0.18
Uric acid (mmol/L)	337.77 ± 87.59	399.63 ± 91.61	387.21 ± 75.39	421.65 ± 85.13	274.12 ± 55.1	324.75 ± 70.91
Scr (mmol/L)	75.79 ± 16.11	81.01 ± 15.96	85.77 ± 12.53*	85.66 ± 13.81*	62.95 ± 9.94	65.22 ± 12.23
BUN (mmol/L)	4.98 ± 1.28	5.25 ± 1.34	5.22 ± 1.28	5.29 ± 1.35	4.67 ± 1.22	5.13 ± 1.33
ALT (mmol/L)	25.77 ± 18.95	37.85 ± 24.96	31.40 ± 21.20	41.13 ± 25.77	18.52 ± 12.23	26.68 ± 17.97
AST (mmol/L)	145.42 ± 20.55	151.66 ± 20.01	155.25 ± 18.53	156.45 ± 18.89	132.77 ± 15.46	135.35 ± 14.29
GGT (mmol/L)	28.47 ± 39.21	57.25 ± 66.01	36.91 ± 47.66	64.47 ± 69.04	17.61 ± 19.58	32.72 ± 46.82
TB (mmol/L)	14.18 ± 5.91	14.46 ± 5.94	15.27 ± 6.33	15.00 ± 6.12	12.77 ± 4.98*	12.63 ± 4.83*
IB (mmol/L)	9.98 ± 4.38	10.42 ± 4.50	10.72 ± 4.74*	10.78 ± 4.67*	9.02 ± 3.66	9.2 ± 3.62
DB (mmol/L)	4.20 ± 1.83	4.04 ± 1.76	4.55 ± 1.94	4.22 ± 1.77	3.75 ± 1.58	3.43 ± 1.6
AVI	12.68 ± 2.78	16.91 ± 2.65	14.06 ± 2.42	17.5 ± 2.41	10.90 ± 2.12	14.89 ± 2.45
WC	78.58 ± 9.06	91.52 ± 7.29	83.20 ± 7.42	93.22 ± 6.42	72.63 ± 7.34	85.72 ± 7.08
WHR	0.85 ± 0.07	0.93 ± 0.05	0.88 ± 0.05	0.94 ± 0.05	0.80 ± 0.06	0.90 ± 0.06
WHtR	0.48 ± 0.05	0.55 ± 0.04	0.50 ± 0.05	0.55 ± 0.04	0.46 ± 0.05	0.55 ± 0.05
Conicity index	1.18 ± 0.08	1.26 ± 0.06	1.20 ± 0.06	1.26 ± 0.06	1.14 ± 0.08	1.24 ± 0.08
BMI	23.04 ± 2.97	27.03 ± 2.79	24.09 ± 2.78	27.38 ± 2.64	21.69 ± 2.64	25.83 ± 2.93
BRI	3.07 ± 0.92	4.47 ± 0.89	3.33 ± 0.84	4.47 ± 0.83	2.73 ± 0.90	4.46 ± 1.08
BAI	26.59 ± 3.15	28.52 ± 3.43	25.53 ± 2.74	27.64 ± 2.79	27.96 ± 3.12	31.53 ± 3.67
ABSI	0.076 ± 0.004	0.079 ± 0.004	0.077 ± 0.004	0.079 ± 0.004	0.075 ± 0.005	0.079 ± 0.005
HBP history (%)	4.63%	23.05%	5.79%	21.24%	3.12%	29.20%
DM history (%)	1.56%	9.42%	2.24%	9.34%	0.69%	9.70%
Smoking (%)	29.81%	43.15%	51.80%	55.51%	1.50%	1.12%
Drinking (%)	44.97%	60.78%	73.22%	76.68%	8.59%	6.71%

*$p > 0.05$, other $p < 0.05$

BMI body mass index, *DBP* diastolic blood pressure, *HDL-c* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *TG* triglyceride, *MetS* metabolic syndrome, *SBP* systolic blood pressure, *AVI* abdominal volume index, *WC* waist circumference, *WHR* waist hip ratio, *WHtR* waist height ratio, *BRI* body roundness index, *ABSI* a body shape index, *BAI* body adiposity index, *TB* total bilirubin, *IB* indirect bilirubin, *DB* direct bilirubin, *ALT* alanine aminotransferase, *AST* aspartate amino transferase, *GGT* gamma-glutamyl transpeptidase, *PP* pulse pressure, *BUN* blood urea nitrogen, *Scr* serum creatinine, *FBG* fast blood glucose

Results

Basic information on cardiovascular risk factors and anthropometric indicators

Table 1 shows baseline characteristics of 76,915 participants with a mean age of 44.36 years ± 12.51 years (59.81% for males and 40.19% for females). Among these, 12,927 individuals (16.81%) are with MetS, while 63,988 subjects (83.19%) are without MetS. The prevalence of MetS was found to be 21.71% in males and 9.5% in females. Participants with MetS are older and have higher values of FBG, TG, LDL-C, TC, SBP, DBP, PP, heart rate, cystatin C, UA, Scr, BUN, ALT, AST, GGT, TB, and IB than those without

MetS. Participants with MetS had lower HDL-C and DB values than those without MetS. Both women and men with MetS had significantly higher mean values for all anthropometric indicators than those without MetS. There was no statistical difference in Scr and IB values between men with and without MetS. There was no statistical difference in TB value between women with and without MetS. Both men and women who have MetS are significantly older than those who do not have MetS. Participants with MetS had higher mean values of anthropometric indices than those without MetS (all $p < 0.05$). Participants with MetS had higher TG, TC, LDL-C, FBG, SBP, DBP, PP, and LDL-C than those without MetS (all $p < 0.05$). Participants with MetS were more likely to have a history of hypertension and diabetes than those without MetS ($p < 0.05$). Interestingly, among female participants, those without MetS have higher smoking and drinking rates than their counterparts with MetS. Among male participants, those with MetS have higher drinking and smoking rates than their counterparts without MetS.

Prevalence of MetS overall and by number of its components across gender and age groups

Table 2 shows that the prevalence of MetS in the study sample is 16.81%, while the value is at 21.71% for men and 9.5% for women. Of the total 76,915 participants, 9527 participants (12.39%) are under 30 years old, 58,655 participants (76.26%) are between 30 and 60 years old, and 8733 participants (11.35%) are over 60 years old. MetS prevalence rate is 4.18% ($n = 398$) in those under 30 years old, 16.83% ($n = 9870$) in those aged 30–60 years old, and 30.45 ($n = 2659$) in those over 60 years old. MetS prevalence rate increases with age ($p < 0.05$). Among the MetS components, increasing WC accounts for 27.40% of the total number of participants ($n = 21,076$), hypertriglyceridemia 31.22% ($n = 24,011$), low HDL-C 15.63% ($n = 12,019$), hypertension 40.36% ($n = 31,042$), and hyperglycemia 21.47% ($n = 16,668$), while 33.38% of the study participants ($n = 25,676$) show no abnormal MetS components (which are increased WC, hypertriglyceridemia, decreased HDL-C, high blood pressure, elevated glycemia). As the number of MetS abnormal components increases, the percentage within the population falls. There are statistically significant differences in the prevalence of MetS across genders and age groups. The prevalence of MetS increases with the increase of age.

Distribution of anthropometric indices in different age groups

The values for all anthropometric indices increase with the increase in age within our study sample, for both the male and female population, with statistically significant differences across age groups ($p < 0.001$; Table 3). There were significant differences among different age groups ($p < 0.001$; Table 3).

Table 2 Relationship between the MetS prevalence overall and by number of its components across genders and age groups

Gender	Age	*n*	MetS prevalence	MetS component numbers					
				0	1	2	3	4	5
Male	< 30	4727	349 (7.38%)	2109 (44.62%)	1397 (29.55%)	722 (15.27%)	342 (7.24%)	125 (2.64%)	32 (0.68%)
	30–60	35,709	8146 (22.81%)	7827 (21.92%)	9435 (26.42%)	8534 (23.90%)	6165 (17.26%)	2976 (8.33%)	772 (2.16%)
	≥ 60	5567	1494 (26.84%)	660 (11.86%)	1671 (30.02%)	1626 (29.21%)	1067 (19.17%)	449 (8.07%)	94 (1.69%)
	Total	46,003	9989 (21.71%)	10,596 (23.03%)	12,503 (27.18%)	10,882 (23.65%)	7574 (16.46%)	3550 (7.72%)	898 (1.95%)
Female	< 30	4800	49 (1.02%)	3571 (74.40%)	918 (19.13%)	227 (4.73%)	59 (1.23%)	23 (0.48%)	2 (0.04%)
	30–60	22,946	1724 (7.51%)	11,200 (48.81%)	6245 (27.22%)	3202 (13.95%)	1480 (6.45%)	625 (2.72%)	194 (0.85%)
	≥ 60	3166	1165 (36.80%)	309 (9.76%)	702 (22.17%)	886 (27.98%)	762 (24.07%)	368 (11.62%)	139 (4.39%)
	Total	30,912	2938 (9.50%)	15,080 (48.78%)	7865 (25.44%)	4315 (13.96%)	2301 (7.44%)	1016 (3.29%)	335 (1.08%)
Total	< 30	9527	398 (4.18%)	5680 (59.62%)	2315 (24.30%)	949 (9.96%)	401 (4.21%)	148 (1.55%)	34 (0.36%)
	30–60	58,655	9870 (16.83%)	19,027 (32.44%)	15,680 (26.73%)	11,736 (20.01%)	7645 (13.03%)	3601 (6.14%)	966 (1.65%)
	≥ 60	8733	2659 (30.45%)	969 (11.10%)	2373 (27.17%)	2512 (28.76%)	1829 (20.94%)	817 (9.36%)	233 (2.67%)
	Total	76,915	12,927 (16.81%)	25,676 (33.38%)	20,368 (26.48%)	15,197 (19.76%)	9875 (12.84%)	4566 (5.94%)	1233 (1.60%)

MetS metabolic syndrome

Table 3 Distribution of anthropometric indices in different age groups

		Total		Male		Female	
		n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
AVI	< 30	9527	11.67 ± 3.07	4727	13.52 ± 2.93	4800	9.85 ± 1.88
	30–60	58,655	13.53 ± 3.13	35,709	14.98 ± 2.73	22,946	11.28 ± 2.27
	≥ 60	8733	14.31 ± 2.97	5567	14.8 ± 2.89	3166	13.44 ± 2.89
	Total	76,915	13.39 ± 3.18	46,003	14.81 ± 2.8	30,912	11.28 ± 2.45
CI	< 30	9527	1.14 ± 0.07	4727	1.17 ± 0.06	4800	1.11 ± 0.07
	30–60	58,655	1.19 ± 0.08	35,709	1.22 ± 0.06	22,946	1.15 ± 0.07
	≥ 60	8733	1.24 ± 0.08	5567	1.24 ± 0.07	3166	1.23 ± 0.09
	Total	76,915	1.19 ± 0.08	46,003	1.22 ± 0.07	30,912	1.15 ± 0.08
WC	< 30	9527	74.94 ± 10.1	4727	81.23 ± 8.98	4800	68.75 ± 6.76
	30–60	58,655	81.24 ± 9.81	35,709	85.92 ± 8.02	22,946	73.95 ± 7.66
	≥ 60	8733	83.85 ± 8.94	5567	85.39 ± 8.56	3166	81.14 ± 8.96
	Total	76,915	80.76 ± 10.03	46,003	85.38 ± 8.31	30,912	73.88 ± 8.27
WHR	< 30	9527	0.81 ± 0.07	4727	0.85 ± 0.05	4800	0.77 ± 0.05
	30–60	58,655	0.86 ± 0.07	35,709	0.90 ± 0.05	22,946	0.81 ± 0.06
	≥ 60	8733	0.90 ± 0.06	5567	0.91 ± 0.06	3166	0.88 ± 0.07
	Total	76,915	0.86 ± 0.07	46,003	0.89 ± 0.06	30,912	0.81 ± 0.07
WHtR	< 30	9527	0.45 ± 0.05	4727	0.48 ± 0.05	4800	0.43 ± 0.04
	30–60	58,655	0.50 ± 0.05	35,709	0.51 ± 0.05	22,946	0.47 ± 0.05
	≥ 60	8733	0.53 ± 0.06	5567	0.52 ± 0.05	3166	0.53 ± 0.06
	Total	76,915	0.49 ± 0.06	46,003	0.51 ± 0.05	30,912	0.47 ± 0.06
BMI	< 30	9527	21.93 ± 3.47	4727	23.65 ± 3.48	4800	20.24 ± 2.49
	30–60	58,655	23.92 ± 3.20	35,709	25.02 ± 2.97	22,946	22.21 ± 2.77
	≥ 60	8733	24.23 ± 3.09	5567	24.40 ± 3.01	3166	23.92 ± 3.21
	Total	76,915	23.71 ± 3.29	46,003	24.8 ± 3.07	30,912	22.08 ± 2.93
BRI	< 30	9527	2.59 ± 0.92	4727	2.96 ± 0.94	4800	2.21 ± 0.72
	30–60	58,655	3.33 ± 1.00	35,709	3.62 ± 0.92	22,946	2.87 ± 0.93
	≥ 60	8733	3.91 ± 1.11	5567	3.81 ± 1.02	3166	4.07 ± 1.25
	Total	76,915	3.30 ± 1.05	46,003	3.58 ± 0.96	30,912	2.89 ± 1.05
BAI	< 30	9527	25.66 ± 3.06	4727	24.80 ± 3.00	4800	26.51 ± 2.88
	30–60	58,655	26.91 ± 3.12	35,709	26.02 ± 2.79	22,946	28.30 ± 3.11
	≥ 60	8733	28.34 ± 3.91	5567	26.82 ± 3.08	3166	31.02 ± 3.79
	Total	76,915	26.92 ± 3.28	46,003	25.99 ± 2.88	30,912	28.3 ± 3.35
ABSI	< 30	9527	0.075 ± 0.004	4727	0.076 ± 0.004	4800	0.074 ± 0.005
	30–60	58,655	0.077 ± 0.004	35,709	0.078 ± 0.004	22,946	0.075 ± 0.005
	≥ 60	8733	0.079 ± 0.005	5567	0.079 ± 0.005	3166	0.079 ± 0.005
	Total	76,915	0.077 ± 0.005	46,003	0.078 ± 0.004	30,912	0.075 ± 0.005

ABSI a body shape index, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *C index* conicity index, *MetS* metabolic syndrome, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Correlation between anthropometric indices and cardiovascular risk factors

Table 4 shows results from Pearson correlation analysis between anthropometric indicators and components of the MetS. We find that the values of AVI, WHR, WHtR, C index, BMI, BRI, BAI, and ABSI all correlate with cardiovascular risk factors ($p < 0.05$). All the correlations are statistically significant, but the correlation coefficients are small. Among anthropometric indices, AVI shows the greatest positive correlation with DBP, TG, and VLDL, and the greatest negative correlation with HDL-C. WHtR shows the greatest correlation with TC, SBP, and LDL-C, and WHR shows the greatest correlation with FBG.

Multiple linear regression analysis of MetS components and anthropometric indices

After adjusting for age, alcohol consumption, and smoking, anthropometric indices AVI, WHR, WHtR, C

Table 4 Correlation between anthropometric indices and cardiovascular risk factors

	AVI	WHR	WHtR	CI	BMI	BRI	BAI	ABSI
SBP	0.385	0.366	0.392	0.302	0.380	0.390	0.155	0.190
DBP	0.395	0.347	0.354	0.269	0.387	0.351	0.083	0.151
FBG	0.235	0.252	0.248	0.208	0.213	0.249	0.082	0.150
TC	0.164	0.186	0.196	0.162	0.159	0.190	0.097	0.119
TG	0.359	0.352	0.333	0.266	0.343	0.328	0.058	0.165
HDL-C	−0.485	−0.452	−0.414	−0.327	−0.471	−0.403	−0.019	−0.181
LDL-C	0.219	0.233	0.236	0.193	0.216	0.227	0.087	0.130
VLDL	0.359	0.352	0.333	0.266	0.343	0.328	0.058	0.165

$p < 0.05$ for all values

ABSI a body shape index, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *C index* conicity index, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglyceride, *VLDL* very low-density lipoprotein, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Table 5 Multiple linear regression analysis of MetS components and anthropometric indices

β	SBP	DBP	Glucose	TC	TG	HDL-C	LDL-C
AVI	0.109	0.136	0.065	−0.061	0.097	−0.295	0.148
WHR	0.065	0.095	0.065	−0.062	0.104	−0.265	0.146
WHtR	0.105	0.107	0.060	−0.047	0.111	−0.274	0.149
CI	0.042	0.073	0.048	−0.039	0.078	−0.193	0.106
BMI	0.140	0.135	0.055	−0.055	0.100	−0.313	0.160
BRI	0.109	0.106	0.064	−0.048	0.112	−0.264	0.143
BAI	0.073	0.028	0.011	0.016*	0.058	−0.074	0.048
ABSI	−0.006*	0.030	0.032	−0.024*	0.050	−0.092	0.056

*$p > 0.05$, elsewhere $p < 0.05$

ABSI a body shape index, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *C index* conicity index, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglyceride, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Table 6 ROC curve analysis of different anthropometric indices to predict MetS components in male participants

	Abdominal obesity		Hypertriglyceridemia		Hyperglycemia		Hypertension		Low-HDL	
	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI
AVI	1.000	1.000–1.000	0.686	0.681–0.691	0.630	0.624–0.635	0.651	0.646–0.656	0.664	0.658–0.67
WHR	0.878	0.875–0.882	0.672	0.668–0.677	0.649	0.643–0.654	0.635	0.630–0.640	0.639	0.632–0.645
WHtR	0.955	0.953–0.956	0.683	0.678–0.687	0.650	0.644–0.655	0.658	0.653–0.663	0.653	0.647–0.66
CI	0.878	0.874–0.881	0.622	0.616–0.627	0.616	0.61–0.622	0.609	0.603–0.614	0.585	0.579–0.592
BMI	0.900	0.897–0.903	0.688	0.683–0.693	0.621	0.616–0.627	0.648	0.643–0.653	0.677	0.671–0.683
BRI	0.955	0.953–0.956	0.683	0.678–0.687	0.650	0.644–0.655	0.658	0.653–0.663	0.653	0.647–0.66
BAI	0.771	0.767–0.776	0.607	0.602–0.613	0.595	0.589–0.601	0.612	0.607–0.617	0.590	0.583–0.597
ABSI	0.738	0.734–0.743	0.557	0.551–0.562	0.576	0.570–0.582	0.558	0.552–0.563	0.521	0.514–0.528

$p < 0.05$

ABSI a body shape index, *AUC* area under the curve, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CI* confidence interval, *C index* conicity index, *HDL-C* high-density lipoprotein cholesterol, *MetS* metabolic syndrome, *ROC* receiver operating characteristic, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Table 7 ROC curve analysis of different anthropometric indices to predict MetS components in female participants

	Abdominal obesity		Hypertriglyceridemia			Hyperglycemia			Hypertension		Low-HDL	
	AUC	95% CI	AUC	95% CI	AUC		95% CI	AUC		95% CI	AUC	95% CI
AVI	1.000	1.000–1.000	0.736	0.729–0.743	0.699		0.691–0.707	0.688		0.681–0.695	0.693	0.685–0.701
WHR	0.932	0.929–0.935	0.739	0.732–0.746	0.699		0.69–0.707	0.679		0.672–0.686	0.686	0.678–0.694
WHtR	0.980	0.978–0.981	0.747	0.74–0.754	0.712		0.704–0.72	0.701		0.694–0.708	0.691	0.683–0.699
CI	0.923	0.919–0.926	0.690	0.682–0.697	0.663		0.655–0.672	0.647		0.640–0.654	0.633	0.624–0.641
BMI	0.906	0.902–0.91	0.724	0.716–0.731	0.690		0.682–0.699	0.687		0.680–0.694	0.696	0.688–0.704
BRI	0.980	0.978–0.981	0.747	0.74–0.754	0.712		0.704–0.72	0.701		0.694–0.708	0.691	0.683–0.699
BAI	0.813	0.807–0.819	0.651	0.643–0.66	0.646		0.637–0.654	0.647		0.640–0.654	0.606	0.597–0.615
ABSI	0.820	0.814–0.825	0.631	0.622–0.639	0.614		0.605–0.623	0.597		0.590–0.604	0.577	0.568–0.586

$p < 0.05$

ABSI a body shape index, *AUC* area under the curve, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CI* confidence interval, *C index* conicity index, *HDL-C* high-density lipoprotein cholesterol, *MetS* metabolic syndrome, *ROC* receiver operating characteristic, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Table 8 Logistic analysis of MetS and anthropometric indices

	B	Sig	Exp(*B*)	95% CI for EXP(*B*)	
				Lower	Upper
AVI	0.584	0.000	1.793	1.774	1.813
WHR	21.962	0.000	3.452E + 09	2.156E + 09	5.526E + 09
WHtR	30.188	0.000	1.289E + 13	7.185E + 12	2.314E + 13
BMI	0.460	0.000	1.585	1.570	1.599
CI	14.614	0.000	2.223E + 06	1.560E + 06	3.166E + 06
BRI	1.484	0.000	4.412	4.287	4.540
BAI	0.257	0.000	1.293	1.283	1.302
ABSI	121.589	0.000	6.391E + 52	4.378E + 50	9.330E + 54

ABSI a body shape index, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CI* confidence interval, *C index* conicity index, *MetS* metabolic syndrome, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

index, BMI, and BRI are all associated with cardiovascular risk factors (Table 5; $p < 0.001$), while ABSI and BAI are not. BAI values correlate with all cardiovascular risk factors with the exception of TC. ABSI values correlate with all the cardiovascular risk factors with the exception of SBP and TC. Anthropometric indices are negatively correlated with HDL-C. With the exception of BAI and ABSI, all the other indices are negatively correlated with TC.

ROC curve analysis of different anthropometric indices to predict MetS components

Tables 6 and 7 show results from the ROC analysis results of anthropometric indices predicting the presence of MetS components. These component indicators are abdominal obesity, hypertriglyceridemia, hyperglycemia, hypertension, and low HDL-cholesterol. AVI had an obvious differential effect on abdominal obesity, with an AUC value of 1.00 for both sexes. In addition, the values of WHR, WHtR, C index, BMI, and BRI are also able to identify abdominal obesity with AUC values above 0.90, while the AUC values for BAI and ABSI are above 0.80, but below 0.90. For hypertriglyceridemia, the highest AUC values correspond to BMI for men, WHtR, and BRI for women (0.688 for men, 0.747 for women). For hyperglycemia, the highest AUC values for males and females correspond to WHtR and BRI (0.650 for males, 0.712 for females). For hypertension, the highest AUC values for men and women correspond to BRI and WHtR (0.658 for men, 0.701 for women). For low HDL-C, the highest AUC for men and women correspond to BMI (0.677 for men, 0.696 for women).

Table 9 ROC curve analysis of anthropometric indicators for MetS in the general population

	AUC	*p*	95% confidence interval	
			Lower bound	Upper bound
AVI	0.888	0.000	0.885	0.891
WHR	0.842	0.000	0.838	0.845
WHtR	0.874	0.000	0.871	0.877
BMI	0.862	0.000	0.859	0.865
CI	0.811	0.000	0.808	0.815
BRI	0.874	0.000	0.871	0.877
BAI	0.779	0.000	0.776	0.783
ABSI	0.745	0.000	0.741	0.749

ABSI a body shape index, *AVI* abdominal volume index, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CI* confidence interval, *C index* conicity index, *MetS* metabolic syndrome, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Logistic analysis of MetS and anthropometric indices

Results from binary Logistic regression analysis (Table 8) show that anthropometric values in the study population independently correlate with MetS even after adjusting for age, gender, drinking, and smoking.

ROC curve analysis of anthropometric indicators for MetS in the general population

Table 9 shows that AVI among all the anthropometric indices has the largest AUC and thus the strongest ability to predict MetS.

ROC curve analysis of anthropometric indices to identify MetS aged ≥ 60 years

Results from ROC curve analysis in Table 10 and Fig. 1A, and Table 11 and Fig. 1B show that AVI has the largest AUC in those aged ≥ 60 years. Among men over the age of 60 years, an AVI cutoff of 16 predicted MetS with a sensitivity of 74.70% and a specificity of 84.90%. The area under the ROC curve is 0.84 ($p < 0.001$). Among women over the age of 60 years, an AVI cutoff of 12.8 predicted MetS with a sensitivity of 90.13% and a specificity of 63.72%. The area under the ROC curve is 0.80 ($p < 0.001$).

ROC curve analysis of anthropometric indices to identify MetS in participants aged 30–60 years

Results from ROC curve analysis in Table 12 and Fig. 1C, and Table 13 and Fig. 1D show that AVI has the largest area under the curve in participants aged 30–60 years. Among men in this age group, an AVI cutoff of 16.0 predicts MetS with a sensitivity of 80.44% and a specificity of 82.36%. The area under the ROC curve is 0.85 ($p < 0.001$). Among women aged 30–60 years, an AVI cutoff of 12.82 predicted MetS with a sensitivity of 87.72% and a specificity of 83.47%. The area under the ROC curve is 0.90 ($p < 0.001$).

ROC curve analysis of anthropometric indices to identify MetS in participants aged < 30 years old

Results from ROC curve analysis in Table 14 and Fig. 1E, and Table 15 and Fig. 1F show that AVI has the largest AUC in the participants aged < 30 years. Among men in this age group, an AVI cutoff of 16.22 predicts MetS with a sensitivity of 87.97% and a specificity of 88.65%. The area under the ROC curve is 0.92 ($p < 0.001$). Among women aged < 30 years, an AVI cutoff of 12.79 predicts MetS with a sensitivity of 95.92% and a specificity of 93.79%. The area under the ROC curve is 0.97 ($p < 0.001$).

AVI showed the strongest ability to predict MetS among different genders and age groups.

Discussion

This is the first large-scale cross-sectional study comparing eight anthropometric indices (AVI, WHR, WHtR, CI, BMI, BRI, BAI, ABSI) with cardiovascular risk factors and MetS. Our results show that AVI, among the eight indices

Table 10 ROC curve analysis of anthropometric indices to identify MetS in men aged ≥ 60 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.60	> 16	0.84	< 0.0001	74.70	84.90	64.47	90.15	4.95	0.30
WHR	0.39	> 0.91	0.76	< 0.0001	73.29	65.36	43.70	86.96	2.12	0.41
WHtR	0.50	> 0.537	0.82	< 0.0001	73.43	76.41	53.31	88.69	3.11	0.35
CI	0.37	> 1.24	0.75	< 0.0001	73.76	63.49	42.56	86.84	2.02	0.41
BMI	0.44	> 25.08	0.79	< 0.0001	72.62	71.62	48.42	87.70	2.56	0.38
BRI	0.50	> 4.09	0.82	< 0.0001	73.16	76.90	53.74	88.65	3.17	0.35
BAI	0.27	> 27.39	0.68	< 0.0001	60.44	66.49	39.82	82.09	1.80	0.59
ABSI	0.12	> 0.07	0.59	< 0.0001	92.97	18.83	29.58	87.96	1.15	0.37

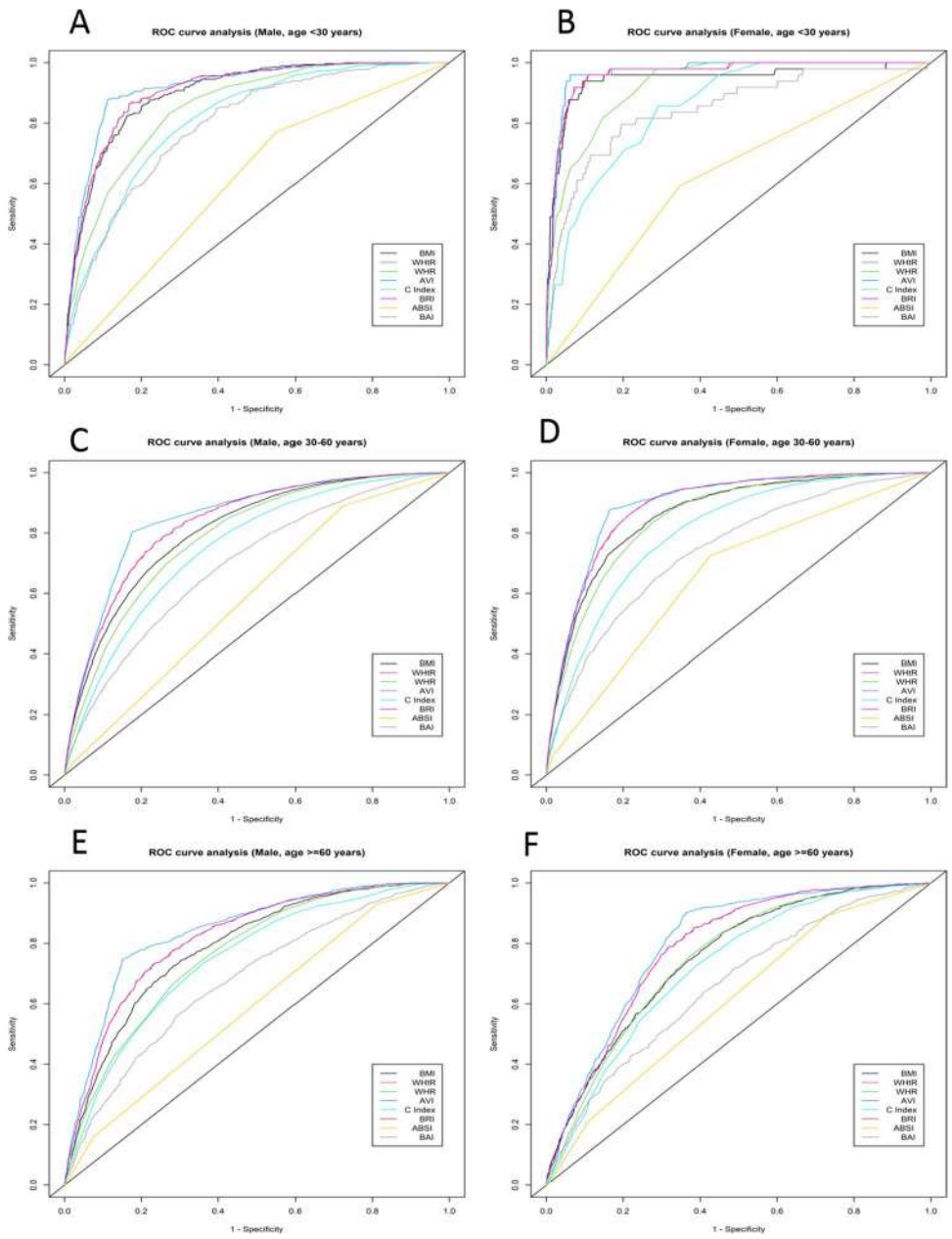

Fig. 1 **A**–**F** ROC curve analysis of anthropometric indices to identify MetS across gender and age group

examined, correlates more strongly with cardiovascular risk factors and MetS, and it has the strongest ability to predict MetS. This result provides a new means by which to screen for cardiovascular risk factors and MetS.

This study reveals that the prevalence rate of MetS was 16.81% in a sample of 76,915 participants, which is similar to the results in a group of Chinese Singaporeans (16.8%) [18]. This may be attributed to the fact that both studies focus on participants of Chinese ethnicity. It is reported that Indians and Malays have higher MetS prevalence rates than the Chinese. In our study, among the population which meets the criteria for MetS, the most commonly occurring component of MetS is hypertension (40.36%), while the least common is reduced HDL-cholesterol (15.63%). The same components are identified in the study by Ceolin and colleagues in a group of 60–100 year-olds from Brazil. This is the same as the results of Ceolin et al [19].

In our study sample, the AVI of those under the age of 30 years has the largest area under the ROC curve in both male and female populations, consistent with the results of Perona and colleagues [20]. Their study identifies AVI as well as WC as the most powerful discriminating anthropometric indicators of MetS in Spanish adolescents. Studies have shown that WC and AVI can predict MetS in adolescents, but only when using the International Diabetes Federation Diagnostic Criteria [21]. In line with these findings, in

Table 11 ROC curve analysis of anthropometry indices to identify MetS in women aged ≥ 60 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.54	> 12.8	0.80	< 0.0001	90.13	63.72	59.12	91.73	2.48	0.15
WHR	0.38	> 0.87	0.75	< 0.0001	76.14	62.32	54.05	81.77	2.02	0.38
WHtR	0.47	> 0.531	0.78	< 0.0001	78.80	68.37	59.19	84.71	2.49	0.31
CI	0.34	> 1.22	0.72	< 0.0001	73.05	60.57	51.89	79.43	1.85	0.44
BMI	0.38	> 22.99	0.75	< 0.0001	83.78	53.97	51.45	85.11	1.82	0.30
BRI	0.47	> 3.96	0.78	< 0.0001	78.80	68.37	59.19	84.71	2.49	0.31
BAI	0.23	> 30.68	0.66	< 0.0001	64.21	58.77	47.55	73.82	1.56	0.61
ABSI	0.16	> 0.07	0.61	< 0.0001	89.36	26.74	41.53	81.19	1.22	0.40

ABSI a body shape index, *AVI* abdominal volume index, *AUC* area under the curve, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CA* crude accuracy, *C index* conicity index, LR^+ positive likelihood ratio, LR^- negative likelihood ratio, *MetS* metabolic syndrome, *NPV* negative predictive value, *PPV* positive predictive value, *SEN* sensitivity, *SPE* specificity, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

Table 12 ROC curve analysis of anthropometric indices to identify MetS in men aged 30–60 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.63	> 16.04	0.85	< 0.0001	80.48	82.34	57.39	93.45	4.56	0.24
WHRR	0.43	> 0.9	0.79	< 0.0001	77.39	65.92	40.16	90.80	2.27	0.34
WHtR	0.53	> 0.528	0.83	< 0.0001	76.49	76.77	49.32	91.70	3.29	0.31
CI	0.38	> 1.22	0.75	< 0.0001	75.12	63.24	37.65	89.58	2.04	0.39
BMI	0.47	> 25.53	0.81	< 0.0001	77.90	68.87	42.51	91.34	2.50	0.32
BRI	0.53	> 3.9	0.83	< 0.0001	76.72	76.57	49.18	91.76	3.27	0.30
BAI	0.29	> 26.48	0.70	< 0.0001	63.95	64.91	35.01	85.90	1.82	0.56
ABSI	0.17	> 0.07	0.59	< 0.0001	88.94	27.77	26.68	89.47	1.23	0.40

Table 13 ROC curve analysis of anthropometric indices to identify MetS in women aged 30–60 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.71	> 12.82	0.90	< 0.0001	87.82	83.47	30.15	98.83	5.31	0.15
WHR	0.56	> 0.83	0.85	< 0.0001	83.35	72.47	19.74	98.17	3.03	0.23
WHtR	0.65	> 0.497	0.89	< 0.0001	86.08	78.53	24.57	98.58	4.01	0.18
CI	0.47	> 1.16	0.80	< 0.0001	81.73	64.94	15.92	97.77	2.33	0.28
BMI	0.57	> 23.24	0.86	< 0.0001	83.87	73.44	20.42	98.25	3.16	0.22
BRI	0.65	> 3.3	0.89	< 0.0001	86.08	78.53	24.57	98.58	4.01	0.18
BAI	0.37	> 29.13	0.74	< 0.0001	70.71	66.13	14.50	96.53	2.09	0.44
ABSI	0.30	> 0.07	0.66	< 0.0001	72.51	57.36	12.14	96.25	1.70	0.48

ABSI a body shape index, *AVI* abdominal volume index, *AUC* area under the curve, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CA* crude accuracy, *C index* conicity index, LR^+ positive likelihood ratio, LR^- negative likelihood ratio, *MetS* metabolic syndrome, *NPV* negative predictive value, *PPV* positive predictive value, *SEN* sensitivity, *SPE* specificity, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

our study, we find similar patterns in people under 30 years old using NCEP-ATP diagnostic criteria for MetS.

In a study on postmenopausal women in Brazil, researchers find that anthropometric indices including WC, WHtR, BMI, BAI, and CI have different degrees of correlation with MetS [22]. Compared with the percentage of body fat derived from dual-energy X-ray absorptiometry (DXA), which is considered as the gold standard, indicators of central obesity (such as WC and WHtR) are more strongly correlated with MetS. In that study, their cutoff values for predicting MetS were 88 cm for WC, 0.57 cm/cm for WHtR, 26.85 kg/m^2 for BMI, 36.34% for BAI, and 1.24 units for C index. In our study, although we do not employ DXA to measure body fat composition, similarly, we find ABSI and WHtR to be more strongly correlated with MetS. The cutoff value of predicting MetS in women over 60 years old is 79 cm for WC, 0.531 cm/cm for WHtR, 22.99 kg/m^2 for BMI, 30.68% for BAI, and 1.22 units for C index. The area

Table 14 ROC curve analysis of anthropometric indices to identify MetS in male aged < 30 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.77	> 16.22	0.92	< 0.0001	87.97	88.65	38.19	98.93	7.75	0.14
WHR	0.56	> 0.87	0.85	< 0.0001	83.09	72.82	19.59	98.18	3.06	0.23
WHtR	0.70	> 0.513	0.91	< 0.0001	86.53	83.12	29.01	98.72	5.13	0.16
CI	0.48	> 1.2	0.81	< 0.0001	75.93	72.50	18.04	97.42	2.76	0.33
BMI	0.66	> 26.17	0.90	< 0.0001	82.52	83.67	28.72	98.36	5.05	0.21
BRI	0.70	> 3.6	0.91	< 0.0001	86.53	83.12	29.01	98.72	5.13	0.16
BAI	0.45	> 25.25	0.80	< 0.0001	84.81	60.37	14.57	98.03	2.14	0.25
ABSI	0.22	> 0.07	0.61	< 0.0001	77.08	44.88	10.03	96.09	1.40	0.51

Table 15 ROC curve analysis of anthropometric indices to identify MetS in female aged < 30 years

	Youden index	Cutoff	AUC	*p*	Sen	Spe	PPV	NPV	LR^+	LR^-
AVI	0.90	> 12.79	0.97	< 0.0001	95.92	93.79	61.86	99.55	15.45	0.04
WHR	0.69	> 0.79	0.93	< 0.0001	97.96	71.35	26.42	99.70	3.42	0.03
WHtR	0.85	> 0.483	0.96	< 0.0001	95.92	89.12	48.08	99.52	8.82	0.05
CI	0.57	> 1.14	0.86	< 0.0001	85.71	70.85	23.59	97.93	2.94	0.20
BMI	0.84	> 23.37	0.95	< 0.0001	93.88	90.49	50.90	99.29	9.87	0.07
BRI	0.85	> 3.05	0.96	< 0.0001	95.92	89.12	48.08	99.52	8.82	0.05
BAI	0.60	> 28.76	0.85	< 0.0001	79.59	80.47	29.97	97.41	4.08	0.25
ABSI	0.25	> 0.07	0.63	< 0.0001	59.18	65.40	15.23	93.85	1.71	0.62

ABSI a body shape index, *AVI* abdominal volume index, *AUC* area under the curve, *BAI* body adiposity index, *BMI* body mass index, *BRI* body roundness index, *CA* crude accuracy, *C index* conicity index, LR^+ positive likelihood ratio, LR^- negative likelihood ratio, *MetS* metabolic syndrome, *NPV* negative predictive value, *PPV* positive predictive value, *SEN* sensitivity, *SPE* specificity, *WC* waist circumference, *WHR* waist-hip ratio, *WHtR* waist-to-height ratio

under the curve of AVI is superior to that of WHtR, showing a stronger ability for predicting MetS.

In this study, we also assess the ability of anthropometric indicators to distinguish MetS. The results suggest that all anthropometric indexes are capable of significantly differentiating individuals with MetS from those without MetS. Since there is a strong correlation between insulin resistance and dyslipidemia in visceral obesity, it is important to have a high discriminability of obesity index in predicting MetS [23]. Different studies have evaluated anthropometric indicators that best predict metabolic risk factors in different ethnic groups, but the results are inconsistent. There is no consensus on the best predictor of MetS or its major components. For instance, previous studies have shown that WC can predict MetS better than BMI, WHR, and WHtR [24] [21]. However, a cross-sectional study of the Chinese adult population reports that WC, WHR, and BMI are equivalent indicators in distinguishing MetS [25]. Another study concludes that BMI may be a better indicator for screening Chinese men aged 40 and over for obesity, dyslipidemia, and other risk factors, while WHtR may be more suitable for Chinese women, especially those aged 70 and over [26]. Our study indicates that AVI was a better predictor of MetS than BMI in men and women of all ages.

Young people tend to be more active than older people are, and a greater proportion of their weight is attributable to muscle rather than fat. Thus, BMI is not an effective measure of body fat composition, and it is unlikely to be used as a MetS screening indicator in younger populations. A prospective cohort study on the 1552 Qatari adult population demonstrates that WC has better predictive power for MetS compared to other indices such as BMI and WHR [27]. Quaye et al.'s study [28] in a population of 160 apparently healthy normoglycemic normotensive adults showed a better correlation between BMI and WC, with MetS and other cardiovascular risk factors than do AVI, BAI, and C index. The AUCs that BMI and WC predict MetS and its components, as they are larger than that of AVI, BAI, and C index. However, after accounting for gender differences, the AUCs that AVI and C index predict MetS in women, while BMI is still the optimal indicator in men. In this study, some anthropometric indicators are found to be associated with cardiovascular risk factors, which are similar to the results of Yang, et al. [24], Sinaga et al. [29], Tian et al. [30], Lawrence Quaye et al. [28], among other studies. However, our study notes that the AUC of AVI is the largest among all indices examined, and is the best predictor for the MetS. The AUC of C index predicts MetS in all females, but its

value is lower than that of BMI. Such as, AVI is the optimal indicator to predict MetS.

Mamtani and Kuljiarni [31] found no correlation between CI and fasting and postprandial glucose, while in this study, it was found that CI was correlated with fasting blood glucose, with a correlation coefficient of 0.208 ($p < 0.05$), which was still correlated after adjusting for age, gender, smoking, and drinking. Motamed [32] and Wang [33] et al. found that CI and AVI performed better in women after gender stratification.

In the AVI formula, when WC is lower than HC, an increase in HC will lead to an increase in AVI [32]. Since HC of women is larger than WC, an increase in HC usually leads to an increase in AVI. Thus, the predictive ability of AVI for the two genders will be different. As shown in this study, the ability of AVI in women to predict MetS is greater than that in men. AVI demonstrates good differentiation ability in MetS screening. As such, in addition to the more classic obesity indicators of WC, WHR, and WHtR, the newer AVI can also be considered as an appropriate obesity index for MetS screening. Previous studies show that AVI is closely associated with impaired glucose tolerance and type 2 diabetes [32]. The obesity patterns we calculated for AVI are similar to the truncated cones of lower-body obesity in women and upper body obesity in men. The advantage of estimating abdominal volume with AVI is that it predicts what is likely to happen with both patterns. Meanwhile, the belly volume underestimated by the AVI formula is likely to be minimal and does not affect the overall volume estimate.

Mamtani and Kulkarni [31] report no correlation between C index and fasting or postprandial glucose. However, in this study, we find that the C index is correlated with fasting blood glucose, with a correlation coefficient of 0.208 ($p < 0.05$), even after adjusting for age, gender, smoking, and drinking status. Both Motamed et al. [32] and Wang et al. [33] find that C index and AVI perform better in predicting MetS in women after accounting for gender differences.

Differences in results between studies can be attributed to differences in anthropometric characteristics between ethnicities and nationalities. With regard to ethnic differences, a study on a Kazak sample of adults in Xinjiang China suggests that WHtR was a better indicator screening MetS in Kazak men and women [34], compared to our finding of AVI to be the best predictor of MetS in men and women. These differences may be attributed to racial differences and the use of different diagnostic criteria [35, 36]. Concerning differences between countries, a study of Korean men at MetS screening reports that WHtR shows greater discrimination compared to BMI. They find WHR to be the best screening tool and is more effective than either BMI or WC in screening for adult metabolic risk factors [37], compared with AVI in our study. The San Antonio Heart Study [38] in non-Hispanic whites and Mexican–American reports that BMI and WC have similar predictive effects for MetS development. The variations of findings may be related to different characteristics of obesity based on ethnicity [39].

AVI has a good differentiation ability in MetS screening. Therefore, besides WC, WHR, and WHtR, AVI can also be considered as the appropriate obesity index for MetS screening. AVI is extrapolated from anthropometry and is closely related to impaired glucose tolerance and type 2 diabetes [8].

Moreover, studies in China showed that the overall burden of CVD in China has significantly increased, with obvious regional differences [40]. In this study, we note that AVI has a strong MetS discrimination ability in a sample of urban residents in west China. Taken together the advantages of AVI and results from our study, it is of great practical significance to be able to use AVI for MetS screening in Chengdu metropolitan area.

In our study, we note that the means of BAI in women are higher than that of men; the BAI values increase with the increase of age, with the highest value over 60 years of age. The reason may come from changes in body composition in postmenopausal women. Decreased estrogen production leads to increased age-related obesity and metabolic disorders. Women of this age group show less physical activity, which may lead to decreased thigh muscle mass and increased visceral fat accumulation [41]. In the normal distribution of male and female adipose tissue, women tend to accumulate excess fat in the lower buttocks (thighs and buttocks). Later, they tend to accumulate excess fat in the upper body, giving rise to central obesity [42]. In addition, women naturally store fat under the skin, while men store fat in the gut.

In our study, we report that ABSI shows the lowest AUC across genders and different age groups in predicting MetS, indicating that ABSI is the weakest predictor of MetS in the population. Zhou et al. [17] find that ABSI and C index are the weakest indicators of MetS in middle-aged and elderly maintenance hemodialysis patients in China. This is similar to our findings on ABSI. Similarly, Zhang et al.'s study [43] report that ABSI shows the lowest AUC in discriminating MetS and most cardiometabolic risk factors. Therefore, ABSI could not be used as an alternative index.

Our study utilizes an anthropometric approach to predict the optimal cutoff point for MetS, which can be easily used in clinical practice. We find that the critical value of other anthropometric indicators with exception of ABSI can be used to identify MetS. Additionally, some anthropometric indicators, such as BAI, predict MetS in women more strongly than in men. As such, applying the measures selectively, such as using the BAI for women only, taking into account gender differences in clinical practice is crucial and more efficient.

This study has some limitations. First, this is a cross-sectional study that provides less evidence over time than

results from longitudinal or randomized epidemiological studies. The cross-sectional nature of the design does not allow for the direction of the assessment of risk factors and health outcomes. Further cohort studies are needed to confirm the findings. Second, because the study is conducted in a single ethnic group, caution should be exercised in extending these results to other ethnic groups. Well-designed longitudinal studies across different ethnic groups are needed to extend our findings. Despite the limitations, we believe our study sample size is substantially large, to contribute significantly to the current literature on identifying anthropometric indices that can most effectively predict MetS.

Conclusion

AVI, WHR, WHtR, BMI, C index, and BAI are anthropometric indices significantly associated with cardiovascular risk factors. Anthropometric indices are useful screening tools for MetS or its components, and cardiovascular risk factors. Of all the anthropometric indices studied, AVI is the best index in distinguishing MetS.

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Author contribution Qinqin Wu and Hu Nie Designed the study, executed the study, analyzed the results, and contributed to the drafting of the manuscript. Qinqin Wu and Ken Qin Analyzed and interpreted the data in the revised version. Qinqin Wu and Youjuan Wang Contributed to designing the study and discussion of results, and the final manuscript. All authors have read and approved the final manuscript.

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Data availability The data used in this study were collected from the Health Management Center of West China Hospital. The data belongs to West China hospital. Those data are not publicly obtainable.

Declarations

Ethics approval and consent to participate This study was approved by the Ethics Committee of West China Hospital of Sichuan University. As this is a retrospective research, informed consent was not essential according to the Ethical Guidelines for Epidemiological Research. The study was allowed by the Ethics Committee of West China, Sichuan University.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Ma LY, Chen WW, Gao RL, Liu LS, Zhu ML, Wang YJ, et al. China cardiovascular diseases report 2018: an updated summary. J Geriatr Cardiol. 2020;17(1):1–8. https://doi.org/10.11909/j.issn.1671-5411.2020.01.001.
2. Cleeman JI, Grundy SM, Becker D, Clark LT, Cooper RS, Denke MA, et al. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Jama-Journal of the American Medical Association. 2001;285(19):2486–97. https://doi.org/10.1001/jama.285.19.2486.
3. Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;20(2):12. https://doi.org/10.1007/s11906-018-0812-z.
4. Gonzalez-Chávez A, Chávez-Fernández JA, Elizondo-Argueta S, González-Tapia A, León-Pedroza JI, Ochoa C. Metabolic syndrome and cardiovascular disease: a health challenge. Arch Med Res. 2018;49(8):516–21. https://doi.org/10.1016/j.arcmed.2018.10.003.
5. Perissinotto E, Pisent C, Sergi G, Grigoletto F. Anthropometric measurements in the elderly: age and gender differences. Br J Nutr. 2002;87(2):177–86. https://doi.org/10.1079/bjn2001487.
6. Steen B. Body composition and aging. Nutr Rev. 1988;46(2):45–51. https://doi.org/10.1111/j.1753-4887.1988.tb05386.x.
7. Almeida NS, Rocha R, Cotrim HP, Daltro C. Anthropometric indicators of visceral adiposity as predictors of non-alcoholic fatty liver disease: a review. World J Hepatol. 2018;10(10):695–701. https://doi.org/10.4254/wjh.v10.i10.695.
8. Guerrero-Romero F, Rodriguez-Moran M. Abdominal volume index. An anthropometry-based index for estimation of obesity is strongly related to impaired glucose tolerance and type 2 diabetes mellitus. Arch Med Res. 2003;34(5):428–32. https://doi.org/10.1016/s0188-4409(03)00073-0.
9. Patil VC, Parale GP, Kulkarni PM, Patil HV. Relation of anthropometric variables to coronary artery disease risk factors. Indian J Endocr Metab. 2011;15(1):31–7. https://doi.org/10.4103/2230-8210.77582.
10. Ruperto M, Barril G, Sanchez-Muniz FJ. Conicity index as a contributor marker of inflammation in haemodialysis patients. Nutr Hosp. 2013;28(5):1688–95. https://doi.org/10.3305/nh.2013.28.5.6626.
11. Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, et al. A better index of body adiposity. Obesity. 2011;19(5):1083–9. https://doi.org/10.1038/oby.2011.38.
12. Krakauer NY, Krakauer JC. A new body shape index predicts mortality hazard independently of body mass index. PLoS One. 2012;7(7):e39504. https://doi.org/10.1371/journal.pone.0039504.
13. Thomas DM, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, et al. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. Obesity. 2013;21(11):2264–71. https://doi.org/10.1002/oby.20408.
14. Nevill AM, Duncan MJ, Lahart IM, Sandercock GR. Scaling waist girth for differences in body size reveals a new improved index associated with cardiometabolic risk. Scand J Med Sci Sports. 2017;27(11):1470–6. https://doi.org/10.1111/sms.12780.
15. Lee DY, Lee MY, Sung KC. Prediction of mortality with a body shape index in young Asians: comparison with body mass index and waist circumference. Obesity. 2018;26(6):1096–103. https://doi.org/10.1002/oby.22193.
16. Wu Q, Qin K, Wang Y. Central obesity is associated with helicobacter pylori infection: a large-scale cross-sectional retrospective

study in West China. Int J Diabetes Dev Ctries. 2019;40(1):52–60. https://doi.org/10.1007/s13410-019-00765-8.
17. Zhou CM, Zhan L, Yuan J, Tong XY, Peng YH, Zha Y. Comparison of visceral, general and central obesity indices in the prediction of metabolic syndrome in maintenance hemodialysis patients. Eating and Weight Disorders-Studies on Anorexia Bulimia and Obesity. 2020;25(3):727-34. doi:10.1007/s40519-019-00678-9.
18. Nurjono M, Lee J. Waist circumference is a potential indicator of metabolic syndrome in Singaporean Chinese. Ann Acad Med Singapore. 2013;42(5):241–5.
19. Ceolin J, Engroff P, Mattiello R, Schwanke CHA. Performance of Anthropometric Indicators in the Prediction of Metabolic Syndrome in the Elderly. Metabolic Syndrome and Related Disorders. 2019;17(4):232-9. doi:10.1089/met.2018.0113.
20. Perona JS, Rio-Valle JS, Ramirez-Velez R, Correa-Rodriguez M, Fernandez-Aparicio A, Gonzalez-Jimenez E. Waist circumference and abdominal volume index are the strongest anthropometric discriminators of metabolic syndrome in Spanish adolescents. European Journal of Clinical Investigation. 2019;49(3):e13060. doi:10.1111/eci.13060.
21. Perona JS, Schmidt-RioValle J, Fernandez-Aparicio A, Correa-Rodriguez M, Ramirez-Velez R, Gonzalez-Jimenez E. Waist Circumference and Abdominal Volume Index Can Predict Metabolic Syndrome in Adolescents, but only When the Criteria of the International Diabetes Federation are Employed for the Diagnosis. Nutrients. 2019;11(6):1370. doi:10.3390/nu11061370.
22. Gadelha AB, Myers J, Moreira S, Dutra MT, Safons MP, Lima RM. Comparison of adiposity indices and cut-off values in the prediction of metabolic syndrome in postmenopausal women. Diabetes Metab Syndr. 2016;10(3):143–8. https://doi.org/10.1016/j.dsx.2016.01.005.
23. Despres J-P, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881–7. https://doi.org/10.1038/nature05488.
24. Yang YJ, Park HJ, Won KB, Chang HJ, Park GM, Kim YG, et al. Relationship between the optimal cut-off values of anthropometric indices for predicting metabolic syndrome and carotid intima-medial thickness in a Korean population. Medicine. 2019;98(42):e17620. https://doi.org/10.1097/md.0000000000017620.
25. Ko K-P, Oh D-K, Min H, Kim C-S, Park J-K, Kim Y, et al. Prospective study of optimal obesity index cutoffs for predicting development of multiple metabolic risk factors: the Korean genome and epidemiology study. J Epidemiol. 2012;22(5):433–9. https://doi.org/10.2188/jea.JE20110164.
26. He YH, Chen YC, Jiang GX, Huang HE, Li R, Li XY, et al. Evaluation of anthropometric indices for metabolic syndrome in Chinese adults aged 40 years and over. Eur J Nutr. 2012;51(1):81–7. https://doi.org/10.1007/s00394-011-0195-2.
27. Bener A, Yousafzai MT, Darwish S, Al-Hamaq AOAA, Nasralla EA, Abdul-Ghani M. Obesity index that better predict metabolic syndrome: body mass index, waist circumference, waist hip ratio, or waist height ratio. J Obes. 2013. https://doi.org/10.1155/2013/269038.
28. Quaye L, Owiredu W, Amidu N, Dapare PPM, Adams Y. Comparative Abilities of Body Mass Index, Waist Circumference, Abdominal Volume Index, Body Adiposity Index, and Conicity Index as Predictive Screening Tools for Metabolic Syndrome among Apparently Healthy Ghanaian Adults. Journal of Obesity. 2019;2019:8143179. doi:10.1155/2019/8143179.
29. Sinaga M, Worku M, Yemane T, Tegene E, Wakayo T, Girma T, et al. Optimal cut-off for obesity and markers of metabolic syndrome for Ethiopian adults. Nutr J. 2018;17(1):109. https://doi.org/10.1186/s12937-018-0416-0.
30. Tian T, Zhang J, Zhu Q, Xie W, Wang Y, Dai Y. Predicting value of five anthropometric measures in metabolic syndrome among Jiangsu Province, China. BMC Public Health. 2020;20(1):1317. https://doi.org/10.1186/s12889-020-09423-9.
31. Mamtani MR, Kulkarni HR. Predictive performance of anthropometric indexes of central obesity for the risk of type 2 diabetes. Arch Med Res. 2005;36(5):581–9. https://doi.org/10.1016/j.arcmed.2005.03.049.
32. Motamed N, Sohrabi M, Poustchi H, Maadi M, Malek M, Keyvani H, et al. The six obesity indices, which one is more compatible with metabolic syndrome? A population based study. Diabetes Metab Syndr. 2017;11(3):173–7. https://doi.org/10.1016/j.dsx.2016.08.024.
33. Wang H, Liu A, Zhao T, Gong X, Pang T, Zhou Y, et al. Comparison of anthropometric indices for predicting the risk of metabolic syndrome and its components in Chinese adults: a prospective, longitudinal study. BMJ Open. 2017;7(9):e016062. https://doi.org/10.1136/bmjopen-2017-016062.
34. Zhang XH, Zhang M, He J, Yan YZ, Ma JL, Wang K, et al. Comparison of anthropometric and atherogenic indices as screening tools of metabolic syndrome in the Kazakh adult population in Xinjiang. Int J Environ Res Public Health. 2016;13(4):428. https://doi.org/10.3390/ijerph13040428.
35. Gharipour M, Sadeghi M, Dianatkhah M, Bidmeshgi S, Ahmadi A, Tahri M, et al. The cut-off values of anthropometric indices for identifying subjects at risk for metabolic syndrome in Iranian elderly men. Journal of obesity. 2014;2014:907149. https://doi.org/10.1155/2014/907149.
36. Llinas MG, Janer PE, Agudo SG, Casquero RG, Gonzalez IC. Usefulness in nursing of different anthropometric and analytical indices to assess the existence of metabolic syndrome with the NCEP ATP III and IDF criteria in Spanish Mediterranean population. Medicina Balear. 2017;32(1):26–34. https://doi.org/10.3306/medicinabalear.32.01.26.
37. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obesity Reviews. 2012;13(3):275-86. doi:10.1111/j.1467-789X.2011.00952.x.
38. Han T, Williams K, Sattar N, Hunt K, Lean M, Haffner S. Analysis of obesity and hyperinsulinemia in the development of metabolic syndrome: San Antonio Heart Study. Obes Res. 2002;10:923–31. https://doi.org/10.1038/oby.2002.126.
39. Guzman de la Garza FJ, Salinas-Martinez AM, Gonzalez-Guajardo E, Palmero-Hinojosa MG, Castro-Garza J, Ramirez-Zuniga JC, et al. Threshold values of sagittal abdominal diameter for the detection of cardio-metabolic risk factors in Northeastern Mexico: a cross-sectional study. Nutricion Hospitalaria. 2016;33(3):268. https://doi.org/10.20960/nh.268.
40. Zhang GS, Yu CH, Luo LS, Li YC, Zeng XY. Trend analysis of the burden of ischemic heart disease in China, 1990 to 2015. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2017;51(10):915-21. doi:10.3760/cma.j.issn.0253-9624.2017.10.009.
41. Arthur FK, Adu-Frimpong M, Osei-Yeboah J, Mensah FO, Owusu L. Prediction of metabolic syndrome among postmenopausal Ghanaian women using obesity and atherogenic markers. Lipids Health Dis. 2012;11:101. https://doi.org/10.1186/1476-511x-11-101.
42. Williams CM. Lipid metabolism in women. Proceedings of the Nutrition Society. 2004;63(1):153–60. https://doi.org/10.1079/pns2003314.
43. Zhang J, Zhu WH, Qiu LF, Huang LJ, Fang LZ. Sex- and age-specific optimal anthropometric indices as screening tools for metabolic syndrome in Chinese adults. Int J Endocrinol. 2018. https://doi.org/10.1155/2018/1067603.

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ORIGINAL ARTICLE

Leptins: association and clinical correlation in pre-diabetics

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Abstract

Background /purpose Leptin—a protein hormone that plays vital role in the regulation of glucose homeostasis and insulin sensitivity, involved in regulating the circadian rhythms of gonadotropic, thyrotropic and adrenal axes in the endocrine system. This cross-sectional study was undertaken to correlate serum leptin levels and other demographic and biochemical parameters between prediabetic cases and control subjects of Kashmiri population. Furthermore, association of serum leptin levels with various demographic and biochemical parameters along with insulin resistance in pre-diabetic subjects was elucidated. In our study, pre-diabetic cases ($n = 212$) and healthy controls ($n = 196$) were enrolled.

Methods All study subjects underwent anthropometric assessment. Serum samples of all study subjects were analysed for biochemical parameters employing the principle of spectrophotometry and leptin levels using sandwich enzyme-linked immunosorbent assay (ELISA). Insulin levels of prediabetic cases were deduced by chemiluminescent micro particle immunoassay (CMIA) technology and thereby calculating their insulin resistance.

Results The cases had statistically increased leptin levels, lipid profile and obesity as compared to controls ($p \leq 0.05$). Our study, showed a positive correlation between elevated leptin levels and increased lipid levels (total cholesterol, triglycerides; TG and very low density lipoproteins-cholesterol; VLDL-C) in prediabetics ($p < 0.001$).

Conclusion Beyond adiposity, leptin levels may increase the risk of pre-diabetes in Kashmiri population. Since the secretion, estimation and measurements of leptins are modest in contrast to insulin levels; therefore, leptin levels can also be included in the routine measurement as an indicator of metabolic syndrome and/or T2DM.

Keywords HOMA IR · Insulin sensitivity · Leptin · Pre-diabetes · Type 2 diabetes mellitus

Introduction

Leptin is an adipokine, a hormone comprising of 167 amino acids produced in the adipocytes and to a lesser extent in other tissues including the placenta of a pregnant woman. Primarily responsible for regulating the body weight by the attainment of balance between food intake and energy expenditure, under normal physiological conditions, leptin plays vital role in the regulation of glucose homeostasis and insulin sensitivity. It has also been seen that leptin has multiple roles in the immune and endocrine systems in bone formation, fertility, tissue remodelling and inflammation [1]. Leptin is also involved in regulating the circadian rhythms of the gonadotropic, thyrotropic and adrenal axes in the endocrine system [2, 3].

Located on chromosome 7 in humans, leptin was first identified as the product of a gene designated OB (obese) in the lab mice. Mouse models with mutant (inactive) form of the gene (ob/ob) exhibited metabolic disturbances similar to as seen in diabetics: constant state of starvation, increased cortisol levels, inability to stay warm, severe obesity, reproductive failure and insulin-resistance. When injected with leptin, such mutant ob/ob mice ate less, lost weight and increased their locomotor activity and thermogenesis [4–6].

Leptin regulates the energy expenditure and the intake of food by way of communicating with central nervous

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system via its receptor Ob-Rb [4–6]. Acting on the receptors in the arcuate nucleus of the hypothalamus that is known to regulate the feeding behaviour, leptin in turn causes the release of anorexigenic peptides, including α-Melanocyte-stimulating hormone that is formed from its polypeptide precursor POMC (pro-opio melanocortin). POMC acts in the brain to eat less. Leptin also stimulates the nervous system thereby increasing blood pressure and heart rate. It also leads to uncoupling of mitochondrial oxidative phosphorylation with consequent thermogenesis [7].

As per the World Health Organization (WHO), prediabetes has been defined as a state of intermediate hyperglycemia using two parameters, impaired fasting glucose (IFG) of 6.1–6.9 mmol/l (110–125 mg/dl) and impaired glucose tolerance (IGT) of 7.8–11.0 mmol/l (140–200 mg/dl) after taking 75 g of oral glucose load based on a 2-h oral glucose tolerance test (OGTT) [8]. American Diabetes Association (ADA) has same cut-off value for IGT (140–200 mg/dl) but the cut-off value for IFG is lower (100–125 mg/dl) and has additional criteria of glycated hemoglobin (HbA1C) (5.7–6.4%) for defining prediabetes [9].

For the perfect regulation of energy homeostasis in the brain, the functions of leptin and insulin signalling are very critical. Although, the relationship between leptin and insulin has been studied but is sometimes controversial. Some studies have not been able to show the direct effect of leptin on the energy homeostasis, whereas some researchers have focussed on the relationship between the two that share the properties of adiposity signals. Secretion of both leptin as well as insulin is affected by the total amount of fat deposits including the short-term changes in the energy balance. Leptin reduces appetite and affects both insulin resistance and lipid metabolism, and these effects are independent of energy intake in humans [10]. Recombinant leptin (metreleptin) has been shown to improve insulin sensitivity and decreases hepatic and circulating triglycerides independent of food intake [10]. Mechanisms governing the role of insulin are fully elucidated but secretion of leptin is not fully understood. Insulin secretion is actively stimulated in response to meals whereas the secretion of leptin is not [11]. Obese individuals have been found to have elevated concentrations of leptin as well as insulin, and they appear to be resistant to the loss of appetite [11, 12]. There are controversial results from the studies relating leptin levels and diabetes [13–16].

In this context, the present study was undertaken to correlate serum leptin levels between cases and controls and study the association of elevated leptin levels with various demographic and biochemical characteristics of pre-diabetic and healthy individuals belonging to the ethnic population of Kashmir.

Material and methods

Study design

It was a cross-sectional study conducted by the Department of Biochemistry in collaboration with the Department of Medicine, Government Medical College Srinagar and Associated SMHS and Super Speciality Hospital.

Study subjects and sample size

A total of two hundred and twelve ($n = 212$) pre-diabetic patients, (both obese and non-obese) attending Department of Medicine, Government Medical College Srinagar and Associated SMHS and Super Speciality Hospital, were enrolled for the present study over a period of 1 and a half years from June 2018 to December 2019. Prediabetes criteria used in this study are in confirmation with ADA guidelines; Fasting glucose: 100–125 mg/dl (6.1–7.0 mmol/l) or 2-h glucose following ingestion of 75 g glucose load: 140–200 mg/dl (7.8–11.0 mmol/l) [8, 9]. Controls ($n = 196$) were randomly selected from a pool of healthy volunteers who visited the hospital for health check-up during the same period and enrolled in the study. All the controls were having fasting blood sugar levels of < 100 mg/dl and oral glucose tolerance test (OGTT) i.e. glucose levels after 2 h of ingestion of 75 g glucose load: < 140 mg/dl.

Anthropometric measurement

All prediabetic patients and healthy controls underwent anthropometric assessment that included measurement of weight and height. Weight was measured without shoes using digital scales in an upright position and recorded to the nearest of 0.1 kg. Height was measured again without shoes in standing position using stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as per the formula:

$$BMI\ in^{Kg}/_{m^2} = \frac{Weight(Kg)}{Height\left(m^2\right)}$$

BMI is an index of adiposity and calculated was classified as per international classification given by WHO [17]. Individual with BMI of < 18.5 kg/m^2 was considered underweight; 18.5–24.99 kg/m^2 as normal; 25–29.99 kg/m^2 as preobese; 30–34.99 kg/m^2 as obese class I; and 35–39.99 kg/m^2 as obese class II [17].

Sample collection, lipid profile and estimation of blood glucose

05 ml of venous blood sample was drawn from each prediabetic patient and control subject after an overnight fast of at least 08–12 h. Samples were centrifuged, and estimation of glucose (by hexokinase G-6-PDH method), total cholesterol (cholesterol dehydrogenase method), TG (by coupled enzymatic reactions), low density lipoprotein-cholesterol and high density lipoprotein-cholesterol (LDL-C and HDL-C using cholesterol esterase, cholesterol oxidase, peroxidase and 4-aminoantipyrine) was done. Samples were processed and analysed on ARCHITECT-C-4000 fully automated biochemistry analyser (Abbott, USA) in the Biochemistry Diagnostic Laboratory, SMHS Hospital Srinagar within 1–2 h after collection. VLDL levels were calculated using conventional Friedewald formula [18]. The normal values of included parameters were as follows: fasting blood glucose, 100–126 mg/dl; total cholesterol, ≤ 200 mg/dl; TG, ≤ 200 mg/dl; LDL-C, ≤ 120 mg/dl; HDL-C, ≥ 40 mg/dl (M) and ≥ 50 mg/dl (F) and VLDL-C, ≤ 30 mg/dl.

Estimation of fasting insulin levels

Insulin levels (μIU/ml) of prediabetic patients were measured using chemiluminescent micro particle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Insulin Kit used was ARCHITECT insulin reagent kit (Abbott, USA), and samples were quantitatively analysed on *ARCHITECT i1000* fully automatic immunoassay analyser (Abbott, USA) within few hours from sample collection following the package-insert instructions. Insulin levels of > 5 μIU/ml are considered to be normal [19].

Insulin resistance (IR)

HOMA-IR (homeostatic model assessment–insulin resistance) was used to evaluate insulin resistance of prediabetic patients using the formula:

$$HOMA - IR = \frac{fasting\ serum\ insulin(\mu IU/ml) \times fasting\ plasma\ glucose(mg/dl)}{405}$$

[20]

Following HOMA score was used as reference for classification of insulin resistance: (a) $< 1.9 =$ insulin sensitive, (b) 1.9 to 2.9 = low IR and (c) $> 2.9 =$ significant IR [20].

Estimation of serum leptin levels

Serum separated from the samples was stored at − 20 °C till the estimation of leptin levels. Serum leptin levels (ng/ml) of prediabetic patients and control subjects were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using Direct Biochem Diagnostic kit (DBC Inc., Canada) with 0.5 ng/ml sensitivity following the package instructions. 3.7 to 11.1 ng/ml was taken as reference normal range for female gender and 2.0 to 5.6 ng/ml for males of all age groups. The sensitivity of the leptin assay was less than 0.1 ng/ml [21].

Statistical analysis

The data was described as mean ± SD. Analysis was done by Student's *t* test, Mann–Whitney *U* test and *F* test (ANOVA). Multivariate analysis was done for the correlating leptin levels between cases and controls using multiple logistic regression analysis. $p \leq 0.05$ was considered significant. Data was analysed using SPSS v. 20.0.

Results

Characteristics of cases and controls

A total of two hundred and twelve ($n = 212$) prediabetic patients were taken for the study. These included 12.2% of patients with ≤ 18 years of age and 87.8% patients with > 18 years of age. The mean age of cases was 37.28 ± 19.7 with a range of 15.0–70.0 years as compared to 37.4 ± 19.8 with a range of 15.0–78.0 years in controls ($p = 0.1$). One hundred fifty-two (71.7%) were females and 60 (28.3%) were males. Cholesterol levels were normal in 73.6% patients as compared to only 26.4% of patients having elevated cholesterol levels. Triglycerides were also normal in 87.7% of patients. On evaluation of BMI, most of the patients were falling in pre-obese category (41.5%). Almost 91% of enrolled patients had mild HOMA IR. Leptin levels were normal in 80.0% of patients and elevated in 20.0% of patients. Apart from cases, a total of 196 controls were also included for the study which were matched for age, gender and dwelling with cases ($p > 0.05$). All the controls were non-diabetic with normal lipid profile and normal BMI. Only 2.0% of controls had elevated leptin levels. Demographic and biochemical characteristics of the study subjects are given in Table 1.

Leptin levels in cases vs controls

When leptin levels were compared between cases and controls, 80.0% and 20.0% of cases had normal and elevated leptin levels respectively as compared to 98.0% and 2.0% of controls with normal and elevated leptin levels respectively. This difference was found to be statistically significant ($p < 0.001$) (Table 1). The mean leptin levels of cases in ng/ml was 6.28 ± 2.92 as compared to 5.52 ± 1.98 in controls ($p = 0.02$). Leptin levels in cases and controls of female gender were 6.5 ± 2.91 and 5.8 ± 1.96

Table 1 Demographic and biochemical characteristics of pre-diabetic cases and controls included in the study

Characteristics	Cases ($n=212$)		Controls ($n=196$)		χ^2	p value
	n	%	*n*	%		
Gender						
Male	60	28.3	51	26.0	0.27	0.6
Female	152	71.7	145	74.0		
Age group						
≤ 18 years	26	12.2	25	12.8	0.02	0.9
> 18 years	186	87.8	171	87.2		
Dwelling						
Rural	104	49.1	96	49.0	0.01	1.0
Urban	108	50.9	100	51.0		
BMI (Kg/m^2)						
Normal	76	35.8	196	100	29.5	**≤ 0.05**
Underweight	02	1.0	00	00		
Preobese	88	41.5	00	00		
Obese class I	24	11.3	00	00		
Obese class II	22	10.4	00	00		
Total cholesterol (mg/dl)						
Normal	156	73.6	196	100	58.05	**< 0.001**
Elevated	56	26.4	00	00		
TG (mg/dl)						
Normal	128	60.3	196	100	95.7	**< 0.001**
Elevated	84	39.7	00	00		
LDL-C (mg/dl)						
Normal	122	57.5	196	100	104.6	**< 0.0001**
Elevated	90	42.5	00	00		
HDL-C (mg/dl)						
Normal	135	63.6	196	100	85.7	**< 0.001**
Low	77	36.4	00	00		
VLDL-C (mg/dl)						
Normal	138	65.1	196	100	81.5	**< 0.0001**
Elevated	74	34.9	00	00		
Leptin levels (ng/ml)						
Normal	170	80.0	192	98.0	32.1	**< 0.0001**
Elevated	42	20.0	04	2.0		
Insulin (µIU/ml)						
Normal	190	89.6				-
Low	22	10.4				
HOMA IR						
Insulin sensitive	166	78.3				-
Low IR	26	12.2				
Significant IR	20	9.5				

TG, triglycerides; *LDL-C*, low-density lipoproteins-cholesterol; *HDL-C*, high-density lipoproteins-cholesterol; *VLDL-C*, very low-density lipoproteins-cholesterol; *BMI*, body mass index*; HOMA IR*, homeostatic model assessment–insulin resistance

The bold signifies statistically significant p values

respectively ($p=0.004$) and male gender were 5.55 ± 2.85 and 4.6 ± 1.74 respectively ($p<0.0001$); therefore, a statistical significance was noted between leptin levels of cases and controls with or without stratification of gender (Table 2).On multivariate analysis using multiple logistic regression, leptin levels were significantly elevated

Table 2 Levels of various biochemical parameters of pre-diabetic cases and controls in terms of mean ± SD

Parameters	Cases mean ± SD (range)	Controls mean ± SD (range)	*p* value
Fasting blood sugar(mg/dl)	119.6 ± 2.36 (112.0–125.0)	83.1 ± 4.4 (70.0–92.0)	< 0.0001
BMI (Kg/m^2)	27.1 ± 5.5 (18.2–51.9)	21.6 ± 1.47 (18.6–24.5)	< 0.0001
Total cholesterol (mg/dl)	182.5 ± 39.1 (96.0–258.0)	159.6 ± 11.9 (123.0–189.0)	< 0.0001
TG (mg/dl)	220.0 ± 62.5 (117.0–358.0)	173.4 ± 12.5 (150.0–195.0)	< 0.0001
HDL-C (mg/dl)	46.5 ± 8.8 (27.0–78.0)	59.9 ± 8.2 (41.0–69.0)	< 0.0001
LDL-C (mg/dl)	122.4 ± 35.0 (45.0–198.0)	72.0 ± 2.3 (27.0–102.0)	< 0.0001
VLDL-C (mg/dl)	43.81 ± 12.4 (23.4–71.6)	34.6 ± 2.5 (30.0–39.0)	< 0.0001
Leptin levels (ng/ml)	6.28 ± 2.92 (2.7–15.2)	5.52 ± 1.98 (2.2–11.2)	0.002
Leptin levels in females (ng/ml)	6.5 ± 2.91 (2.7–15.2)	5.8 ± 1.96 (2.2–10.03)	0.004
Leptin levels in males (ng/ml)	5.55 ± 2.85 (2.7–14.7)	4.6 ± 1.74 (2.2–11.2)	< 0.0001
Insulin levels (µIU/ml)	10.8 ± 1.2 (0.27–55.26)	-	
HOMA IR	1.47 ± 0.7 (0.03–13.6)	-	

TG, triglycerides; *LDL-C*,low-density lipoproteins-cholesterol; *HDL-C*, high-density lipoproteins-cholesterol; *VLDL-C*, very low-density lipoproteins-cholesterol, *BMI*, body mass index, *HOMA IR*, homeostatic model assessment–insulin resistance

Table 3 Association of serum leptin levels as continuous variable between prediabetic cases and controls

Serum leptin levels in prediabetic cases vs. controls			
Model 1*	**Model 2****	**Model 3*****	**Model 4******
1.92 (1.6–2.17)	1.96 (1.7–2.22)	0.53 (0.458–0.615)	0.57 (0.451–0.721)
0.52 (0.46–0.599)	0.51 (0.45–0.58)	1.8 (1.6–2.3)	1.7 (1.4–2.2)
$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

*Unadjusted OR (95% CI) and *p* value

**OR (95% CI) and *p* value adjusted for gender, age and dwelling

***OR (95% CI) and *p* value adjusted for gender, age, dwelling and BMI

****OR (95% CI) and *p* value adjusted for gender, age, dwelling, BMI and lipid parameters

in prediabetic cases compared to controls when adjusted for age, gender, BMI and lipid parameters ($p < 0.0001$) (Table 3).

Mean levels of biochemical parameters in cases vs controls

The mean fasting blood sugar levels (mg/dl) of cases was 119.6 ± 2.36 (112.0–125.0) as compared to 83.1 ± 4.4 (70.0–92.0) in controls, and the difference was statistically significant (< 0.0001). The mean BMI (Kg/m^2) of the cases was 27.1 ± 5.5 (18.2–51.9) as compared to 21.6 ± 1.47 (18.6–24.5) in controls (< 0.0001). A statistical significance was noted between cases and controls in terms of mean levels of total cholesterol, TG, LDL-C, HDL-C and VLDL-C ($p < 0.0001$). The mean fasting insulin levels (µIU/ml) of cases was 10.8 ± 1.2 (0.27 to 55.26). Mean HOMA

Table 4 Association of elevated leptin levels with various demographic and biochemical characteristics of pre-diabetics included in the study

Characteristics	Cases $n=212$ (%)	Leptin levels of cases ($n=212$)		OR (95% CI)	p value
		Normal 170 (80.0%)	**Elevated 42 (20.0%)**		
Gender					
Male	60 (28.3)	48 (28.2)	12 (28.6)	Ref	0.95
Female	152 (71.1)	122 (71.8)	30 (71.4)	0.98 (0.46–2.1)	
Age group					
≤ 18 years	26 (12.2)	20 (11.7)	06 (14.2)	Ref	0.7
> 18 years	186 (87.8)	150 (88.3)	36 (85.8)	0.8 (0.3–2.1)	
Dwelling					
Rural	104 (49.1)	88 (51.7)	16 (38.0)	Ref	0.1
Urban	108 (50.9)	82 (48.3)	26 (62.0)	1.73 (0.87–3.5)	
BMI (Kg/m^2)					
Normal	76 (35.8)	58 (34.2)	18 (42.9)	Ref	
Underweight	02 (1.0)	01 (0.6)	01 (2.4)	3.2 (0.2–54.1)	0.8
Preobese	88 (41.5)	72 (42.4)	16 (38.1)	0.7 (0.3–1.5)	0.4
Obese class I	24 (11.3)	18 (10.5)	06 (14.2)	1.07 (0.3–3.1)	0.8
Obese class II	22 (10.4)	21 (12.3)	01 (2.4)	0.2 (0.02–1.4)	0.06
Total cholesterol (mg/dl)					
Normal	156 (73.6)	140 (82.3)	16 (38.0)	Ref	**< 0.001**
Elevated	56 (26.4)	30 (17.7)	26 (62.0)	7.4 (3.5–15.9)	
TG (mg/dl)					
Normal	128 (60.3)	120 (70.5)	08 (19.0)	Ref	**< 0.001**
Elevated	84 (39.7)	50 (29.5)	34 (81.0)	10.08 (4.4–24.7)	
LDL-C (mg/dl)					
Normal	122 (57.5)	102 (60.0)	20 (47.6)	Ref	0.1
Elevated	90 (42.5)	68 (40.0)	22 (52.4)	1.6 (0.8–3.2)	
HDL-C (mg/dl)					
Normal	116 (54.7)	93 (54.7)	23 (54.7)	Ref	0.4
Low	96 (45.3)	77 (45.3)	19 (45.3)	1.0 (0.5–1.9)	
VLDL-C (mg/dl)					
Normal	138 (65.1)	128 (75.2)	10 (23.8)	Ref	**< 0.001**
Elevated	74 (34.9)	42 (24.8)	32 (76.2)	9.6 (4.4–22.1)	
Insulin (µIU/ml)					
Normal	190 (89.6)	154 (90.5)	36 (85.7)	Ref	0.3
Low	22 (10.4)	16 (19.5)	06 (14.3)	1.6 (0.5–4.3)	
HOMA IR					
Insulin sensitive	166 (78.3)	134 (78.8)	32 (76.2)	Ref	
Low IR	26 (12.2)	18 (10.6)	08 (19.0)	0.5 (0.2–1.3)	0.1
Significant IR	20 (9.5)	18 (10.6)	02 (4.8)	2.1 (0.5–9.7)	0.2

TG, triglycerides; *LDL*, low-density lipoproteins; *HD*L, high-density lipoproteins; V*LDL*, very low-density lipoproteins; *BMI*, body mass index; *HOMA IR*, homeostatic model assessment–insulin resistance

The bold signifies statistically significant p values

IR was found to be 1.47 ± 0.7 (0.03 to 13.6). Levels of various biochemical parameters of pre-diabetic cases and controls in terms of mean ± SD are described in Table 2.

Stratification analysis with respect to leptin levels

Table 4 depicts the association of elevated leptin levels with various demographic and biochemical characteristics of

prediabetic patients included in the study. It was observed that 17.7% (30 of 170) of patients with normal leptin levels were having elevated cholesterol levels as compared to 62.0% (26 of 42) of patients with elevated leptin levels along with elevated total cholesterol levels ($p < 0.001$). Out of 170 patients with normal leptin levels, only 50 (29.5%) patients were found to have elevated TG levels as compared to 34 out of 42 (82.1%) patients with elevated TG level and elevated leptin levels ($p < 0.001$). 24.8% (42 of 170) of patients with normal leptin levels were having elevated VLDL-C (very low density lipoprotein-cholesterol) levels compared to 76.2% (32 of 42) of patients with elevated leptin levels having elevated VLDL-C levels ($p = 0.04$). We did not find any significant association between elevated leptin levels and other demographic and biochemical characteristics of study patients ($p > 0.05$) (Table 4). Association of elevated leptin levels with various demographic and biochemical characteristics of controls (healthy individuals) is contained in Table 5. Significance of elevated leptin levels was not noted with any of the demographic and biochemical parameters of control subjects.

Discussion

Diabetes is one of the most common chronic diseases with 425 million people living with it in 2017 [22] and is the fourth leading cause of death in the developed world [22].

Table 5 Association of elevated leptin levels with various demographic and biochemical characteristics of controls (healthy individuals) included in the study

Characteristics	Controls $n = 196$ (%)	Leptin levels of controls ($n = 196$)		OR (95% CI)	p value
		Normal 192 (100.0%)	**Elevated 04 (00.0%)**		
Gender					
Male	51 (26.0)	51 (26.5)	00 (00.0)	Ref	0.7
Female	145 (74.0)	141 (73.5)	04 (100.0)	1.8 (0.2–16.0)	
Age group					
≤ 18 years	25 (12.8)	25 (13.0)	00 (00.0)	Ref	0.9
> 18 years	171 (87.2)	167 (87.0)	04 (100.0)	0.7 (0.08–6.8)	
Dwelling					
Rural	96 (49.0)	95 (49.4)	01 (25.0)	Ref	0.6
Urban	100 (51.0)	97 (50.6)	03 (75.0)	2.9 (0.3–28.7)	
BMI (Kg/m^2)					
Normal	196 (100.0)	192 (100.0)	04 (100.0)	Ref	
Underweight	00 (00.0)	00 (100.0)	00 (100.0)	38.6 (2.1–70.8)	0.06
Preobese	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	0.06
Obese class I	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	0.06
Obese class II	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	0.06
Total cholesterol (mg/dl)					
Normal	196 (100)	192 (100.0)	04 (100.0)	Ref	0.06
Elevated	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	
TG (mg/dl)					
Normal	196 (100.0)	192 (100.0)	04 (100.0)	Ref	0.06
Elevated	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	
LDL-C (mg/dl)					
Normal	196 (100.0)	192 (100.0)	04 (100.0)	Ref	0.06
Elevated	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	
HDL-C (mg/dl)					
Normal	196 (100.0)	192 (100.0)	04 (100.0)	Ref	0.06
Low	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	
VLDL-C (mg/dl)					
Normal	196 (100.0)	192 (100.0)	04 (100.0)	Ref	0.06
Elevated	00 (00.0)	00 (00.0)	00 (00.0)	38.6 (2.1–70.8)	

TG, triglycerides; *LDL*, low-density lipoproteins; *HDL*, high-density lipoproteins; *VLDL*, very low-density lipoproteins; *BMI*, body mass index; HOMA IR, homeostatic model assessment–insulin resistance

Pathological dysregulations eventually leading to diabetes are in most of the individuals preceded by prediabetes, which being an early phase and opportunity for the adoption of preventive interventions [23]. Prediabetes will progress to overt type 2 diabetes (T2DM) in approximately 25% of subjects within 3–5 years, and as many as 70% of individuals with prediabetes will develop overt diabetes within their lifetime [23]. In the USA, an estimated 88 million adults aged 18 years or older had prediabetes in 2018 [24]. People with prediabetes are also at an increased risk of developing cardiovascular diseases (CVD) [23].

Largely formed by white adipose cells, the adipokine-leptin has a multiplicity of central and peripheral actions that are mainly associated with the regulation of energy balance food intake and metabolism [25, 26]. From the time when the leptin was discovered in 1994 to its latest approval as a licenced medicine in the USA and Japan, much remains to be done in order to explain the role of this adipose-derived hormone in biotic functions and to optimise its use for the benefit of human health. For this purpose, studying different sections of the population is imperative in addressing the factors affecting the levels of leptin in various ethnic populations.

To the best of our understanding, globally, this is one of the few case–control studies to elucidate the relationship of leptin levels with various demographic and biochemical parameters including insulin resistance of prediabetic cases and healthy controls [27–30]. In addition, this is probably the first study of this kind from Kashmir valley (North India). Leptin levels were elevated in cases as compared to controls as per our observation, and we found a significant association between elevated leptin levels and increased risk of prediabetes in both men and women ($p \leq 0.05$) (Tables 1 and 2). Although not many studies have been undertaken to understand the relationship between leptin levels and prediabetes, our finding is consistent with previous studies done so far [28–30]. It is also consistent with some longitudinal studies on the effects of increased leptin concentrations on diabetes in Caucasian and Japanese American populations [31, 32]. Leptin may play a role in the pathophysiology of diabetes, possibly by suppressing insulin secretion [33]. Although some studies have reported no association between plasma leptin levels and diabetes [34], the inverse relation has also been reported by two studies [35–37].

On stratification with respect to demographic factors like gender, age and dwelling, interestingly, the leptin levels were not significantly associated with gender ($p > 0.05$). Although, there is considerable variability in serum leptin concentrations among individuals, the gender difference and pattern of sexual dimorphism in serum leptin levels is well established with circulating leptin levels systematically higher in females than in males [38, 39]. In vitro studies showed that both estradiol and dexamethasone increased leptin release in incubations of adipose tissue samples obtained from female, but not male, donors [40]. Previous studies have described the negative correlation of testosterone with serum leptin levels independent of body fatness [41]. We did not find any significant association of age and dwelling with leptin levels of prediabetic cases ($p > 0.05$). Based on previous studies, which equally reported that leptin is reduced [42, 43], unchanged [44, 45] or even increased [46] during ageing, the relationship between age and leptin is not yet clear. It is well established that ageing affects body composition, such as reduced muscle strength and increased fat depots, which might be related at least in part to changes in serum leptin levels and/or its pattern of secretion [47]. No previous study has reported the correlation between leptin levels and dwelling per se.

In our study, elevated leptin levels were significantly associated with elevated cholesterol, TG and VLDL levels ($p \leq 0.05$). It is well-established that leptin affects lipid metabolism, but whether these effects are a result of direct leptin action on the liver has not been fully addressed. Hepatic leptin action can modulate the amount of triglycerides available for incorporation into each VLDL particle through the effects of leptin on increasing fatty acid oxidation [48]. Previously, leptin levels were found to be significantly and positively correlated with serum lipoproteins [49]. Both leptin and adiponectin levels have been shown to affect atherosclerotic changes in T2DM [50]. Furthermore, there is an excess triglyceride accumulation in adipose tissue, muscle pancreas and liver due to attenuation of leptin sensitivity in the brain resulting in impaired insulin sensitivity and secretion [51]. In one of the previous studies, the leptin-prediabetes associations were independent of lipid levels [30].

Higher levels of leptins (hyperleptinemia) are positively correlated with BMI and other measures of obesity and this state is commonly referred to as leptin resistance [52–54]. But interestingly, on evaluation of BMI in our study, most of the patients were falling in pre-obese category (41.5%) but no significant association of elevated leptin levels was associated with obesity (BMI) thus emphasising the fact that leptin-prediabetes associations cannot be totally explained by comprehensive measures of adiposity.

Although in our study, we did not find any correlation between insulin levels and leptins but a positive correlation of leptin levels with fasting insulin levels in humans have been found previously [55, 56]. Leptin production is further stimulated due to decreased inhibition of insulin secretion resulting in hyperinsulinemia. In line with our study, the studies conducted by Mancini et al. and Erturk et al. reported no significant correlation between leptin and HOMA IR [57, 58]. In contradiction to our study, most studies found a significant association between HOMA IR and leptin in their populations [59–61]. However, some studies reported inconsistent results [62, 63].

Towards positive side, our study included prediabetic patients instead of diabetics which minimised several sources of confusing variables, such as unmeasured metabolic alterations, drug treatment effect or other subclinical pathology as a result of diabetes that might make secretion and effects of leptin ambiguous. Although this is among the largest study of this kind in Kashmiri population, the sample size of our study is modest, and large sample size would be needed to further consolidate the results.

Conclusion

Our findings strengthen the concept that there is an obvious significant correlation between increased mean leptin levels, increased mean lipid levels and mean BMI with risk of prediabetes when compared to controls suggesting that leptin could be an additional biomarker for identifying individuals at high risk of prediabetes among Kashmiri population. In prediabetics, increased leptin levels are significantly associated with dyslipidemia but not with other markers of obesity such as BMI suggesting that leptin levels influence adiposity to a significant extent in prediabetics. Continuous follow-up of these subjects will help to estimate the usefulness of leptin as a predictor of prediabetes and progress to type 2 diabetes.

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Author contribution RA has designed the study, drafted the manuscript and approved the final version of manuscript for publication; MSK has done acquisition and analysis/interpretation of data, drafted the manuscript and approved the final version of manuscript for publication; MHB has done acquisition of data, revised the manuscript and approved the final version of manuscript for publication; IS has done data analysis, revised the manuscript and approved the final version of manuscript for publication; SR and SM have conceptualised the study, revised it critically, recruited the patients and provided the overall logistics and lab facilities.

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Data availability The raw and supplementary data is available and could be produced after institutional clearance.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The study was approved by the Ethical Clearance Committee of Government Medical College and Associated Hospitals (Ref. No. 140/ETH/GMC/ICM, dated: 22–05-2019) and in compliance with the World Medical Association's Declaration of Helsinki. Information as per the self-designed questionnaire was collected from study subjects after getting a pre-informed consent from them to participate in the study.

Consent for publication All contributing authors have given their consent for publication.

Competing interests The authors declare no competing interests.

References

1. Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med. 2010;152:93–100.
2. Berglund ED, Vianna CR, Donato J, Kim MH, Chuang JC, et al. Direct leptin action on POMC neurons regulates glucose homeostasis and hepatic insulin sensitivity in mice. J Clin Invest. 2012;122:1000–10009.
3. Qulsum A, Akbar M, Ruhi A, Majid S, Sabah R, et al. Polymorphisms in the 3'UTR of the human leptin gene and their role in hypertension. Mol Med Rep. 2012;5:1058–62.
4. Friedman JM. A tale of two hormones. Nat Med. 2010;16:1100–6.
5. Bates SH, Myers MJ. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol Metab. 2003;14:447–52.
6. Harvey J, Ashford ML. Leptin in the CNS: much more than a satiety signal. Neuropharmacology. 2003;44:845–54.
7. David L. Nelson, Michael M. Cox. Lehninger principles of biochemistry 6th edition.
8. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva: World Health Organization 2006; 1-50
9. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37:S81–90.
10. Brown RJ, Valencia A, Startzell M, Cochran E, Walter PJ, Garraffo HM, et al. Metreleptin-mediated improvements in insulin sensitivity are independent of food intake in humans with lipodystrophy. J Clin Investig. 2018;128:3504–16.
11. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet induced obesity. J Clin Invest. 2000;105:1827–32.
12. Chen SC, Kochan JP, Campfield LA, Burn P, Smeyne RJ. Splice variants of the OB receptor gene are differentially expressed in brain and peripheral tissues of mice. J Recept Signal Transduct Res. 1999;19:245–66.
13. Maahs DM, Hamman RF, D'Agostino R, Dolan LM, Imperatore G, et al. The association between adiponectin/leptin ratio and diabetes type: the SEARCH for Diabetes in Youth Study. J Pediatr. 2009;155:133–5.
14. Soderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, et al. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. Int J Obes (Lond). 2007;31:1126–33.
15. Anna MD, Ursula HN, Maria MG, Timothy JK. The glucoregulatory actions of leptin. Mol Metab. 2017;6:1052–65.
16. Schmidt MI, Duncan BB, Vigo A, Pnakow JS, Couper D, et al. Leptin and incident type 2 diabetes: risk or protection? Diabetologia. 2006;49:2086–96.

17. Roohi A, Tehseen H, Samia R. To study the prevalence of metabolic syndrome in the adult population of Kashmir. Int J Sci Res. 2018;7:60–3.
18. Jaakko N, Ville-Petteri M, Jukka H, Leena T, Yrjö H, et al. Estimation of VLDL, IDL, LDL, HDL2, apoA-I, and apoB From the Friedewald inputs–apoB and IDL, but not LDL, are associated with mortality in type 1 diabetes. Ann Med. 2009;41:451–61.
19. Melmed S, Polonsky KS, Larsen PR, Kronenberg HM. Williams Textbook of Endocrinology. 13th ed. Saunders Philadelphia: Elsevier; 2016.
20. Michael V, Daniel K, Ingrid F, Hans-Georg P, Klaus GP, et al. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. Clin Biochem. 2007;40:964–8.
21. Weisner G, Mario V, Greg C, Douglas S, David K, et al. Leptin is released from the human brain: influence of adiposity and gender. J Clin Endocrinol Metab. 1999;84:2270–4.
22. Pouya S, Inga P, Paraskevi S, Belma M, Suvi K, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
23. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet. 2012;379:2279–790.
24. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2020. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2020.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia. 1985;28:412–9.
26. Margetic S, Gazzola C, Pegg GG, mHill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord. 2002;26:1407–33.
27. Bungau S, Behl T, Tit DM, Banica F, Bratu OG, Diaconu CC, et al. Interactions between leptin and insulin resistance in patients with prediabetes, with and without NAFLD. Exp Ther Med. 2020;20:197.
28. Yunna J, Ibiye O, Jim W, Sotonte E, Samuel D. Adiponectin levels predict prediabetes risk: the pathobiology of prediabetes in A Biracial Cohort (POP-ABC) study. BMJ Open Diabetes Res Care. 2016;4:1–8.
29. Hossain IA, Akter S1, Rahman MK, Ali L. Specific association of serum leptin and insulinemic indices with non-alcoholic fatty liver disease in prediabetic subjects. PLOSOne 2005; 10: e0142165
30. Guoying W, Xin L, Katherine KC, Shanchun Z, Binyan W, et al. Prediabetes is not all about obesity: association between plasma leptin and prediabetes in lean rural Chinese adults. Eur J Endocrinol. 2010;163:243–9.
31. Welsh P, Murray HM, Buckley BM, de Craen AJ, Ford I, et al. Leptin predicts diabetes but not cardiovascular disease: results from a large prospective study in an elderly population. Diabetes Care. 2009;32:308–10.
32. McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, et al. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. Diabetes Care. 1999;22:65–70.
33. Fischer SM, Hanefeld SM, Haffner C, Fusch U, Schwanebeck C, et al. Insulin- resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. Acta Diabetol. 2002;39:105–10.
34. Sun Q, Van Dam RM, Meigs JB, Franco OH, Mantzoros CS, et al. Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in U.S. women: a prospective study. Diabetes. 2010;59:611–8.
35. Tatti L, Masselli A, Buonanno P, Mauro Di, Strollo F. Leptin levels in diabetic and nondiabetic subjects. Endocrine. 2001;15:305–8.
36. Abdelgadir M, Elbagir M, Eltom M, Berne C, Ahren B. Reduced leptin concentrations in subjects with type 2 diabetes mellitus in Sudan. Metabolism. 2002;51:304–6.
37. Matsuda M, Liu Y, Mahankali S, Pu Y, Mahankali A, et al. Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes. 1999;48:1801–6.
38. Mazusaki H, Ogawa Y, Isse N, et al. Human obese gene expression: adipocyte specific expression and regional differences in the adipose tissue. Diabetes. 1995;44(588–858):40.
39. Montague CT, Prins JB, Sanders L, et al. Depot-related gene expression in human subcutaneous and omental adipocytes. Diabetes. 1998;47:1384–91.
40. Casabiell X, Pineiro V, Peino R, et al. Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. J Clin Endocrinol Metab. 1998;83:2149–55.
41. Vettor R, Depergola G, Pagano C, et al. Gender differences in serum leptin in obese people. Relationships with testosterone, body fat distribution and insulin sensitivity. Eur J Clin Invest. 1997;27:1016–24.
42. Rosembaum M, Nicolson M, Hirsch J, Heymsfield SB, Dympna G, Chu F, Leibel RL. Effects of gender, body composition, and menopause on plasma concentrations of leptin. J Clin Endocrinol Metab. 1996;81:3424–7.
43. Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab. 1996;81:3909–13.
44. Perry HM, Morley JE, Horowitz M, et al. Body composition and age in African-American and Caucasian women: relationship to plasma leptin levels. Metabolism. 1997;46:1178–84.
45. Ahren B, Pacini G. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. J Clin Endocrinol Metab. 1998;83:3350–6.
46. Baumgartner RN, Walters DL, Morley JE, Patrick P, Montoya GP, Gary P. Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. Metabolism. 1999;48:378–84.
47. Lamberts SWJ, van den Beld AW, van der Lely AJ. The endocrinology of aging. Science. 1997;278:419–24.
48. Huang W, Metlakunta A, Dedousis N, Ortmeyer HK, Stefanovic-Racic M, et al. Leptin augments the acute suppressive effects of insulin on hepatic very low-density lipoprotein production in rats. Endocrinology. 2009;150:2169–74.
49. Fethi BS, Wala GH, Faika BM, Mohamed Chiheb BR, Omrane B, et al. Correlations of oxidized low density lipoprotein with insulin, leptin and risk of cardiovascular disease in a group of diabetic obese Tunisian women. J Obes Weight Loss Ther. 2015;5:1–9.
50. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, et al. Correlation of the leptin: adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia. 2009;52:2345–9.
51. Unger RH. Lipotropic diseases. Ann Rev Med. 2002;53:319–36.
52. Brzechffa PR, Jakımıuk AJ, Agarwal SK, Weitsman SR. Serum immunoreactive leptin concentrations in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1996;84:4166–9.
53. Mendonca HC, Montenegro RM, Foss MC, Silva De Sa MF, Ferriani RA. Positive correlation of serum leptin with estradiol levels in patients with polycystic ovary syndrome. Braz J Med Biol Res. 2004;37:729–36.

54. Fritz MA, Speroff L. Clinical gynaecology endocrinology and infertility. 8th ed. New Delhi: Wolters Klumer, Lippikott Williams, Wilkins Publisher; 2011
55. Schwartz MW, Prigeon RL, Kahn SE, Nicolson M, Moore J, et al. Evidence that plasma leptin and insulin levels are associated with body adiposity via different mechanisms. Diabetes Care. 1997;20:1476–81.
56. Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. Am J Physiol Endocrinol Metab. 2000;278:E1–14.
57. Mancini F, Cianciosi A, Marchesini RG, Facchinetti F, Cesare B, et al. Endothelial function and its relationship to leptin, homocysteine, and insulin resistance in lean and overweight eumenorrheic women and polycystic patients. Fertil Steril. 2009;91:2537–44.
58. Erturk E, Kuru N, SavciTuncel E, Ersoy C, Imamoglu S. Serum Leptin levels correlate with obesity parameters but not with hyperinsulinism in women with polcystic ovary syndrome. Fertil Steril. 2004;82:1364–8.
59. Mente A, Razak F, Blankenberg S, Vuksan V, Davis AD, et al. Ethnic variation in adiponectin and leptin levels and their association with adiposity and insulin resistance. Diabetes Care. 2010;33:1629–34.
60. Esteghamati A, Khalilzadeh O, Anvari M, Rashidi A, Mokhtari M, et al. Association of serum leptin levels with homeostasis model assessment–estimated insulin resistance and metabolic syndrome: the key role of central obesity. Metab Syndr Relat Disord. 2009;7:447–52.
61. Huang KC, Lin RCY, Kormas N, Lee LT, Chen CY, et al. Plasma leptin is associated with insulin resistance independent of age, body mass index, fat mass, lipids, and pubertal development in nondiabetic adolescents. Int J Obes. 2004;28:470–5.
62. Nakhjavani M, Esteghamati A, Tarafdari AM, Nikzamir A, Ashraf H, et al. Association of plasma leptin levels and insulin resistance in diabetic women: a cross-sectional analysis in an Iranian population with different results in men and women. Gynecol Endocrinol. 2011;27:14–9.
63. Panahloo A, Mohamed AV, Pinkney J, Goodrick S, Coppack S, et al. Relationships between plasma leptin and insulin concentrations, but not insulin resistance, in non-insulin dependent diabetes mellitus. Diabetes. 1997;46:1340–1340.

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ORIGINAL ARTICLE

Association between diabetes and acute lymphocytic leukemia, acute myeloid leukemia, non-Hopkin lymphoma, and multiple myeloma

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Abstract

Objective Diabetes increases the risk for cancers. However, whether it is associated with hematologic malignancies is not clear. The present study investigated the association between diabetes and acute lymphocytic leukemia (ALL), acute myeloid leukemia (ML), non-Hopkin lymphoma (NHL), and multiple myeloma (MM).

Methods Newly diagnosed adult cancer patients were recruited consecutively from our clinical database. Peoples from a local enterprise were recruited to create a small-scale population-based dataset. We compared the diabetes prevalence between the cancer patients and the local people; an increase in diabetes prevalence in the cancer patients suggests an association between diabetes and the cancer(s).

Results We found that the prevalence of diabetes was 19.7%, 21.3%, 12.5%, and 12.0% in ALL, AML, NHL, and MM, respectively, which was higher than that (9.1%) in the local people. Despite that there were more male than female cancer patients, there were more female than male diabetic patients. The increase in diabetes prevalence occurred in ALL and NHL patients aged 18 to 39 years old as well as in AML patients over 40. In MM patients, the increase in diabetes prevalence (18.6%) occurred only in females. Approximately 70% of the diabetic patients were undiagnosed before the diagnosis of the blood cancer. Approximately half of the pre-existing diabetic patients had anti-diabetic treatment, with over 70% of them still had poor glycemic control.

Conclusions Our results suggest that diabetes is associated with ALL, AML, NHL, and MM, at least in adult patients.

Keywords Diabetes · Acute lymphocytic leukemia · Acute myeloid leukemia · Non-Hopkin lymphoma · Multiple myeloma · Association

Introduction

Both diabetes and cancer are common non-communicable diseases with increasing prevalence. According to the Global Report on Diabetes from the World Health Organization, the prevalence of diabetes has increased from 4.7% in 1980 to 8.5% in 2014, with 422 million adults diagnosed with the disease. It is estimated that 451 million adults had diabetes in 2017, with almost half of all people living with undiagnosed diabetes [1]. Cancer is the second leading cause of death world-wide. According the statistics from the World Health Organization, cancer caused approximately 9.6 million deaths, or one in six deaths, in 2018 [2]. Diabetes and cancer cast enormous socioeconomic burden to societies. More 60 years ago, concurrent diabetes and cancer in patients had already been noted in clinical setting. However, it remained inconclusive of whether the two diseases are associated until recent decades when diabetes and cancer are highly

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prevalent. In the past decades, epidemiological studies showed that diabetes was associated with the occurrence of various cancers such as those in the liver, pancreas, stomach, colorectum, kidney, and breast. It is postulated that the pre-diabetic and diabetic states increase the risk for cancer. However, those presented results were not clear and sometimes conflicting [3]. In relevance to hematologic cancers, more recently, Castillo and co-workers performed a meta-analysis based on 13 case–control and 13 cohort studies, and found that diabetes was associated with NHL ($p < 0.01$), leukemia ($p = 0.02$), and possibly myeloma ($p = 0.08$) [4]. Yan et al. reported that type 2 rather than type 1 diabetes is associated with leukemia [5]. In a large insurance database in Taiwan, diabetes was found to be associated with NHL in both male and female groups [6]. Analysis on databases from Shanghai also revealed that patients with diabetes had an increased risk for NHL [7]. Wang et al. performed a meta-analysis on 35 cohort studies, and found a moderate increase in risk of NHL in types 1 and 2 diabetes, which was more significant in Asians [8]. Data from another meta-analysis supported the view that diabetes increases the risk for NHL, and further pointed out that male diabetic patients were more likely to develop NHL than females [9]. However, there are studies which showed that diabetes does not increase the overall risk for blood cancer, even in large database [10]. Diabetes might not only modify the cancer risk but also the cancer course. Indeed, diabetes and pre-diabetic state are associated with poorer prognosis of chronic lymphocytic leukemia [11], NHL [12], and myeloma [13]. On the other hand, anti-diabetic treatment, in particular by metformin, not only reduces the risk for lymphoma [14] but also improves the prognosis of lymphoma [15] and myeloma [16]. Although it is possible that metformin reduces cancer risk in diabetes by improving the metabolic profile, it has been shown to directly act on cancer cells. The compound inhibits in vitro cancer cell proliferation, migration, and invasion as well as induces apoptosis and autophagy [17], which inhibits the tumorigenesis and tumor progression [18]. In relevance to leukemia, some believed that metformin could be used to treat leukemia [19]. However, there was also evidence that metformin had neutral effect on leukemia, and yet, there was possibility that long-term use of the drug might increase the cancer risk [20].

The published meta-analyses were performed on limited number of original studies completed in different time and geographic location. The heterogeneity may affect the analysis outcome, as differences in genetic composition and life style may produce biases [4]. Anti-diabetic drugs, in particular metformin, are found to modify cancer risk, thus, including study before and after the common use of metformin in meta-analysis may produce bias. Moreover, hematologic cancers are heterogeneous and inclusion of acute lymphocytic or myeloid leukemia in analysis, rather only leukemia, might provide more informative insights. Thus, more original studies are required for a better view on the relationship between diabetes and hematologic malignancies. The present study examined the prevalence of diabetes in newly diagnosed adult patients with acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), NHL, or multiple myeloma (MM) to assess the association between diabetes and the blood malignancies. The subjects were recruited from our region and the influence of the use of metformin was minimized, which was believed to avoid the biases which might be produced by the current meta-analyses.

Research design and methods

Approval for the study was obtained from the Clinical Ethics Committee of Linyi People's Hospital which is the major regional hospital with a catchment population of 1.29 million with typical demographic and social class distributions. Most of the inhabitants are eastern Chinese in origin. Because of the lack of a long-term health care financing policy, the majority of people in this region do not have medical insurance for chronic diseases, such as cancer and diabetes. As a result, most of these patients seek medical care at the public hospitals where only nominal fees are charged. The cancer patients aged from 18 years or above, who were hospitalized between 2015 and 2020, were consecutively recruited. We recruited 1050 local people to create a small-scale population-based survey dataset in 2019.

AML, ALL, and NHL were diagnosed and classified according to the 2016 WHO criteria [21, 22]. MM was diagnosed and classified according to the criteria by the 2014 International Myeloma Working Group [23]. Fasting blood samples were taken for biochemical assays. For the cancer patients, blood samples were taken on the diagnosis of cancer before any clinical treatment. Diabetes was evidenced when fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L) [24]. Those with diabetes history were also included in the diabetes group, even though they had achieved satisfactory glycemic control. In the study, the patients with AML, ALL, NHL, and MM were 622, 416, 789, and 392, respectively. The subjects randomly recruited outside the hospital for the estimation of diabetes prevalence were 1052. Soon after admission of the patients, fasting blood samples were taken to the biochemical analyses. For the subjects recruited from outside the hospital, who were involved in the survey for the estimation of diabetes prevalence, fasting blood samples were taken for the determination of the metabolic profile.

Fasting blood glucose levels were measured using a hexokinase method. Plasma levels of total cholesterol and triglyceride were assayed enzymatically using commercially available reagents (Beckmancoulter; Beckman Instrument, USA). HDL-cholesterol was detected using the selective

Table 1 Demographic data of the adult patients with ALL, AML, NHL, or MM

Cancer type	Clinical parameter	Adult patients with ALL, AML, NHL, or MM				
		Overall ($n=2219$)	Men ($n=1266$)	Women ($n=911$)	Non-diabetic	Diabetic
AML	Age (95% CI)	51.71 (50.48 to 52.93)	52.43 (50.75 to 54.12)	50.89 (49.11 to 52.66)	50.39 (48.98 to 51.81)	57.23 (55.00 to 59.47)***
	Fasting plasma glucose (mmol/L) (95% CI)	6.33 (6.15 to 6.51)	6.26 (6.01 to 6.51)	6.40 (6.14 to 6.67)	5.52 (5.45 to 5.58)	9.63 (9.04 to 10.22)***
	Total cholesterol (mmol/L) (95% CI)	3.71 (3.60 to 3.82)	3.57 (3.43 to 3.72)	3.88 (3.71 to 4.05)*	3.73 (3.60 to 3.85)	3.63 (3.37 to 3.89)
	Low-density lipoprotein (mmol/L) (95% CI)	2.31 (2.23 to 2.40)	2.23 (2.12 to 2.34)	2.42 (2.29 to 2.55)*	2.32 (2.23 to 2.42)	2.26 (2.06 to 2.46)
	High-density lipoprotein (mmol/L) (95% CI)	0.89 (0.86 to 0.92)	0.86 (0.83 to 0.90)	0.93 (0.87 to 0.97)*	0.91 (0.86 to 0.94)	0.83 (0.78 to 0.88)
	Triglycerides (mmol/L) (95% CI)	1.51 (1.41 to 1.61)	1.40 (1.29 to 1.52)	1.65 (1.48 to 1.82)*	1.50 (1.39 to 1.62)	1.57 (1.38 to 1.76)
ALL	Age (95% CI)	41.98 (38.99 to 44.97)	43.29 (38.96 to 47.62)	40.38 (36.22 to 44.54)	41.00 (37.82 to 44.18)	47.57 (38.19 to 56.95)
	Fasting plasma glucose (mmol/L) (95% CI)	5.70 (5.34 to 6.01)	5.82 (5.29 to 6.35)	5.56 (5.06 to 6.06)	5.11 (4.94 to 5.27)	9.28 (8.04 to 10.52)***
	Total cholesterol (mmol/L) (95% CI)	4.08 (3.77 to 4.39)	4.14 (3.72 to 4.56)	4.02 (3.54 to 4.50)	4.08 (3.73 to 4.44)	4.13 (3.28 to 4.98)
	Low-density lipoprotein (mmol/L) (95% CI)	2.60 (2.36 to 2.84)	2.67 (2.38 to 2.96)	2.54 (2.16 to 2.92)	2.59 (2.32 to 2.86)	2.74 (2.11 to 3.37)
	High-density lipoprotein (mmol/L) (95% CI)	0.88 (0.80 to 0.96)	0.84 (0.72 to 0.96)	0.91 (0.81 to 1.02)	0.90 (0.81 to 0.99)	0.81 (0.59 to 1.03)
	Triglycerides (mmol/L) (95% CI)	1.92 (1.40 to 2.43)	1.66 (1.5 to 1.8)	1.74 (1.53 to 1.93)	1.66 (1.52 to 1.80)	1.83 (1.52 to 2.14)
NHL	Age (95% CI)	57.63 (56.22 to 59.03)	57.56 (55.78 to 59.35)	57.73 (55.43 to 60.04)	57.43 (55.88 to 58.98)	58.84 (55.58 to 62.1)
	Fasting plasma glucose (mmol/L) (95% CI)	5.71 (5.53 to 5.88)	5.64 (5.43 to 5.84)	5.83 (5.51 to 6.16)	5.15 (5.08 to 5.23)	9.13 (8.46 to 9.80)***
	Total cholesterol (mmol/L) (95% CI)	4.16 (4.00 to 4.34)	3.99 (3.78 to 4.20)	4.50 (4.25 to 4.75)*	4.21 (4.04 to 4.39)	3.74 (3.25 to 4.23)
	Low-density lipoprotein (mmol/L) (95% CI)	2.69 (2.58 to 2.80)	2.62 (2.48 to 2.76)	2.82 (2.64 to 3.00)	2.71 (2.60 to 2.83)	2.49 (2.17 to 2.81)
	High-density lipoprotein (mmol/L) (95% CI)	1.11 (1.04 to 1.18)	1.11 (1.01 to 1.21)	1.10 (1.01 to 1.20)	1.14 (1.06 to 1.22)	0.83 (0.73 to 0.93)*
	Triglycerides (mmol/L) (95% CI)	1.40 (1.28 to 1.52)	1.44 (1.27 to 1.60)	1.33 (1.17 to 1.49)	1.41 (1.28 to 1.54)	1.30 (1.00 to 1.61)

Table 1 (continued)

Cancer type	Clinical parameter	Adult patients with ALL, AML, NHL, or MM				
		Overall ($n=2219$)	Men ($n=1266$)	Women ($n=911$)	Non-diabetic	Diabetic
MM	Age (95% CI)	61.69 (60.73 to 62.65)	61.24 (59.91 to 62.57)	62.34 (60.97 to 63.7)	61.75 (60.7 to 62.8)	61.28 (58.92 to 63.63)
	Fasting plasma glucose (mmol/L) (95% CI)	5.77 (5.57 to 5.97)	5.79 (5.50 to 6.07)	5.74 (5.47 to 6.01)	5.25 (5.18 to 5.33)	9.54 (8.46 to 10.63)***
	Total cholesterol (mmol/L) (95% CI)	3.82 (3.63 to 4.01)	3.62 (3.41 to 3.84)	4.12 (3.76 to 4.45)*	3.84 (3.63 to 4.04)	3.71 (3.20 to 4.21)
	Low-density lipoprotein (mmol/L) (95% CI)	2.34 (2.19 to 2.49)	2.20 (2.03 to 2.36)	2.55 (2.28 to 2.81)*	2.35 (2.19 to 2.51)	2.24 (1.88 to 2.61)
	High-density lipoprotein (mmol/L) (95% CI)	0.96 (0.92 to 1.01)	0.94 (0.88 to 1.00)	0.99 (0.93 to 1.06)	0.97 (0.92 to 1.01)	0.90 (0.74 to 1.06)
	Triglycerides (mmol/L) (95% CI)	1.40 (1.28 to 1.51)	1.32 (1.16 to 1.47)	1.51 (1.33 to 1.69)	1.35 (1.24 to 1.47)	1.74 (1.20 to 2.27)*

Date are expressed in mean (95% confidence interval)

*$p<0.05$; ***$p<0.001$, compared between males and females or diabetic and non-diabetic patients

inhibition method. LDL-cholesterol was measured using a surfactant method. All biochemical variables were measured using an autoanalyzer (Beckmancoulter AU5821; Beckman Instrument, USA) at the biochemical laboratory of the Linyi People's Hospital. Fasting blood samples were centrifuged (3000 rpm, 10 min) to obtain serum fraction. Exactly 1.2-µl serum was loaded onto the automatic analyzing machine each time, separately, for the quantification of glucose, HDL-C, LDL-C, and total cholesterol, using the primary and secondary wavelengths at 340 nm and 660 nm, 600 nm and 700 nm, and 540 nm and 600 nm, respectively. Exactly 1.6-µl serum was loaded to determine the level of triglyceride at the primary and secondary wavelengths at 660 and 800 respectively.

All data for continuous variables are expressed as mean (95% confidence interval) and were assessed using Student's *t*-test. χ^2 test was performed for analyzing proportions. The statistical analyses were performed using the Statistical Package for Social Sciences version 26 (SPSS, Chicago). A p value <0.05 was considered to be statistically significant. We also confirmed all methods were performed in accordance with the relevant guidelines and regulations.

Results

Table 1 shows the clinical data of the patients with ALL, AML, NHL, or MM. The prevalence of diabetes was 19.7%, 21.3%, 12.5%, and 12.0% in ALL, AML, NHL, and MM patients, respectively (Table 2; Fig. 1), which was higher than that (9.1%) in our local people and that (8.7% and 9.7%) in two recent national surveys (Table 3; Fig. 2). As shown in Table 2 and Fig. 1, there were more male than female patients in all the four cancer groups, with the male to female ratio of 1.24:1, 1.13:1, 1.74:1, and 1.43:1 in the ALL, AML, NHL, and MM patients, respectively. In contrast, the prevalence of diabetes was higher in the female than male cancer patients, although the difference did not reach a statistically significant level upon our sample size. Moreover, the increase in diabetes prevalence occurred in ALL and NHL patients aged 18 to 39 years old, and in AML patients aged over 40. In MM patients, the increase in diabetes prevalence (18.6%) occurred in female patients, rather than in a specific age group. In the cancer patients, approximately 70% of the diabetes cases were undiagnosed before the diagnosis of the cancer. Approximately half of the pre-existing diabetic patients had anti-diabetic treatment, with over 70% of them still had poor glycemic control.

Discussion

AML is the most common leukemia in adults, with more male than female patients [26], which accounts for around 1.3% of all new cancer cases in the USA in 2015 [27]. The development of ALL shows a bi-model feature, with one peak at children and the other at around 50 years old. In a Chinese population, the incidence of ALL was 0.63 per

Table 2 Prevalence of diabetes in the patients with ALL, AML, NHL, or MM

		ALL				AML				NHL				MM			
		n	Self-reported (%)	FPG≧7 (%)	Self-reported or FPG≧7 (%)	n	Self-reported (%)	FPG≧7 (%)	Self-reported or FPG≧7 (%)	*n*	Self-reported (%)	FPG≧7 (%)	Self-reported or FPG≧7 (%)	*n*	Self-reported (%)	FPG≧7 (%)	Self-reported or FPG≧7 (%)
Total		416	5.3 (3.15 to 7.45)	18.7 (14.95 to 22.45)	19.7 (15.9 to 23.5)***	622	6.4 (4.5 to 8.3)	19.7 (16.6 to 22.8)	21.3 (18.1 to 24.5)***	789	3.4 (2.1 to 4.7)	11.4 (9.2 to 13.6)	12.5 (10.2 to 14.8)**	392	4.3 (2.3 to 6.3)	12.0 (8.8 to 15.2)	14.3 (10.8 to 17.8)**
Men		229	4.4 (1.74 to 7.06)	17.5 (12.58 to 22.42)	18.3 (13.3 to 23.3)**	330	5.2 (2.8 to 7.6)	17.5 (13.4 to 21.6)	19.4 (15.1 to 23.7)***	476	3.8 (2.1 to 5.5)	10.2 (7.5 to 12.9)	11.1 (8.3 to 13.9)	231	2.6 (0.5 to 4.7)	10.0 (6.1 to 13.9)	11.7 (7.6 to 15.8)
Women		184	6.5 (2.94 to 10.06)	20.6 (14.76 to 26.44)	21.7 (15.7 to 27.7)***	292	7.9 (4.8 to 11.0)	21.9 (17.2 to 26.6)	23.6 (18.7 to 28.5)***	274	3.3 (2.1 to 5.4)	12.9 (8.9 to 12.9)	13.9 (9.8 to 18.0)**	161	6.8 (2.9 to 10.7)	14.9 (9.4 to 20.4)	18.6 (12.6 to 24.6)***
Age	18–39	124	1.6 (0.08 to 3.16)	12.9 (7.00 to 18.8)	13.7 (7.7 to 19.8)***	143	—	5.6 (1.8 to 9.4)	5.6 (1.8 to 9.4)	100	3.0 (0.1 to 6.0)	7.0 (2.0 to 12.0)	7.0 (2.0 to 12.0)*	7	—	—	—
	40–59	110	3.6 (0.12 to 7.08)	18.2 (10.99 to 25.41)	19.1 (11.8 to 26.5)*	255	7.5 (4.3 to 10.7)	21.6 (16.5 to 26.7)	23.1 (17.9 to 28.3)***	293	2.0 (0.4 to 3.6)	10.9 (7.3 to 14.5)	11.6 (7.9 to 15.3)	153	3.3 (0.5 to 6.1)	11.8 (6.7 to 16.9)	12.4 (7.2 to 17.6)
	>60	173	9.2 (4.89 to 13.51)	23.7 (17.4 to 30.0)	24.9 (18.5 to 31.3)	224	9.4 (5.6 to 13.2)	26.3 (20.5 to 32.1)	29.5 (23.5 to 35.5)*	357	5 (2.7 to 7.3)	14.3 (10.7 to 17.9)	16.2 (12.4 to 20.0)	232	5.2 (2.3 to 8.1)	12.5 (8.2 to 16.8)	15.9 (11.2 to 20.6)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, compared with the prevalence of diabetes in local people (Table 3)

FPG fasting plasma glucose

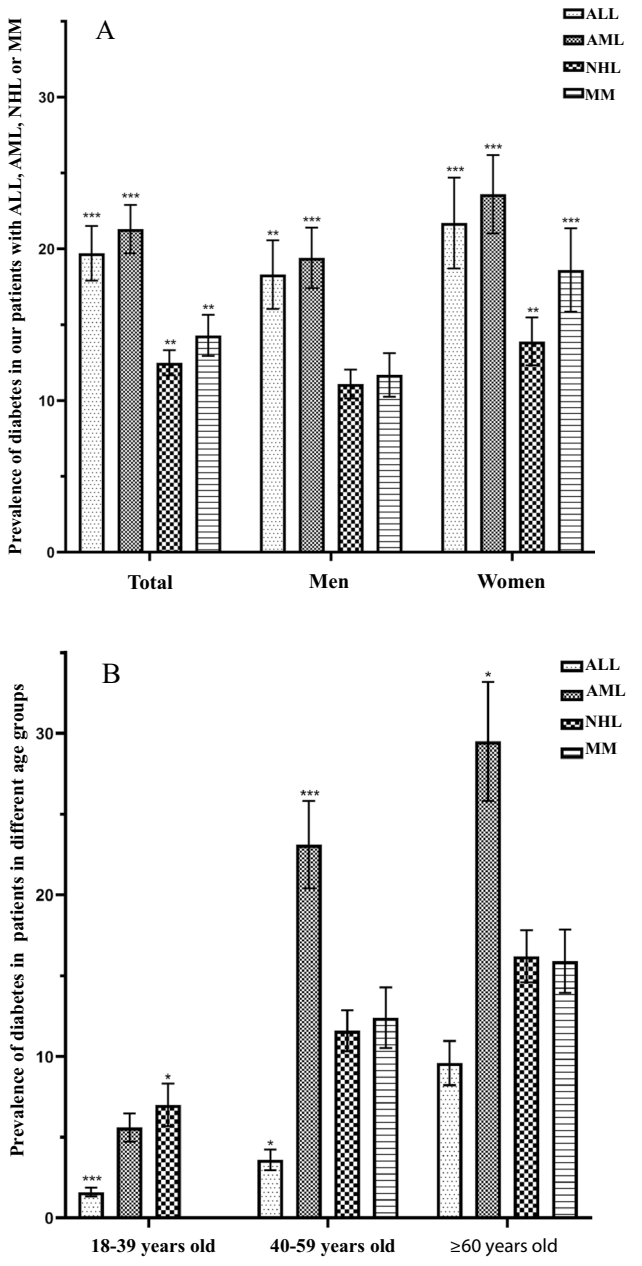

Fig. 1 Prevalence of diabetes in our adult patients with ALL, AML, NHL, or MM. **A** The prevalence of diabetes in all patients as well as male and female patients. **B** The prevalence of diabetes in our patients according to different age groups. $*p<0.05$, $***p<0.001$, compare to the prevalence of diabetes in our survey in our local population and those reported in two national surveys (Fig. 2). The diagnosis criteria of diabetes used in our study and the national surveys are the same

100,000 people, with the male:female ratio of approximately 1.5:1 [28]. Although AML in adults is less common compared with ALL, it represents a devastating clinical condition. The increase in prevalence and improved therapy

Table 3 Prevalence of diabetes in population-based subjects

		Local population-based subjects			Published national surveys	
	n	Self-reported (%)	FPG ≥ 7 mmol/L (%)	Self-reported or FPG ≥ 7 mmol/L (%)	Survey 1 [24]	Survey 2 [25]
Total	1052	2.7 (1.7 to 3.7)	8.0 (6.4 to 9.6)	9.1 (7.4 to 10.8)	8.7	9.7
Men	601	3.5 (2.0 to 5.0)	9.7 (7.3 to 12.1)	11 (8.5 to 13.5)	9.7	10.6
Women	451	1.6 (0.4 to 2.8)	5.8 (3.6 to 8.0)	6.7 (4.4 to 9.0)	7.7	8.8
Age 18–39	447	0.9 (0 to 1.8)	1.6 (0.4 to 2.8)	2.5 (1.1 to 3.9)	2.6	3.2
40–59	415	4.3 (2.3 to 6.3)	9.4 (6.6 to 12.2)	10.4 (7.5 to 13.3)	11.1	11.5
> 60	188	6.9 (3.3 to 10.5)	20.2 (14.5 to 25.9)	20.2 (14.5 to 25.9)	20.4	20.4

Date are expressed in prevalence (95% confidence interval) or prevalence alone

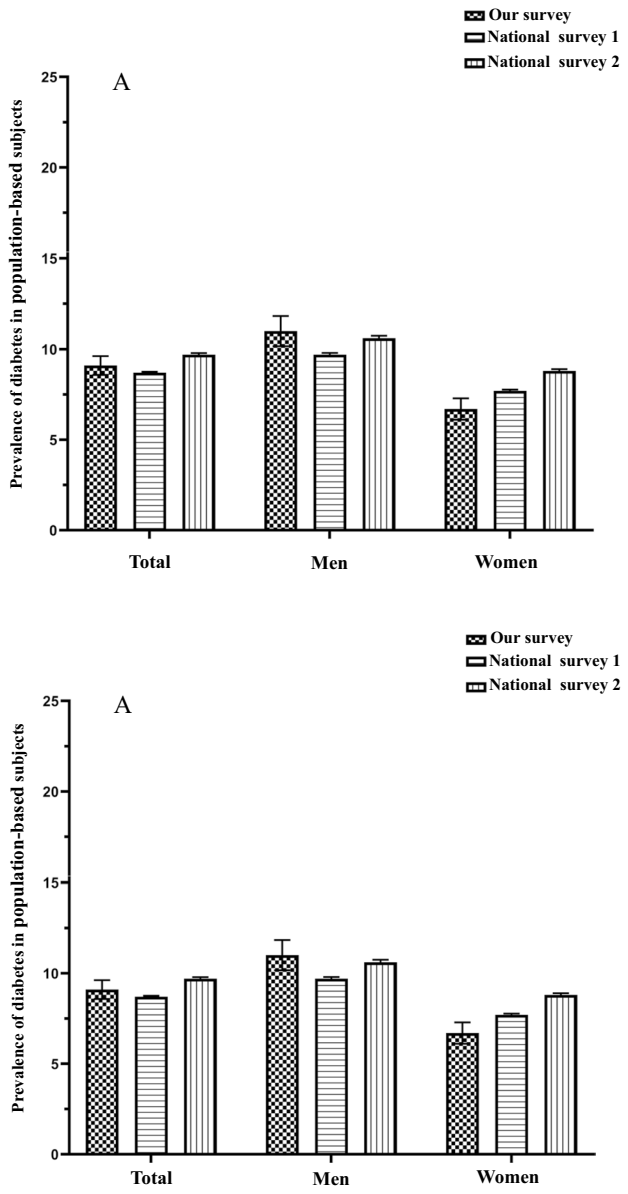

Fig. 2 The prevalence of diabetes in our local population and in national surveys. **A** The prevalence of diabetes in our local population and those reported in the two national surveys. **B** The prevalence of diabetes in our local population and reported in a national survey according to the age groups. There was no significant difference in the prevalence of diabetes between our local population and the population recruited for the national surveys. The numbers of male and female subjects were available from the national survey 1 but not 2; thus, only the data of diabetes prevalence of diabetes in different age groups from the national survey 1 were shown and used in the statistical analysis

outcome has increased the number of patients with NHL. From a population-based database in Taiwan, the incidence of MM is estimated to be 1.83 per 100,000 people, with more male than female patients [29]. Despite that diabetes is now known to increase the risk for various cancers, whether the metabolic disease is associated with these hematologic malignancies still remains inconclusive. Our results showed that diabetes prevalence was increased in all of the patients with ALL, AML, NHL, or MM, suggesting that diabetes is associated with the four hematologic malignancies, in particular ALL and AML. In our study, most diabetes cases were undiagnosed before the diagnosis of the blood cancer, and majority of the patients with diabetes history had poor glycemic control, which indicates that our results are not confounded by anti-diabetic treatment, but may truly reflect the impact of metabolic disorder on the risk for the blood cancers. The people living with undiagnosed diabetes are at serious risk for developing cancer, including the hematologic malignancies.

Similar to the previous observations in the literature, we found that there were more male than female patients with the blood cancers, suggesting an increased susceptibility to the blood cancers in males. In contract, in the ALL, AML, NHL, and MM patients, there were more female than male diabetic patients, although the difference did not reach a statistically significant level upon the sample size. However, the female:male ratio in the diabetic patients was significantly increased compared to that in the non-diabetic cancer patients ($p=0.004$), if patients with ALL, AML, NHL, or MM were combined. In a report on pancreatic cancer, the authors showed that the number of female diabetic patients was significantly higher than that of diabetic males ($p=0.008$) [30]. Thus, the association of diabetes with cancer might have a female preponderance.

Commonly, researchers analyze cancer prevalence in diabetes to assess the impact of diabetes on cancer risk. In the present study, we analyzed the diabetes prevalence in cancer patients to reflect the disease association. Such strategy, although uncommon, did have been employed by investigators [30], which can also produce informative data. There are possible biases in our analysis. Firstly, the sample size is limited, which may not be adequate for revealing the disease association correctly, and may be able for fully documenting the characters of the association (i.e., female preponderance).

Whether and how diabetes promotes cancer development remains unknown. Overweight and lifestyle were both confounding factors, and high glucose and insulin use were speculated to favor tumorigenesis via various mechanisms. TET2, a tumor suppressor which can repress leukemia progression, is a substrate of the AMP-activated kinase (AMPK) which phosphorylates TET2 at serine 99 and thereby stabilizes it for tumor repression. However, in the case of diabetes, increased glucose level impedes AMPK-mediated phosphorylation at serine 99, which results in the destabilization and degradation of TET2, which links diabetes to cancer [31]. In genetically modified experimental models, centrosome amplification is sufficient to cause tumorigenesis.

It would be interesting to investigate whether centrosome amplification is a biological link between diabetes and blood malignancies, since centrosome amplification occurs in cells with centrosome amplification can form tumors in recipient animals and mice spontaneously develop tumors, suggesting that centrosome amplification can cause tumorigenesis. Centrosome amplification occurs in myelodysplastic syndromes [32], leukemia [33], lymphoma [34], and myeloma [35]. Diabetes lowers the level of leukemia inhibitory factor that can induce differentiation and inhibits proliferation of leukemic cells [36, 37], which is also candidate mechanism. Alternatively, the association between diabetes and leukemia might reflect their shared etiological mechanisms rather than a promoting effect of diabetes on tumorigenesis. Let-7 is a family of non-coding microRNAs that include Let-7a to g, Let-7i, and miR-98. In leukemia, several let-7 family members (let-7a to f) were upregulated and associated with a high grate of the cancer [38, 39]. On the other hand, let-7 can act as an essential factor to regulate glucose metabolism [40]. Lin28/let-7 is a self-renewal system [41], in which let-7 is inhabited by Lin28. In muscle cells, both Lin28a knockdown or let-7 over-expression result in insulin resistance and impaired glucose [40]. Thus, Let-7 might be a common etiological factor for diabetes and leukemia.

Conclusion

Our results showed that many diabetic patients remained undiagnosed with poor glycemic profile. Diabetes was over presented in patients with ALL, AML, NHL, and MM, which was more obvious in the female than that in the male cancer patients. It is suggested that diabetes increases the risk for these hematologic cancers in adults, in particular in the females.

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Author contribution J.Z. Zhao, YC Lu, Y.M. Wang, B.L. Xiao, and H.Y. Li collected the data. J.Z. Zhao and Y.C. Lu prepared Tables 1, 2, and 3. S.C. Lee and L.J. Wang applied for research grants, designed the study, interpreted the data, and wrote the manuscript. All authors reviewed the manuscript.

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Data availability The data are available from the corresponding authors.

Declarations

Statement of ethics Approval for the study was obtained from the Clinical Ethics Committee of Linyi People's Hospital. This research is strictly complying with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All of the patients and their guardians have given their written informed consent.

Conflict of interest The authors declare no competing interests.

References

1. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
2. WHO. Cancer overview. https://www.who.int/health-topics/cancer#tab=tab_1
3. Abudawood M. Diabetes and cancer: a comprehensive review. J Res Med Sci. 2019;24:94.
4. Castillo JJ, Mull N, Reagan JL, et al. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. Blood. 2012;119:4845–50.
5. Yan P, Wang Y, Fu T, et al. The association between type 1 and 2 diabetes mellitus and the risk of leukemia: a systematic review and meta-analysis of 18 cohort studies. Endocr J. 2021;68:281–9.
6. Tseng CH. Diabetes and non-Hodgkin's lymphoma: analyses of prevalence and annual incidence in 2005 using the National Health Insurance database in Taiwan. Ann Oncol. 2012;23:153–8.
7. Yang WS, Li HL, Xu HL, et al. Type 2 diabetes and the risk of non-Hodgkin's lymphoma: a report from two population-based cohort studies in China. Eur J Cancer Prev. 2016;25:149–54.
8. Wang Y, Liu X, Yan P, et al. Association between type 1 and type 2 diabetes and risk of non-Hodgkin's lymphoma: a meta-analysis of cohort studies. Diabetes Metab. 2020;46:8–19.
9. Xu J, Wang T. Association of diabetes mellitus with non-Hodgkin lymphoma risk: a meta-analysis of cohort studies. Hematology. 2019;24:527–32.
10. Khan AE, Gallo V, Linseisen J, et al. Diabetes and the risk of non-Hodgkin's lymphoma and multiple myeloma in the European Prospective Investigation into Cancer and Nutrition. Haematologica. 2008;93:842–50.
11. Gao R, Man TS, Liang JH, et al. Diabetes mellitus is associated with inferior prognosis in patients with chronic lymphocytic leukemia: a propensity score-matched analysis. Cancer Res Treat. 2020;52:189–206.
12. Lin SY, Hsieh MS, Chen LS, et al. Diabetes mellitus associated with the occurrence and prognosis of non-Hodgkin's lymphoma. Eur J Cancer Prev. 2007;16:471–8.

13. Chou YS, Yang CF, Chen HS, et al. Pre-existing diabetes mellitus in patients with multiple myeloma. Eur J Haematol. 2012;89:320–7.
14. Tseng CH. Metformin is associated with a lower risk of non-Hodgkin lymphoma in patients with type 2 diabetes. Diabetes Metab. 2019;45:458–64.
15. Wynn A, Vacheron A, Zuber J, et al. Metformin associated with increased survival in type 2 diabetes patients with pancreatic cancer and lymphoma. Am J Med Sci. 2019;358:200–3.
16. Wu W, Merriman K, Nabaah A, et al. The association of diabetes and anti-diabetic medications with clinical outcomes in multiple myeloma. Br J Cancer. 2014;111:628–36.
17. Zou G, Bai J, Li D, et al. Effect of metformin on the proliferation, apoptosis, invasion and autophagy of ovarian cancer cells. Exp Ther Med. 2019;18:2086–94.
18. De Santi M, Baldelli G, Diotallevi A, et al. Metformin prevents cell tumorigenesis through autophagy-related cell death. Sci Rep. 2019;9:66.
19. Biondani G, Peyron JF. Metformin, an anti-diabetic drug to target leukemia. Front Endocrinol (Lausanne). 2018;9:446.
20. Tseng CH. Metformin use and leukemia risk in patients with type 2 diabetes mellitus. Front Endocrinol (Lausanne). 2020;11:541090.
21. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
22. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:2375–90.
23. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-548.
24. Li Y, Teng D, Shi X, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. BMJ. 2020;369:m997.
25. Yang W, Lu J, Weng J, et al. Prevalence of diabetes among men and women in China. N Engl J Med. 2010;362:1090–101.
26. Shysh AC, Nguyen LT, Guo M, Vaska M, Naugler C, Rashid-Kolvear F. The incidence of acute myeloid leukemia in Calgary, Alberta, Canada: a retrospective cohort study. BMC Public Health. 2017;18:94.
27. Tamamyan G, Kadia T, Ravandi F, et al. Frontline treatment of acute myeloid leukemia in adults. Crit Rev Oncol Hematol. 2017;110:20–34.
28. Clinical Collaborative Group of Fujian Medical Association Hematological B. Incidence of newly diagnosed adult acute leukemia in Fujian province from 2011 to 2012. Zhonghua Xue Ye Xue Za Zhi. 2015;36:733–8.
29. Chen JH, Chung CH, Wang YC, Hsu SN, Huang WY, Chien WC. Prevalence and mortality-related factors of multiple myeloma in Taiwan. Plos One. 2016;11:e0167227.
30. Trna J, Dite P, Adamcova A, Crawford BJ, Hermanova M. Diabetes mellitus in pancreatic cancer patients in the Czech Republic: sex differences. Exp Diabetes Res. 2012;2012:414893.
31. Wu D, Hu D, Chen H, et al. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. Nature. 2018;559:637–41.
32. Nolte F, Giehl M, Haass W, et al. Centrosome aberrations in bone marrow cells from patients with myelodysplastic syndromes correlate with chromosomal instability. Ann Hematol. 2013;92:1325–33.
33. Neben K, Giesecke C, Schweizer S, Ho AD, Kramer A. Centrosome aberrations in acute myeloid leukemia are correlated with cytogenetic risk profile. Blood. 2003;101:289–91.
34. Kramer A, Schweizer S, Neben K, et al. Centrosome aberrations as a possible mechanism for chromosomal instability in non-Hodgkin's lymphoma. Leukemia. 2003;17:2207–13.
35. Kryukova E, Kryukov F, Hajek R. Centrosome amplification and clonal evolution in multiple myeloma: short review. Crit Rev Oncol Hematol. 2016;98:116–21.
36. Zarei R, Nikpour P, Rashidi B, Eskandari N, Aboutorabi R. Evaluation of diabetes effects on the expression of leukemia inhibitory factor and vascular endothelial growth factor a genes and proteins at the time of endometrial receptivity after superovulation in rat model. Adv Biomed Res. 2019;8:66.
37. Gearing DP, Gough NM, King JA, et al. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). Embo j. 1987;6:3995–4002.
38. Garzon R, Volinia S, Liu CG, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008;111:3183–9.
39. Marcucci G, Mrozek K, Radmacher MD, Garzon R, Bloomfield CD. The prognostic and functional role of microRNAs in acute myeloid leukemia. Blood. 2011;117:1121–9.
40. Zhu H, Shyh-Chang N, Segre AV, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell. 2011;147:81–94.
41. Copley MR, Babovic S, Benz C, et al. The Lin28b-let-7-Hmga2 axis determines the higher self-renewal potential of fetal haematopoietic stem cells. Nat Cell Biol. 2013;15:916–25.

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ORIGINAL ARTICLE

New results for monogenic diabetes with analysis of causative genes using next-generation sequencing: a tertiary centre experience from Turkey

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Abstract

Background Although monogenic diabetes accounts for a small proportion of diabetes cases, accurate diagnosis may significantly change treatment. This study aimed to contribute to knowledge about the genotype-phenotype relationship in monogenic diabetes.

Methods This study used data from a tertiary centre in Turkey. Genetic analysis outcomes for 36 patients were evaluated. The panel included 23 genes related to maturity-onset diabetes of the young (MODY), neonatal diabetes, and some genes related to hyperglycemic hypoglycemia. The next-generation sequencing method was used after DNA isolation from the peripheral blood.

Results Mutations were identified in 19 (52.8%) of 36 patients. Of the 19 mutations, 7 (36.8%) were new mutations. A total of 20 cases met the MODY clinical criteria, and mutations were identified in 11 (55%) of them. In total, nine patients had more than one mutation. Mutations were identified on the *ABCC8* ($n = 7$), *PDX1* ($n = 6$), *GLIS3* ($n = 6$), *ZFP57* ($n = 5$), *GCK* ($n = 4$), *HNF1A* ($n = 3$), *GLUD* ($n = 3$), and *HNF4A*, *KLF11, NKX2-2*, and *INSR* genes ($n = 1$ each).

Conclusion Our findings highlight a broad clinical and genetic spectrum of MODY, and genetic analysis may provide a better understanding of diabetes and improve the individualised treatment approach.

Keywords MODY · Neonatal diabetes · Monogenic diabetes · Next-generation sequencing

Introduction

Nearly 90% of diabetics have type 2 diabetes mellitus (DM) and 5–10% have type 1 DM. Type 1 and type 2 DM have no single genetic cause. Variations causing an increase in risk, and some reducing risk, were identified, and a multigenetic effect was reported. However, a genetic variation may be identified as the true cause of DM in 2–5% of diabetics [1–4]. Monogenic DM cases comprise maturity-onset diabetes of the young (MODY), neonatal DM (NDM), and some rare diabetic syndromes. The most common is MODY. NDM is rare, as reported in 1 per 90,000 live births in Europe [5]. Monogenic diabetic forms should be suspected in cases without classic type 2 DM or type 1 DM clinical findings or diabetics with intense family history, and genetic studies should be planned.

NDM is generally defined as DM emerging in the first 6 months after birth. Temporary or permanent types may be present. The most common causes of NDM are *KCNJ11*, *ABCC8*, and *INS* heterozygous gene mutations. Homozygote *INS* mutations may create a clinical course very similar to type 1 DM [6]. Some other genes had also been identified to cause NDM. NDM has a lower heritability rate than MODY [7].

The term "MODY" was first used in 1975 [8]. However, mutations responsible for this DM form were revealed in the 1990s [9]. With the use of new-generation sequencing devices in recent years, recognising genes related to disease has increased. Finally, 14 MODY-related genes were

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identified. However, not all the 14 forms of 'MODY' are indeed true MODY.

Whether some rare MODY types like MODY 7,8,9,11 are indeed MODY is debatable [10].

Especially for diabetics under 45 years old, the frequency of MODY reaches up to 5%, and 80% of these cases were misdiagnosed as type 1 DM or type 2 DM [11]. Genetic confirmation of the diagnosis of MODY may lead to managing more appropriate treatment approaches. While some monogenic DM forms respond to sulfonylurea treatment very well, newer drugs such as DPP4 and SGLT2 inhibitors are also being studied to manage MODY [10]. Identification of new MODY mutations and reporting of their clinical data contribute to a better understanding of the MODY phenotypes and may provide developing more improved therapeutic approaches.

In this article, we present analysis results of a gene panel including 23 genes and the clinical characteristics of cases. This study aimed to contribute to knowledge about the genotype-phenotype relationship in monogenic DM.

Methods

This single-centre study included 36 patients whose gene analysis was performed between January 2018 and December 2019. Analyses were performed in a University Medical Genetic laboratory with the new-generation sequencing method after DNA isolation from the peripheral blood. Results were assessed with Ion reporter v. 5.6 and IGV software. The panel included genes related to MODY and NDM and some genes related to hyperinsulinemic hypoglycemia.

The pathogenicity of the mutations was classified according to the American College of Medical Genetics and Genomics criteria [12]. The Clinvar, HGMD, and Varsome databases were also used to assess mutations. In silico analysis was also performed using SIFT, MutationTaster, Human Splicing Finder, Mutation Assessor, and Polyphen2software. GERP score, DANN score, and GnomAD frequency were also employed for the assessment of variations.

In this study, the following 23 genes were investigated: *HNF1A, GCK, HNF4A, PDX1, HNF1B, NEUROD1, KLF11, ZFP57, PAX4, INS, BLK, ABCC8, KCNJ11, RFX6, HADH, SLC16A1, FOXP3, G6PC2, NEUROG3, GLIS3, NKX2-2, GLUD1*, and *INSR*.

The clinical history and laboratory results of the patients were obtained from the hospital records. MODY's clinical diagnosis was defined as follows: DM diagnosis at a young age, positive family history (autosomal dominant inheritance observed in at least two or three generations), absence of C-peptide negativity, and lack of β-cell autoimmunity [13].

Statistical data analysis used SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). The mean and standard deviation values were used for descriptive statistics.

Results

Thirty-six patients referred for the genetic study were assessed in this study. Of all the participants, 25 were female (69.5%), and 11 were male (30.5%). The mean age was 31.4 ± 15.5 years.

Thirty patients had analysis requested with suspicion of monogenic DM, two paediatric patients had hypoglycemia suspicion, and the reason for analysis could not be identified in four patients.

Nineteen different variants were detected in 19 of 36 cases. Nine patients had more than one variant. Seven variants were novel. Thirteen of the mutations were interpreted as variant of uncertain significance, likely pathogenic or pathogenic. A total of 20 cases met the MODY clinical criteria, and mutations were identified in 11 (55%) of them. Four mutation-positive cases with diabetes did not meet all of the MODY criteria. The other four patients with the mutation also did not fully meet the DM diagnostic criteria. Of the 16 patients with negative mutation analysis, nine met the clinical criteria for MODY and were considered to have mutation-negative MODY (Figure 1). One patient with hyperinsulinemic hypoglycemia was identified to have a mutation of the HADH gene, but he was not evaluated in the analyses of this study.

Mutations were present on *HNF1A* in three patients, *HNF4A* in one, *KLF11* in one, *PDX1* in six, *ABCC8* in seven, *GLIS3* in six, *XFP57* in five, *GCK* in four, *GLUD1* in three, *NKX2-2* in one, and *INSR* in one. The clinical features of the cases are shown in Table 1, and database analyses of the detected mutations are shown in Table 2.

Glucokinase (GCK) mutation

Four patients had *GCK* mutations. One of the patients was 28 years old and was diagnosed with DM at age 13. The HbA1c value was 6.8% with diet alone. The two other patients were a mother and daughter in family B. Both were not using pharmacologic agents for DM treatment. The mother had HbA1c value of 6.9%, while the daughter had 7.1%. The fourth patient had *GCK* and *ABCC8* mutations. The patient was 4 years old and diagnosed with DM at age 2. HbA1c value was 6.4% with diet alone. There was no family history of DM.

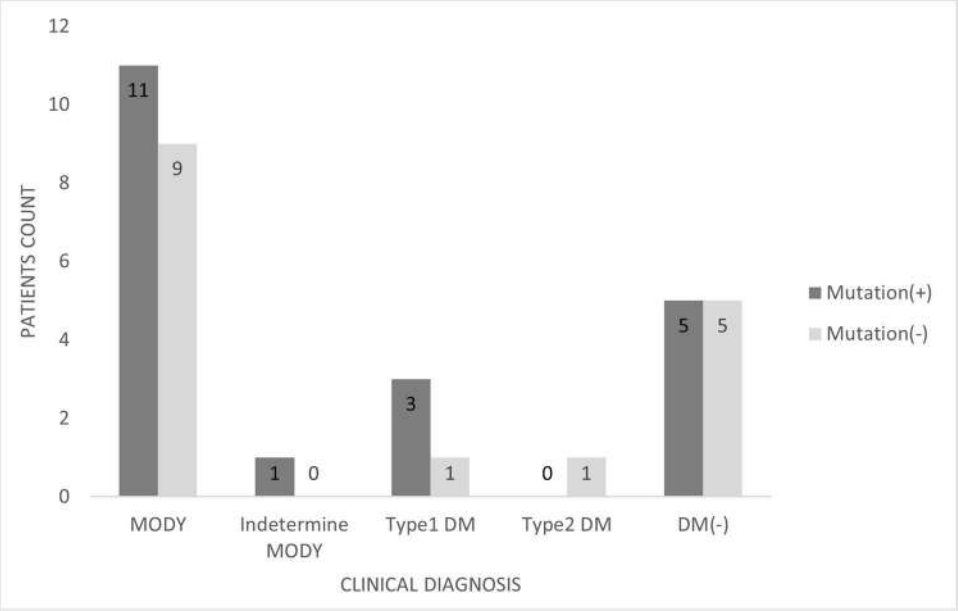

Fig. 1 Distribution of analyzed cases according to mutation status and diagnosis

HNF1A mutation

Three patients had *HNF1A* mutation. One of the patients with *HNF1A* mutation had anti-GAD positivity, and the C-peptide was at unmeasurably low levels, so she was diagnosed with type 1 DM. The other two patients with *HNF1A* mutation were a mother and a daughter. The daughter was 33 years old and had no DM with an HbA1c of 5.1%. The mother was 64 years old without DM.

Family A

Family A was identified to have multiple gene mutations. Genetic analysis was performed for seven family members, and six were identified to have mutations. The proband was 23 years old and diagnosed with DM 4 years ago. Her last HbA1c was 5.3% with diet alone. The proband's mother was 46 years old and had DM for 15 years with HbA1c of 6.8% under both insulin and oral anti-diabetic (OAD) treatment. The mother had diabetic polyneuropathy and coronary artery disease. A sibling of the proband, diagnosed at age 16, had DM for 5 years. The HbA1c value was 6.5% with insulin and OAD treatment. Another sibling was diagnosed at age 17 and had DM for 8 years, and his HbA1c value was 4.8% with insulin treatment alone. The maternal aunt of the proband was 42 years old and had DM. Another maternal aunt of the proband was 40 years old without a DM diagnosis.

KLF11 mutation

A new mutation was identified on the *KLF11* gene in a 57-year-old woman diagnosed with DM at age 10. Her last HbA1c value was 8.0% with OAD and insulin treatment. She had diabetic proliferative retinopathy and diabetic polyneuropathy. There was a family history of MODY with typical three generations.

NKX2-2 mutation

A new mutation was identified on the *NKX2-2* gene in a 43-year-old patient diagnosed with DM 6 years ago. The HbA1c value was 7.5% with OAD treatment alone. There was a typical family history of MODY.

HNF4A mutation

One patient had *HNF4A* mutation. Simultaneous *GLIS3* mutation was also identified. The patient was 23 years old and diagnosed with DM 7 years ago. The HbA1c value was 10.1% with OAD treatment alone. There was a typical family history of MODY.

ABCC8 mutation

All seven patients with *the ABCC8* mutation also had a mutation in another gene. Three different mutations were identified for the *ABCC8* gene, and two of these were new mutations.

INR mutation

A new mutation was identified for the *INR* gene in a 4-year-old patient with DM diagnosis at age 2. She was receiving basal and bolus insulin treatment, and the last HbA1c value was 7.7%. The mother or father had no DM.

Table 1 Mutation results and clinical data of the patients

Patient number	Variant	Clinic status	Family	Salient features
P1	ABCC8 c.2116+39T>A GLIS3 c.1056G>C GLUD1 c.200T>C PDX1 c.246T>C	DM (+)	A	Age: 23 Tre:Diet HbA1c: %5.3
P2	ABCC8 c.2116+39T>A GLUD1 c.200T>C PDX1 c.246T>C ZFP57 c.1103A>T	DM (+)	A	Age: 16 Tre:OAD+insulin HbA1c: %6.5
P3	GLIS3 c.1056G>C PDX1 c.246T>C ZFP57 c.1103A>T	DM (+)	A	Age: 17 Tre:insulin HbA1c: %5.8
P4	ABCC8 c.2116+39T>A PDX1 c.246T>C ZFP57 c.1103A>T	DM (-)	A	
P5	ABCC8 c.2116+39T>A GLIS3 c.1056G>C PDX1 c.246T>C ZFP57 c.1103A>T	DM (+)	A	Age: 29 Tre:Diet HbA1c: %6.5
P6	ABCC8 c.2116+39T>A GLIS3 c.1056G>C PDX1 c.246T>C ZFP57 c.1103A>T	DM (+)	A	Age: 31 Tre:OAD+insulin Comp: neuropathycoroner arter disease HbA1c: %6.8
P7	GCK c.91A>T p.k31*	DM (+)	B	Age:20 Tre:Diet HbA1c: %6.9
P8	GCK c.91A>T p.k31*	DM (+)	B	Age:6 Tre:Diet HbA1c: %7.1
P9	HNF1A c.1108-27C>T	DM (-)	C	
P10	HNF1A c.1108-27C>T	DM (-)	C	Tre:Diet HbA1c: %5.1
P11	KLF11 c.514G>A	DM (+)	-	Age:10 Tre:Diet+insulin Comp: retinopathy Neuropathy HbA1c: %8
P12	GCK c.895G>C	DM (+)	-	Age: 13 Tre:Diet Comp: neuropathy HbA1c: %6.8
P13	NKX2-2 c.*73G>A	DM (+)	-	Age: 35 Tre:OAD HbA1c: %7.5
P14	HNF4A c.725G>A GLIS3 c.1585C>G	DM (+)	-	Age: 16 Tre:OAD HbA1c: %10.1
P15	ABCC8 c.1259T>G GCK c.547G>A	DM (+)	-	Age: 2 Tre:Diet HbA1c: %6.4
P16	GLIS3 c.893C>A	T1 DM	-	Tre: Insulin HbA1c: %7.8
P17	HNF1A c.1108-27C>T	T1 DM	-	Tre: Insulin HbA1c: %7.5
P18	ABCC8 c.1332+4delC GLUD1 c.1568G>A	DM (-)	-	
P19	INSR c.1777G>T	T1 DM	-	Age: 2 Tre:Insulin HbA1c: %7.7

DM diabetes mellittus**,** *HbA1c* last glycosylated haemoglobin, *Tre* treatment, *Comp* Complicatio

Table 2 Variants detected by sequencing 23 genes in our study including 36 participants

Benign and likely benign variants

Patient no.	Chr	Gene	Status	Nuc change	Protein effect	Mutation type	ClinVar	GnomAD frequency	SIFT	GELP	DANN	insiA MLG/ BNG	Pathogenicity
P2,P3,P4,P5,P6	chr6	ZFP57	Known	c.1103A>T	p.Asp368Val	Missense	2 submis. Benign, likely benign	**0.0221**	Tolerated, damaging	4,21	0,94	1/18	B
P9,P10	chr12	HNF1A	Known	c.1108-27C>T	-	Non-coding	Not reported	**0.00387**	-	2,26	0,36	0/1	B
P13	chr20	NKX2-2	New	c.*73G>A	-	Non-coding	Not reported	0.0129	-	5.54	0.93	0/1	LB
P16	chr9	GLIS3	Known	c.893C>A	p.Ser298Tyr	Missense	3 submis Benign Likely benign	**0.00215**	Damaging	5.59	0.99	9/10	LB
P17	chr12	HNF1A	Known	c.1108-27C>T	-	Non-coding	Not reported	**0.00387**	-	2.26	0.36	0/1	B
P18	chr10	GLUD1	Known	c.1568G>A	p.Arg523His	Missense	1 submis Uncertain significance	**0.0000477**	Tolerated	5.05	0.99	12/8	B

VUS, pathogenic, and likely pathogenic variants

Patient no.	Chr	Gene	Status	Nuc change	Protein effect	Mutation type	ClinVar	GnomAD frequency	SIFT	GELP	DANN	insiA MLG/ BNG	Pathogenicity
P1,P2,P3, P4,P5,P6	chr13	PDX1	New	c.246T>C	p.Leu82=	Synonymous	Not reported	No entry	-	4,96	0,57	0/1	VUS
P1,P2,P4,P5, P6	chr11	ABCC8	New	c.2116+39T>A	-	Non-coding	Not reported	0.000284	-	5,16	0,82	0/1	VUS
P1,P3,P5 P6	chr9	GLIS3	Known	c.1056G>C	p.Leu352=	Synonymous	2 submis Benign NDH syndrome	**0.000338**	-	5,82	0,63	0/1	VUS
P1,P2	chr10	GLUD1	Known	c.200T>C	p.Phe67Ser	Missense	1 submis Likely benign	0.000016	Tolerated	4,01	0,97	13/8	VUS
P7,P8	chr7	GCK	Known	c.91A>T	p.k31*	Nonsense	-	No entry	-	5,26	0,99	7/1	P
P11	Chr2	KLF11	New	c.514G>A	p.Gly172Arg	Missense	Not reported	**0.000012**	Damaging	4,80	0,97	5/16	VUS
P12	chr7	GCK	Known	c.895G>C	p.G299R	Missense	1 submis Pathogenic	No entry	Damaging	4.7	0,99	20/1	P
P14	chr20	HNF4A	Known	c.725G>A	p.Arg242His	Missense	Not reported	No entry	Damaging	4.98	0.99	19/2	LP
P14	chr9	GLIS3	New	c.1585C>G	p.Arg529Gly	Missense	Not reported	No entry	Damaging	5.51	0.99	17/3	VUS
P15	chr11	ABCC8	New	c.1259T>G	p.Leu420Arg	Missense	Not reported	No entry	Damaging	4.78	0.99	18/2	LP
P15	chr7	GCK	Known	c.547G>A	p.Val183Met	Missense	6 submis Pathogenic	No entry	Damaging	5.82	0.99	19/1	P
P18	chr11	ABCC8	Known	c.1332+4delC	-	Deletion Non-coding	5 submis 3 Likely benign 2 VUS	0.00097	-	4.1199	-	-	VUS

Table 2 (continued)

Benign and likely benign variants													
Patient no.	**Chr**	**Gene**	**Status**	**Nuc change**	**Protein effect**	**Mutation type**	**ClinVar**	**GnomAD frequency**	**SIFT**	**GELP**	**DANN**	**insiA MLG/ BNG**	**Pathogenicity**
P19	chr19	INSR	New	c.1777G>T	p.Val593Leu	Missense		0.00000398	Damaging	5.07	0.99	19/1	VUS

Chr chromosome, *Nuc* nucleotide, *insiA* in silico analysis, *MLG* malign, *BNG* benign, *Freq* frequency, *LB* likely benign, *VUS* variant of undetermined significance, *B* benign, *P* pathogenic, *submis* submission

GLIS3 mutation

One patient had a new mutation on the *GLIS3* gene. However, this patient was considered type 1 DM based on clinical findings and undetectable C-peptide levels.

Mutation-negative MODY

Nine patients did not have mutations but with clinical features that met the MODY criteria. The mean age was 40 ± 11.5 years, and the mean age at diagnosis was 29.4 ± 7.9 years. The mean of the last HbA1c value was 10.0% ± 3.1%, and the mean body mass index was 26.6 ± 3.0 kg/m^2.

Discussion

Our study investigated the results of a panel containing genes that might cause monogenic DM from a single centre in Turkey. Of the 36 patients investigated, 19 different mutations were detected, 7 of which were novel. A total of 20 patients fully met the clinical criteria for MODY, and 11(%55) of these patients had mutations. A recent study in Turkey reported a mutation identification rate of 65% in 43 children [14]. However, the mutation identification rate may vary according to the inclusion criteria for the analysis. Expanding the criteria used to request genetic analysis may decrease the positivity rates to 10–20% [13]. A recent multi-centre study identified a lower mutation positivity rate of 17.6% in 204 cases from Mediterranean countries [15].

Although genetic studies on monogenic DM have increased, novel genes could not be identified except for the neonatal period. However, the allelic spectrum of the known genes continues to expand. Some of the new variants detected do not meet all of the classic MODY criteria, adding a new perspective to MODY [16]. These variants suggest that monogenic diabetes, and some other types of diabetes may share a common genetic spectrum. We also identified variants in four diabetic cases that did not fully meet the MODY criteria. However, these variants may be benign or de novo mutations, as well as they can also be considered to support this new perspective. One patient with HNF1A mutation was interpreted as type 1 DM because of C-peptide negativity and anti-GAD antibody positivity. One patient with GLIS3 mutation had intense insulin requirements and low C-peptide level. Another pediatric case with INSR mutation was accepted as type 1 DM because of a lack of family history and clinical findings. One patient had ABCC8 and GCK mutation; however, indeterminate MODY was recognised due to a lack of family history.

Of the mutations identified in our study, four were *GCK* gene mutations. *GCK* mutations cause MODY type 2 and are associated with mild DM. Elevated fasting blood sugar can

be detected since birth; however, no significant progression is expected over the years [9]. Pharmacological treatment for those with *GCK* mutations may not help in lowering blood sugar [18]. The diagnoses were made during childhood in this study, and the serum glucose levels were not very high. Patients had mild progression of DM and were managed with diet and lifestyle changes. Two patients (mother and daughter; P7, P8) had mild DM findings despite having c.91A>T non-sense mutations. Similarly, those with heterozygous non-sense or frameshift mutations of the *GCK* gene may have mild progression of clinical DM [18].

P12, with family history and mild serum glucose elevation, meet the typical MODY type 2 diagnosis. The identified *GCK* c.895G>C variant was previously reported as pathological.

P15 was the case that identified the GCK and ABCC8 mutations together mentioned above but was considered indeterminate MODY due to the absence of family history. The identified *GCK* c.895G>C variant was previously reported as pathological. The *ABCC8* c.1259T>G variant was interpreted as pathogenic in most in silico analyses, but this patient's clinical course was consistent with MODY type 2.

HNF1A mutation was identified in three patients. *HNF1A* mutations cause the frequently encountered MODY type 3 [19]. One of these patients (P17) had been interpreted as type 1 DM due to C-peptide negativity and positive anti-GAD antibody. This patient had *HNF1A* c.1108-27C>T mutation in the intronic region, which was considered benign. The other two patients were a mother and daughter (P9, P10) with *HNF1A* c.1108-27C>T intronic region mutation. One of these patients had pre-DM emerging only with weight gain. These cases did not fully meet the DM diagnostic criteria; analysis was requested because of a family history of severe DM. These two cases were considered in the non-DM group, and this mutation was interpreted as benign because of the lack of obvious DM diagnosis.

We identified the *KLF11* gene mutation, a rare cause of MODY, in P11. *KLF11* regulates *PDX1* transcription in pancreatic beta cells [9]. Our 57-year-old patient had been diagnosed with DM at age ten and did not have adequate blood sugar control despite both OAD and intensive insulin treatment. She also had diabetic neuropathy and proliferative retinopathy. Very few cases were related to *KLF-11* mutation [20]. A study screening patients with obesity reported that one patient had *a KLF11* variant. The 16-year-old patient was reported to have hyperlipidemia accompanied by mild DM progression [21]. Considering that our patient had complicated DM, *KLF11* mutations might be associated with severe disease progression.

In family A, mutations were identified in six of seven patients analysed, and five had DM (P1, P2, P3, P5, P6). Interpreting the phenotype-genotype correlation was difficult because multiple mutations were identified in this family. This family may be considered *PDX1* mutation associated with MODY type 4, but family analysis showed that patient–healthy individual segregation was partly consistent with the *PDX1* c.246T>C variant. This mutation was assessed as VUS. All six family members with the mutation had c.246T>C synonymous variant on the *PDX1* gene. The *PDX1* gene is an important transcription factor gene for pancreas development and beta-cell maturation. MODY type 4 families are scarce. Generally, affected family members are diagnosed at a young age, and the majority may require insulin [10]. Autoantibodies against *PDX1* may also cause type 1 DM [22]. The identified *PDX1* variant was not found in the Clinvar database, and it was assessed as likely benign in the Varsome database. However, it was predicted to affect the splice region in the Human Splicing Finder database.

The *ABCC8* c.2116+39T>A variant identified in five members of the family A (P1, P2, P4, P5, and P6) was in the intronic region and not reported in the Clinvar database. This gene codes sulfonylurea receptor 1 (SUR1) subunits in the ATP-susceptible potassium channel and thus plays a role in regulating insulin secretion [23]. *ABCC8* mutations were first associated with MODY type 12 in 2012, and it may also cause NDM [24]. MODY type 12 families may be obese and overweight. They respond well to sulfonylurea treatment [25]. In this study, the family members had average weight. Additionally, it was predicted not to affect the splice region in the Human Splicing Finder database, and this mutation was assessed as VUS.

The *GLIS3* c.1056G>C variant is also identified in four family A members. *GLIS3* is a member of the GLIS family of Krüppel-like zinc finger transcription factors. It is dominantly expressed in the pancreas, thyroid, and kidneys. Mutations in *GLIS3* cause an NDM syndrome characterised by congenital hypothyroidism and polycystic kidney. Variants are reported at high rates among those with type 1 DM and type 2 DM. It works with the *PDX1* gene in insulin gene transcription control [26]. However, this *GLIS3* gene mutation was not detected in P2, who has moderate DM. Clinvar database reported this mutation in a neonate with DM and congenital hypothyroidism and interpreted as VUS. This variant was not predicted to affect the splice region in the Human Splicing Finder database, and this mutation was assessed as VUS.

Another variant identified in family A was a c.1103A>T missense variant on the *ZFP57* gene. The *ZFP57* gene codes a transcription factor necessary to adequately sustain methylation during early embryonic development. *ZFP57* mutations are reported to be associated with multiple locus-imprinting disorders in transient NDM [27]. However, the phenotype effect of this gene on the juvenile and adult periods is unknown. The *ZFP57* c.1103A>T variant was reported as VUS in the Varsome database and

was predicted to affect the splicing region in the Human Splicing Finder database. However, this variant was not detected in the proband but was detected in the non-diabetic P4. It was interpreted as benign in most in silico analyses.

Additionally, two of the five family A members with DM were identified to have *GLUD1* c.200T>C variant. *GLUD1* ensures the synthesis of glutamate dehydrogenase, a mitochondrial matrix enzyme included in the tricarboxylic acid cycle. Mutations were associated with familial hyperinsulinemic hypoglycemia [28]. Most in silico analyses assessed this mutation as pathogenic.

In family A, possible mutations on other unexamined genes may be associated with DM development. Multiple mutations may also have an additive effect on DM progression [29].

HNF4A c.724G>A mutation was identified in P14. *HNF4A* encodes a nuclear transcription factor. Pathogenic mutations are associated with MODY type 1. *HNF4A* mutations comprised 7.5% of MODY cases in a new study from Mediterranean countries, including Turkey [15]. MODY type 1 cases were generally diagnosed in the young adult period, progressed over time, and may require multiple OAD or insulin treatments [30]. P14 was diagnosed in the adolescent period and had high HbA1c levels despite intense treatment. The identified mutation was interpreted as pathogenic in most in silico analyses. This patient also had c.1585C>G missense mutation on the *GLIS3* gene. This variant was interpreted as pathological in most in silico analyses.

P16 had *GLIS3* c.893C>A variant. This variant is generally interpreted as VUS, while it was assessed as benign in the Clinvar database. This patient was diagnosed with type 1 DM due to intense insulin treatment, C-peptide negativity, and lack of family history.

P13 was identified to have c.*73G>A intronic region mutation on the *NKX2-2* gene. This gene is a transcription factor gene related to pancreas development. Mutations are associated with NDM [31]. This patient met the MODY clinical criteria; however, he was diagnosed with DM in adulthood. This variant is interpreted as LB; however, it is in the 3′ untranslated region and may have acted by altering the gene's RNA stability and expression level.

P18, without DM, had *ABCC8* c.1332+4delC intronic region deletion. Some cases are assessed as benign and VUS in the Clinvar database. According to the Human Splicing Finder prediction algorithm, this mutation is predicted to affect splicing. However, it was not considered to affect DM development because P18 had no DM. This patient was also identified to have *GLUD1* c.1568 G>A missense mutation. A case of hyperammonemia hyperinsulinemia syndrome was reported in the Clinvar database. However, the clinical findings of this patient were not consistent with hypoglycemia.

P19 was identified to have *INSR* c.1777G>T missense mutation. *INSR* gene mutations may cause severe insulin-resistant syndromes and hyperinsulinemic hypoglycemia [32]. However, no clinical case has been reported. P19 was diagnosed with DM at age 2 years and has used insulin since then. Her mother and father did not have DM. Clinical findings were not consistent with insulin resistance. The C-peptide level was slightly low, but autoantibody tests could not be obtained. The diagnosis was not definite for this patient, but she was considered to have type 1 DM.

Although we analysed all 23 MODY-related genes in all cases, our major limitation was the limited number of cases from a single centre. Another limitation is that mutations in the deep intronic regions of the regulatory sequences and large copy changes could not be detected due to the limitation of our method.

Conclusion

This study analysed data from a single centre in Turkey and aimed to contribute to the phenotype-genotype correlation in monogenic DM. We detected some new mutations and reviewed the clinical findings of previously known mutations. We found that a mutation can be detected in only half of clinically diagnosed MODY patients, and some mutation carriers do not have all of the classic MODY traits. These findings highlight a broad clinical and genetic spectrum of monogenic DM. Detecting new genes and new mutations is critical for a better understanding this form of DM and may improve the individualised treatment approach.

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Author contributions E.K.,G.G.O and U.A. collected the data. E.K, E.S.S and U.A. prepared Tables and Figures. E.K, F.S and E.S.S designed the study, interpreted the data, and wrote the manuscript. All authors reviewed the manuscript.

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Data Availability The data are available from the corresponding authors.

Declarations

Statement of ethics Ethics permission was granted by Canakkale 18 Mart University Clinical Research Ethics Committee dated 11.11.2020, decision no. 2020-13. This research is strictly complying with the guidelines for human studies and was conducted.

Conflict of interest The authors declare no competing interests.

Ethical considerations Ethics permission was granted by University Clinical Research Ethics Committee dated 11.11.2020, decision no. 2020-13.

References

1. Irgens HU, Molnes J, Johansson BB, et al. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. Diabetologia. 2013;56(7):1512–9. https://doi.org/10.1007/s00125-013-2916-y.
2. Fendler W, Borowiec M, Baranowska-Jazwiecka A, et al. Prevalence of monogenic diabetes amongst Polish children after a nationwide genetic screening campaign. Diabetologia. 2012;55(10):2631–5. https://doi.org/10.1007/s00125-012-2621-2.
3. Shepherd M, Shields B, Hammersley S, et al. Systematic population screening, using biomarkers and genetic testing, identifies 2.5% of the UK pediatric diabetes population with monogenic diabetes. Diabetes Care. 2016;39(11):1879–88. https://doi.org/10.2337/dc16-0645.
4. Gat-Yablonski G, Shalitin S, Phillip M. Maturity onset diabetes of the young–review. Pediatr Endocrinol Rev PER. 2006;3(Suppl 3):514–20.
5. Iafusco D, Massa O, Pasquino B, et al. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. Acta Diabetol. 2012;49(5):405–8. https://doi.org/10.1007/s00592-011-0331-8.
6. Al-Agha AE, Ahmad IAA, Basnawi FAA, Al-Nasser EYF. A recessive mutation in the insulin gene in neonatal diabetes. Turk J Endocrinol Metab. 2015;19(1):25–7. https://doi.org/10.4274/tjem.2326.
7. Lemelman MB, Letourneau L, Greeley SAW. Neonatal diabetes mellitus: an update on diagnosis and management. Clin Perinatol. 2018;45(1):41–59. https://doi.org/10.1016/j.clp.2017.10.006.
8. Tattersall RB, Fajans SS. A difference between the inheritance of classical juvenile-onset and maturity-onset type diabetes of young people. Diabetes. 1975;24(1):44–53. https://doi.org/10.2337/diab.24.1.44.
9. Anık A, Çatlı G, Abacı A, Böber E. Maturity-onset diabetes of the young (MODY): an update. J Pediatr Endocrinol Metab JPEM. 2015;28(3–4):251–63. https://doi.org/10.1515/jpem-2014-0384.
10. Aarthy R, Aston-Mourney K, Mikocka-Walus A, et al. Clinical features, complications and treatment of rarer forms of maturity-onset diabetes of the young (MODY) - A review. J Diabetes Complicat. 2021;35(1): 107640. https://doi.org/10.1016/j.jdiacomp.2020.107640.
11. Thanabalasingham G, Pal A, Selwood MP, et al. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. Diabetes Care. 2012;35(6):1206–12. https://doi.org/10.2337/dc11-1243.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K. Rehm HL Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24. https://doi.org/10.1038/gim.2015.30.
13. Owen KR. Monogenic diabetes: old and new approaches to diagnosis. Clin Med Lond Engl. 2013;13(3):278–81. https://doi.org/10.7861/clinmedicine.13-3-278.
14. Ağladıoğlu SY, Aycan Z, Çetinkaya S, et al. Maturity onset diabetes of youth (MODY) in Turkish children: sequence analysis of 11 causative genes by next generation sequencing. J Pediatr Endocrinol Metab JPEM. 2016;29(4):487–96. https://doi.org/10.1515/jpem-2015-0039.
15. Vaxillaire M, Bonnefond A, Liatis S, et al. Monogenic diabetes characteristics in a transnational multicenter study from Mediterranean countries. Diabetes Res Clin Pract. 2020;171:108553. https://doi.org/10.1016/j.diabres.2020.108553.
16. Owen KR. Monogenic diabetes in adults: what are the new developments? Curr Opin Genet Dev. 2018;50:103–10. https://doi.org/10.1016/j.gde.2018.04.006.
17. Tattersall R. Maturity-onset diabetes of the young: a clinical history. Diabet Med J Br Diabet Assoc. 1998;15(1):11–4. https://doi.org/10.1002/(SICI)1096-9136(199801)15:1%3c11::AID-DIA561%3e3.0.CO;2-0.
18. Ping Xiao Y, Hua XuX, Lan Fang Y, et al. GCK mutations in Chinese MODY2 patients: a family pedigree report and review of Chinese literature. J Pediatr Endocrinol Metab JPEM. 2016;29(8):959–64. https://doi.org/10.1515/jpem-2015-0354.
19. Valkovicova T, Skopkova M, Stanik J, Gasperikova D. Novel insights into genetics and clinics of the HNF1A-MODY. Endocr Regul. 2019;53(2):110–34. https://doi.org/10.2478/enr-2019-0013.
20. Neve B, Fernandez-Zapico ME, Ashkenazi-Katalan V, et al. Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. Proc Natl Acad Sci U S A. 2005;102(13):4807–12. https://doi.org/10.1073/pnas.0409177102.
21. Kleinberger JW, Copeland KC, Gandica RG, et al. Monogenic diabetes in overweight and obese youth diagnosed with type 2 diabetes: the TODAY clinical trial. Genet Med Off J Am Coll Med Genet. 2018;20(6):583–90. https://doi.org/10.1038/gim.2017.150.
22. Li S-W, Koya V, Li Y, et al. Pancreatic duodenal homeobox 1 protein is a novel beta-cell-specific autoantigen for type I diabetes. Lab Investig J Tech Methods Pathol. 2010;90(1):31–9. https://doi.org/10.1038/labinvest.2009.116.
23. Patch AM, Flanagan SE, Boustred C, Hattersley AT, Ellard S. Mutations in the ABCC8 gene encoding the SUR1 subunit of the KATP channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. Diabetes Obes Metab. 2007;9(Suppl 2):28–39. https://doi.org/10.1111/j.1463-1326.2007.00772.x.
24. Bowman P, Flanagan SE, Edghill EL, et al. Heterozygous ABCC8 mutations are a cause of MODY. Diabetologia. 2012;55(1):123–7. https://doi.org/10.1007/s00125-011-2319-x.
25. Lin L, Quan H, Chen K, Chen D, Lin D, Fang T. ABCC8-related maturity-onset diabetes of the young (MODY12): a report of a Chinese family. Front Endocrinol. 2020;11:645. https://doi.org/10.3389/fendo.2020.00645.
26. Wen X, Yang Y. Emerging roles of GLIS3 in neonatal diabetes, type 1 and type 2 diabetes. J Mol Endocrinol. 2017;58(2):R73–85. https://doi.org/10.1530/JME-16-0232.
27. Touati A, Errea-Dorronsoro J, Nouri S, et al. Transient neonatal diabetes mellitus and hypomethylation at additional imprinted loci: novel ZFP57 mutation and review on the literature. Acta Diabetol. 2019;56(3):301–7. https://doi.org/10.1007/s00592-018-1239-3.
28. Ninković D, Sarnavka V, Bašnec A, et al. Hyperinsulinism-hyperammonemia syndrome: a de novo mutation of the GLUD1 gene in twins and a review of the literature. J Pediatr Endocrinol Metab JPEM. 2016;29(9):1083–8. https://doi.org/10.1515/jpem-2016-0086.
29. Forlani G, Zucchini S, Rocco AD, et al. Double heterozygous mutations involving both HNF1A/MODY3 and HNF4A/MODY1 genes: a case report. Diabetes Care. 2010;33(11). Accessed January 16, 2021. https://cyberleninka.org/article/n/1249234

30. Warncke K, Kummer S, Raile K, et al. Frequency and characteristics of MODY 1 (HNF4A Mutation) and MODY 5 (HNF1B Mutation): analysis from the DPV database. J Clin Endocrinol Metab. 2019;104(3):845–55. https://doi.org/10.1210/jc.2018-01696.
31. Auerbach A, Cohen A, Ofek Shlomai N, et al. NKX2-2 mutation causes congenital diabetes and infantile obesity with paradoxical glucose-induced ghrelin secretion. J Clin Endocrinol Metab. 2020;105(11). https://doi.org/10.1210/clinem/dgaa563
32. Sethi A, Foulds N, Ehtisham S, et al. Heterozygous insulin receptor (INSR) mutation associated with neonatal hyperinsulinemic hypoglycaemia and familial diabetes mellitus: case series. J Clin Res Pediatr Endocrinol. 2020;12(4):420–6. https://doi.org/10.4274/jcrpe.g.,alenos.2019.2019.0106.

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ORIGINAL ARTICLE

A preliminary analysis of mitochondrial DNA atlas in the type 2 diabetes patients

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Abstract

Background Type 2 diabetes (T2D), one of the most common metabolic diseases, is the result of insulin resistance or impaired insulin secretion due to mitochondrial dysfunction. Mitochondrial DNA (mtDNA) polymorphisms play an important role in the physiological and pathological characteristics of T2D. However, their mechanism is poorly understood.

Methods To directly identify candidate mtDNA variants associated with T2D at the genome-wide level, we constructed forty libraries from ten patients with T2D and thirty control individuals for deep sequencing. We characterized their mtDNA atlas, analysed their single nucleotide polymorphisms (MtSNPs) and screened potential mtDNA mutation sites associated with T2D.

Results We found ten mtDNA polymorphisms at nucleotides 489 T > C, 3105AC > A, 3107 N > C, 8701A > G, 9540 T > C, 10398A > G, 10400C > T, 10873 T > C, 12705C > T and 14783 T > C that showed a significant difference between patients and control subjects.

Conclusion Therefore, our results characterize the mtDNA atlas of patients with T2D and further demonstrate that mtDNA variants participate in the pathophysiology of T2D and other diseases. In addition, mtDNA variants may be candidate molecular biomarkers of T2D and may be valuable for early diagnosis of T2D in the future.

Keywords Type 2 diabetes · Mitochondrial DNA · Single nucleotide polymorphisms · Molecular biomarkers

Introduction

Type 2 diabetes (T2D) is one of the most common metabolic diseases that is characterized by sustained hyperglycemia, resulting from impaired insulin secretion or insulin resistance in most patients [1, 2]. It was estimated that approximately 451 million people had diabetes worldwide in 2017, and the number of patients with diabetes will increase to 693 million by 2045, of which T2D accounts for approximately 90% [1, 3]. As a result of physical inactivity and poor diet, the number of children, adolescents, and younger adults with T2D has increased dramatically around the world, and

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about half of all people with T2D are undiagnosed. Recently, some studies indicated that mitochondria regulate insulin secretion from pancreatic β cells, and their dysfunction was associated with the occurrence of T2D [4–7]. Moreover, they have identified a great number of mitochondrial DNA (mtDNA) variants from patients with T2D, which affect the development of diabetes [4].

In practice, mitochondrial dysfunction might also play a role in the pathogenesis of insulin resistance, and their single nucleotide polymorphisms (MtSNPs) were associated with T2D [4, 8, 9]. Some experimental evidence has indicated that the mtDNA 16189C variant may increase the risk of T2D in Asians [9], but the 16,184–16,193 variant of the poly-C tract does not have a major influence on T2D [10]. The mtDNA mutations at 1310, 1438 and 12,026 were associated with type 2 diabetic patients in the Japanese population [11]. Matsunaga and colleagues found that the Mt5178A genotype may be unrelated to the etiology of T2D but has an antiatherogenic effect in type 2 diabetic patients [12]. Tawata's group indicated that the mtDNA mutation at 14,577 T/C is probably a major pathogenic mutation for T2D. Remarkably, mtDNA ND1 gene mutations at nt3316 (G > A), nt3394 (T > C) and 3426 (A > G) might reveal the pathophysiology of T2D together with other genetic and environmental factors [13]. Furthermore, other mtSNPs also have also been implicated in the physiopathological process of T2D, such as C16270T, C16320T, C5178A, and T16189C [2, 14, 15]. However, their mechanism is poorly understood.

Here, to directly identify candidate mtDNA variants associated with T2D at the genome-wide level, we constructed forty libraries from ten patients with T2D and thirty control subjects for deep sequencing. Subsequently, we characterized their mitochondrial DNA atlas and analysed their single nucleotide polymorphisms (MtSNPs) and insertions and deletions (InDels), and screened potential mtDNA variants associated with T2D. Our research finds some differential mutation sites that may be candidate molecular biomarkers of T2D, which may be valuable for early diagnosis of T2D.

Materials and methods

Human samples and DNA extraction

We have clinically identified and diagnosed all participants according to the diabetes diagnostic criteria of the World Health Organization, and they have no positive family history [16]. Then, we selected 10 patients with T2D as the subjects of the study and collected their blood samples. They had an average fasting plasma glucose ≥ 8.02 mmol/L, and blood glycosylated hemoglobin level of ≥ 9.29% (Table 1). The 30 control subjects had no history of T2D and were matched with the disease group in age, region and gender ratio as much as possible. Written informed consent was obtained from all donors. Subsequently, genomic DNA was extracted from all donors using a Magbead Blood DNA Kit (CWBIOTech, CW2361S) according to the manufacturer's methods. The present study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Shenzhen People's Hospital.

Table 1 Clinical and biochemical characteristics of the patients with type 2 diabetes

Characteristic	Value
Gender (males/females)	7/3
Age (years)	58.10 ± 4.00
HbA1c (%)	9.29 ± 0.59
Fasting plasma glucose (mmol/l)	8.02 ± 0.97
Serum C-peptide (μg/L)	2.75 ± 1.73
Total cholesterol (mmol/l)	4.98 ± 0.54
Triglycerides (mmol/l)	2.64 ± 0.49
HDL-C (mmol/l)	0.862 ± 0.06
LDL-C (mmol/l)	2.85 ± 0.30

Mitochondrial DNA sequencing

After total genomic DNA extraction, mitochondrial DNA capture of all participants was performed as previously reported, with minor modifications [17]. Briefly, the genomic DNA was randomly fragmented by a Bioruptor Pico (Diagenode, B01060001) into 150–200-bp fragments. The sheared products were detected by an Agilent 2100 Bioanalyzer system (Agilent, G2939AA). The ends of the DNA fragments were repaired and an Illumina Adaptor was added (Fast Library Prep Kit, iGeneTech, Beijing, China). Pre-PCR was performed after adapter ligation. Products were purified using Agencourt AMPure XP beads (Beckman, p/n A63880). After the sequencing library was constructed, the mitochondrial genome was captured with an AI-Mito-Cap Kit (iGeneTech, Beijing, China) and sequenced on an Illumina NovaSeq 6000 sequencer (Illumina, San Diego, CA) with 150-base paired-end reads.

SNPs and InDels detection and annotation

The advanced bioinformatics analysis began with raw data generated from the NovaSeq6000 platforms. The sequence signatures with adapter ligation and low quality and containing > 5 unknown bases were removed to obtain clean sequences, which were subjected to further analysis. The clean sequences were aligned to the human reference genome (UCSC hg19) by a Burrows-Wheeler Aligner (BWA) [18], and the alignment results were saved in BAM format files. For further advanced analysis of the

final BAM files, single nucleotide polymorphisms (SNPs) were detected by GATK [19] and insertion and deletions (InDels) were detected by SAMtools [20]. Then, the mutation types of SNPs and distribution of InDels were analysed by GraphPad Prism (Version 8.0.1). Finally, ANNOVAR and snpEff software (Version 4.3i) were used for annotation and classification, and after the annotation database was constructed, the mutation sites would be annotated for gene and function according to the corresponding database [21]. The pathogenicity prediction for the mtDNA variants was further analysed using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines (ACMG/AMP guidelines) [22] and dbNSFP at Ensemble VEP (Variant Effect Predictor) [23] which produces prediction scores for different algorithms like SIFT, PolyPhen 2.0, CADD, and FATHMM.

Statistical analysis of candidate mtDNA variants

To systematically analyse potential mtDNA variants associated with T2D at the genome-wide level, the frequency of mtDNA mutation sites between the cases and the control group was evaluated by t test and χ^2 test. Statistical analyses of mtDNA variants were performed using SPSS 21.0, and the level of significance was set at $p < 0.05$.

Results

Analysis of mitochondrial DNA sequencing data

Mitochondrial DNA was captured from ten patients with T2D and sequenced on the NovaSeq6000 platform. The raw reads with adapters, low quality and unknown bases were removed, and we collected clean read data for further bioinformatics analysis. In total, we obtained an average of 1.78-M raw reads and 268.46-Mb raw bases for each individual (Table 2). A Phred quality score is a method to evaluate the quality of sequencing data. Qphred was defined as base mass, and the e indicated the sequencing error rate. They have a relationship as follows: $\text{Qphred} = -10\log10(e)$, and we found that the Qphred was greater than 85% for each sample. Upon further statistical analysis for clean reads, we identified an average of 1.26-M clean reads for each individual, which accurately aligned to the reference genome (UCSC hg19) (Table S1). The distribution of mitochondrial target regions of the ten patients is shown in Table S2.

Profiling of mitochondrial DNA SNPs and InDels

MtDNA polymorphisms are associated with various complex hereditary disorders and may be candidate molecular biomarkers for diseases [2, 4]. We systematically detected SNPs and InDels of ten patients with T2D. In total, we identified 34, 32, 41, 43, 35, 38, 42, 42, 46 and 36 SNPs for T2D-1, T2D-2, T2D-3, T2D-4, T2D-5, T2D-6, T2D-7, T2D-8, T2D-9 and T2D-10, respectively, the majority of which were in target regions. Interestingly, most of SNPs were heterozygous and were G > A and C > T, which contributed to identification of candidate molecular biomarkers (Fig. 1). Similarly, of the InDels, we identified 3, 3, 4, 1, 1, 1, 3, 1, 2 and 5 InDels for T2D-1, T2D-2, T2D-3, T2D-4, T2D-5, T2D-6, T2D-7, T2D-8, T2D-9 and T2D-10, respectively, the majority of which were identified as small insertions or deletions (Fig. 2).

Identification of candidate mtDNA variants

Samples from a total of 10 patients with T2D and 30 controls were used to screen candidate mtDNA variants and aligned to the human reference genome (UCSC hg19). We found mtDNA mutations of T2D patients at nucleotides 489 T > C, 3105AC > A, 3107 N > C, 8701A > G, 9540 T > C, 10398A > G, 10400C > T, 10873 T > C, 12705C > T and 14783 T > C by comparing them with the controls. Their variants were considered SNPs and showed a significant difference. There were seven mutations at nucleotides 489 T > C, 8701A > G, 9540 T > C,

Table 2 Analysis of sequencing data from the ten patients with type 2 diabetes

No	Sample name	Raw reads (M)	Raw bases (Mb)	Clean reads (M)	Clean bases (Mb)	Qcrate (%)
1	T2D-1	1.9	286.86	1.87	254.29	88.65
2	T2D-2	1.56	234.81	1.53	211.31	89.99
3	T2D-3	1.67	252.51	1.65	219.25	86.83
4	T2D-4	1.7	256.29	1.68	228.75	89.25
5	T2D-5	1.66	250.21	1.63	222.49	88.92
6	T2D-6	1.89	284.75	1.86	250.1	87.83
7	T2D-7	1.62	244.63	1.6	217.65	88.97
8	T2D-8	1.9	286.31	1.87	245.72	85.82
9	T2D-9	2.08	313.52	2.05	274.22	87.46
10	T2D-10	1.82	274.72	1.79	248.99	90.63

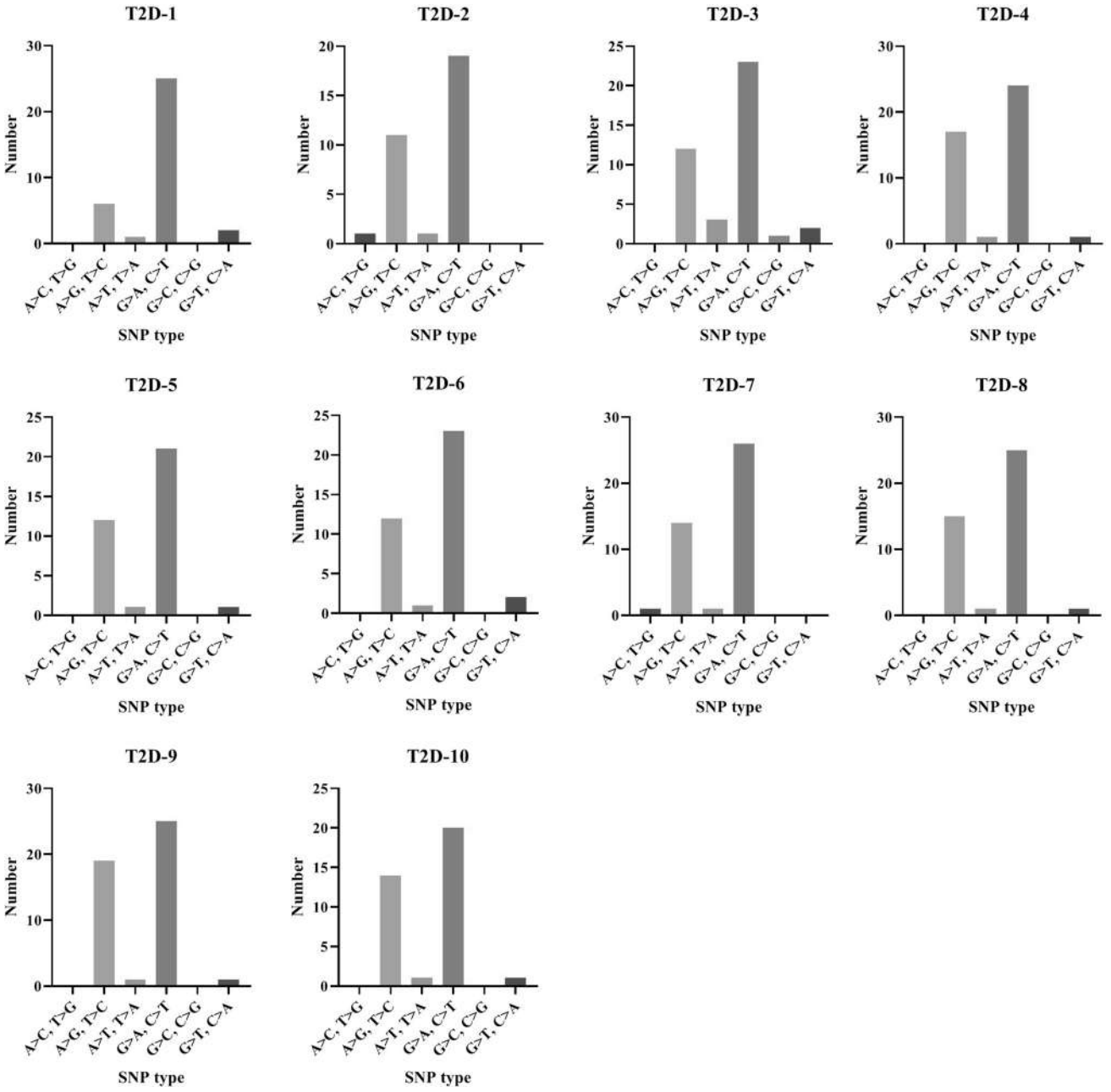

Fig. 1 Profiling of mitochondrial single nucleotide polymorphisms (MtSNPs) of ten patients with T2D. The *x*-axis denotes mtSNP types of patients with T2D, and the *y*-axis indicates the numbers of these mutation types

10398A > G, 10400C > T, 10873 T > C, 12705C > T and 14783 T > C that had significantly higher mutation frequencies among T2D patients, while the polymorphisms 3105AC > A and 3107 N > C had a lower frequency among T2D patients than controls (Table 3). In addition, the pathogenicity prediction results for the ten candidate mtDNA variants are shown in Table 4, and they were reclassified into two categories: "BA1", and "PM2". However, these candidate mtDNA variants are rarely reported to be directly related to type 2 diabetes, and further cohort validation and function are still required.

Discussion

T2D is a complex and multifactorial disease that is affected by the factors of genetic and environmental factors, and is maternally inherited [1, 24–26]. Several studies had reported

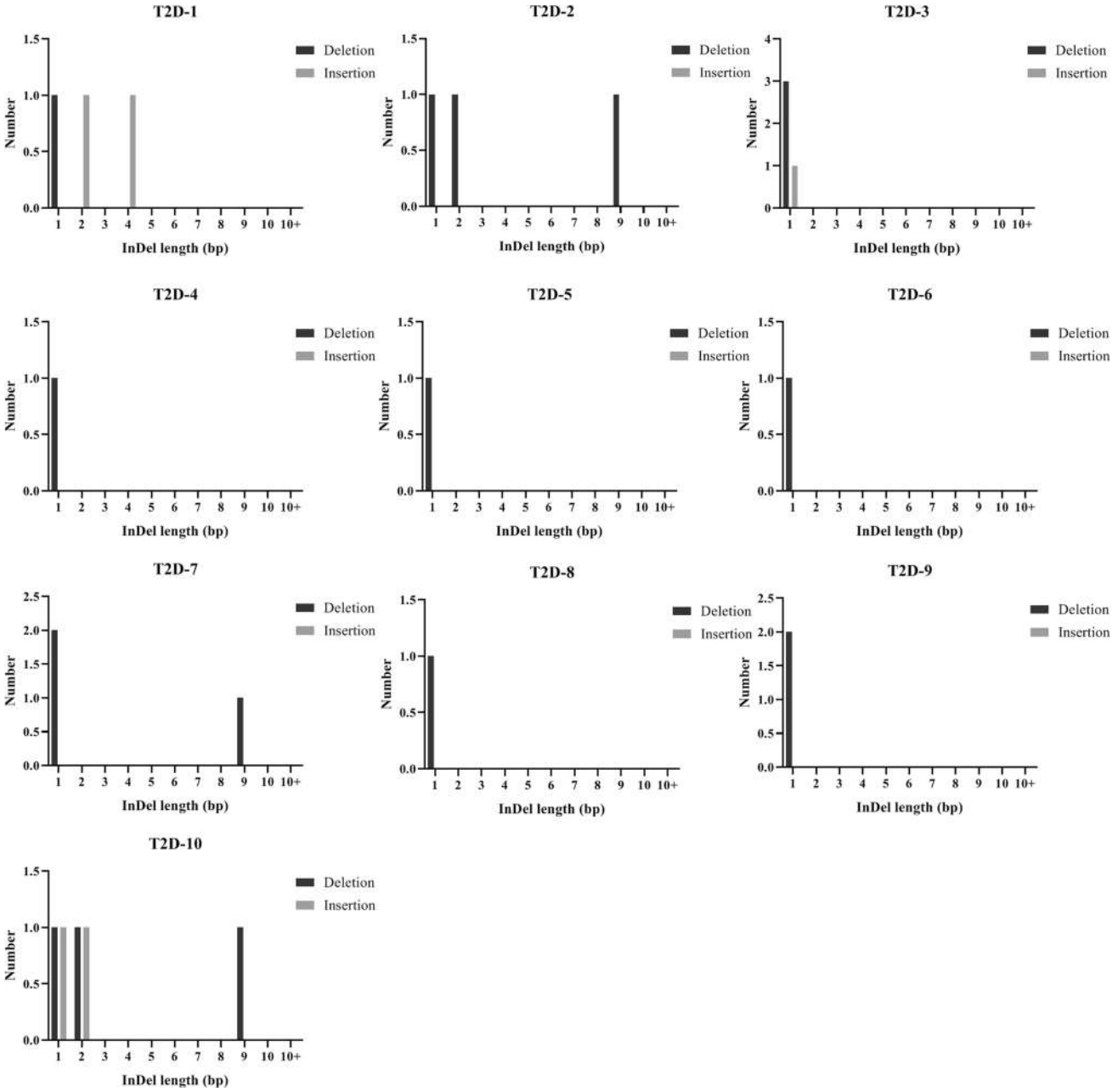

Fig. 2 Profiling of insertions and deletions (InDels) of ten patients with T2D. The horizontal axis represents the position of reads, and the vertical axis indicates the numbers of the mutation types

that mtDNA variants were associated with T2D pathophysiology and were involved in the development of disease [27–29]. Moreover, mtDNA variants can contribute to single and multi-system diseases such as Alzheimer's disease [28], Parkinson disease [30], myoclonic epilepsy and ragged-red fibre disease [31]. In the present study, we identified all mitochondrial DNA polymorphisms from a group of T2D patients and characterized their atlas of SNPs and InDels by target region capture sequencing. With the advancement of high-throughput sequencing technology, target region sequencing is an effective and systemic method to detect specific regions and has been successfully applied in renal oncocytoma and neurodegenerative diseases for mitochondrial DNA sequencing [17, 32]; its capture efficiency is higher than that of traditional methods. Our results further demonstrate that this high-throughput sequencing technology may be a useful tool to study these common inherited diseases.

To further screen the mtDNA variants in T2D, we systematically identified their mitochondrial DNA polymorphisms.

Table 3 The candidate mtDNA variants between controls and patients with type 2 diabetes

Types	Gene name	T2D (*n*, %)	Controls (*n*, %)	χ^2	*p* value*
489 T > C	RNR1	6 (60.00)	6 (20.00)	3.968	0.046
3105AC > A	RNR2	10 (10.00)	16 (53.33)	7.719	0.007
3107 N > C	RNR2	10 (100.00)	16 (53.00)	7.179	0.007
8701A > G	ATP6	6 (60.00)	6 (20.00)	3.968	0.046
9540 T > C	COX3	6 (60.00)	6 (20.00)	3.968	0.046
10398A > G	ND3	8 (80.00)	10 (33.00)	4.848	0.028
10400C > T	ND3	6 (60.00)	6 (20.00)	3.968	0.046
10873 T > C	ND4	6 (60.00)	6 (20.00)	3.968	0.046
12705C > T	ND5	7 (70.00)	9 (30.00)	3.472	0.062
14783 T > C	CYTB	6 (60.00)	6 (20.00)	3.968	0.046

*p value less than 0.05 was considered statistically significant

Then, we found that their SNP types were G > A and C > T, the majority of which were identified as small insertions or deletions, which may be the focus of the next T2D study. We also identified ten mtDNA mutations that showed a significant difference. The polymorphism 3243A > G was first reported in 1992 and proved to be a pathogenetic factor for T2D [25]. However, we did not detect this mtDNA mutation among those that showed a significant difference in our study. Except for the mtDNA polymorphism 489 T > C [33], other polymorphisms are not reported to be related to T2D, including mtDNA polymorphisms 3105AC > A, 3107 N > C, 8701A > G, 9540 T > C, 10398A > G, 10400C > T, 10873 T > C, 12705C > T and 14783 T > C, which may be candidate molecular biomarkers associated with T2D. However, the candidate mtDNA variants were screened based on the ten patients with T2D and thirty control individuals sequencing dataset through bioinformatics analysis. Their disease protective value needs to be verified through the subsequent analysis results from different cohort and platform, and integrally validate its accuracy on the other datasets. In addition, the following are limitations for the samples in this study: firstly, we have limited number of patients with T2D in the department of endocrinology of Shenzhen People's Hospital; secondly, we have very restricted criteria for volunteer recruitment, and all participants were clinically identified and diagnosed according to the diabetes diagnostic criteria of the World Health Organization. People who do not meet those criteria will be excluded in this study. Therefore, this was a reason that the sample size of T2D group is smaller. And this part of the work still needs further study.

Interestingly, some experimental evidence has indicated that these mtDNA polymorphisms have association with the path mechanism of other diseases. The mtDNA polymorphism 8701A > G was previously reported to affect mitochondrial matrix pH and intracellular calcium dynamics, and mtDNA polymorphisms 9540 T > C, 10398A > G, and 12705C > T have a relationship with cancer [34, 35]. The mtDNA polymorphism 10398A > G was involved in the occurrence and development of various diseases such as cancer, neurodegenerative disorders and metabolic diseases [36–39]. This polymorphism had a significant association with systemic lupus erythematosus (SLE) patients, and it was found that T cells of SLE patients were prone to necrosis when ATP is depleted [40]. In addition, Alsbeih and colleagues believe that the nonsynonymous A10398G in ND3 may lead to suboptimum mitochondrial respiratory activity [41], which may provide some support for the susceptibility to T2D. These results suggested that the majority of mtDNA variants functioned in different ways to control various biological processes, affecting the development of T2D.

Conclusion

In summary, our results reveal and characterize the mtDNA atlas of patients with T2D and further support that mtDNA variants participate in the pathophysiology of T2D and other diseases. Our findings further demonstrate that mtDNA

Table 4 The ten candidate mtDNA variants and their pathogenicity prediction scores

Types	Gene name	Gene ID	Mitomap status	Mitomap frequency	SIFT	PolyPhen	CADD	FATHMM	ACMG
489 T > C	RNR1	/	Reported	25.82%	/	/	/	/	BA1
3105AC > A	RNR2	/	/	0.00006	/	/	/	/	PM2
3107 N > C	RNR2	/	/	/	/	/	/	/	PM2
8701A > G	ATP6	ENSG00000198899	/	32.99%	0.39	1.14	−0.52	−0.27	BA1
9540 T > C	COX3	/	/	32.97%	/	/	/	/	/
10398A > G	ND3	ENSG00000198840	Reported	43.97%	0.57	1.99	−0.81	−0.42	BA1
10400C > T	ND3	/	/	21.32%	/	/	/	/	/
10873 T > C	ND4	/	/	32.95%	/	/	/	/	/
12705C > T	ND5	/	Reported	41.12%	/	/	/	/	/
14783 T > C	CYTB	/	Reported	21.31%	/	/	/	/	/

variants may be candidate molecular biomarkers of T2D and may be valuable for early diagnosis of T2D in the future.

Abbreviations T2D: Type 2 diabetes; mtDNA: Mitochondrial DNA; InDels: Insertions and deletions; SNPs: Single nucleotide polymorphisms

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Author contribution DY and LCH conceived and designed the experiments. LCH, TDE, CY and WXB performed the experiments. LCH, XW, CJJ and ZYY analysed the data. LCH, XYY and ZYY drafted the manuscript. LCH, CY and TDE revised the manuscript critically for important intellectual content. XYY and DY obtained the funding. All authors have read and approved the final manuscript.

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Availability of data and material The raw and processed data from mitochondrial DNA sequencing in this study have been deposited with the Gene Expression Omnibus under accession number GSE133958. And all other materials are available from the corresponding author.

Code availability None.

Declarations

Ethics approval The present study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Shenzhen People's Hospital.

Consent to participate Written informed consent was obtained from all participants.

Consent for publication None.

Competing interests The authors declare no competing interests.

References

1. Cho N, Shaw J, Karuranga S, Huang Y, da Rocha FJ, Ohlrogge A, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
2. Charoute H, Kefi R, Bounaceur S, Benrahma H, Reguig A, Kandil M, Rouba H, Bakhchane A, Abdelhak S, Barakat A. Novel variants of mitochondrial DNA associated with Type 2 diabetes mellitus in Moroccan population. Mitochondrial DNA Part A. 2018;29(1):9–13.
3. Holman N, Young B, Gadsby R. Current prevalence of Type 1 and Type 2 diabetes in adults and children in the UK. Diabet Med. 2015;32(9):1119–20.
4. Liao W-Q, Pang Y, Yu C-A, Wen J-Y, Zhang Y-G, Li X-H. Novel mutations of mitochondrial DNA associated with type 2 diabetes in Chinese Han population. Tohoku J Exp Med. 2008;215(4):377–84.
5. Suárez-Rivero J, Villanueva-Paz M, de la Cruz-Ojeda P, de la Mata M, Cotán D, Oropesa-Ávila M, de Lavera I, Álvarez-Córdoba M, Luzón-Hidalgo R, Sánchez-Alcázar J. Mitochondrial dynamics in mitochondrial diseases. Diseases. 2017;5(1):1.
6. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science. 2005;307(5708):384–7.
7. Mulder H, Ling C. Mitochondrial dysfunction in pancreatic β-cells in type 2 diabetes. Mol Cell Endocrinol. 2009;297(1–2):34–40.
8. Patti M-E, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev. 2010;31(3):364–95.
9. Park KS, Chan J, Chuang L-M, Suzuki S, Araki E, Nanjo K, Ji L, Ng M, Nishi M, Furuta H. A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. Diabetologia. 2008;51(4):602–8.
10. Chinnery P, Elliott H, Patel S, Lambert C, Keers S, Durham S, McCarthy M, Hitman G, Hattersley A, Walker M. Role of the mitochondrial DNA 16184–16193 poly-C tract in type 2 diabetes. The Lancet. 2005;366(9497):1650–1.
11. Tawata M, Ohtaka M, Iwase E, Ikegishi Y, Aida K, Onaya T. New mitochondrial DNA homoplasmic mutations associated with Japanese patients with type 2 diabetes. Diabetes. 1998;47(2):276–7.
12. Matsunaga H, Tanaka Y, Tanaka M, Gong J-S, Zhang J, Nomiyama T, Ogawa O, Ogihara T, Yamada Y, Yagi K. Antiatherogenic mitochondrial genotype in patients with type 2 diabetes. Diabetes Care. 2001;24(3):500–3.
13. Yu P, Yu D-m, Liu D-M, Wang K, Tang X-Z. Relationship between mutations of mitochondrial DNA ND1 gene and type 2 diabetes. Chin Med J. 2004;117(7):985–9.
14. Saha SK, Akther J, Huda N, Yasmin T, Alam MS, Hosen MI, Hasan AM, Nabi AN. Genetic association study of C5178A and G10398A mitochondrial DNA variants with type 2 diabetes in Bangladeshi population. Meta Gene. 2019;19:23–31.
15. Kumari T, Vachher M, Bansal S, Bamezai RN, Kumar B. Meta-analysis of mitochondrial T16189C polymorphism for cancer and type 2 diabetes risk. Clin Chim Acta. 2018;482:136–43.
16. Organization WH. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. 2006.
17. Wei W, Keogh MJ, Wilson I, Coxhead J, Ryan S, Rollinson S, Griffin H, Kurzawa-Akanbi M, Santibanez-Koref M, Talbot K. Mitochondrial DNA point mutations and relative copy number in 1363 disease and control human brains. Acta Neuropathol Commun. 2017;5(1):13.
18. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639–45.
19. Bauer DC. Variant calling comparison CASAVA1. 8 and GATK. 2011.
20. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25(16):2078–9.
21. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164–e164.
22. Qu HQ, Wang X, Tian L, Hakonarson H. Application of ACMG criteria to classify variants in the human gene mutation database. J Hum Genet. 2019;64(11):1091–5.
23. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. The ensembl variant effect predictor. Genome Biol. 2016;17(1):1–4.
24. Sun X, Sui W, Wang X, Hou X, Ou M, Dai Y, Xiang Y. Whole-genome re-sequencing for the identification of high contribution susceptibility gene variants in patients with type 2 diabetes. Mol Med Rep. 2016;13(5):3735–46.
25. Van den Ouweland J, Lemkes H, Ruitenbeek W, Sandkuijl L, De Vijlder M, Struyvenberg P, Van de Kamp J, Maassen J. Mutation in mitochondrial tRNA Leu (UUR) gene in a large pedigree with

maternally transmitted type II diabetes mellitus and deafness. Nat Genet. 1992;1(5):368.
26. Wang P-W, Lin T-K, Weng S-W, Liou C-W. Mitochondrial DNA variants in the pathogenesis of type 2 diabetes-relevance of asian population studies. Rev Diabet Stud RDS. 2009;6(4):237.
27. Wallace DC, Zheng X, Lott MT, Shoffner JM, Hodge JA, Kelley RI, Epstein CM, Hopkins LC. Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. Cell. 1988;55(4):601–10.
28. Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochim Biophys Acta BBA Mol Basis Dis. 2010;1802(1):2–10.
29. Meyers DE, Basha HI, Koenig MK. Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. Tex Heart Inst J. 2013;40(4):385.
30. Shoffner JM, Brown MD, Torroni A, Lott MT, Cabell MF, Mirra SS, Beal MF, Yang C-C, Gearing M, Salvo R. Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. Genomics. 1993;17(1):171–84.
31. Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNALys mutation. Cell. 1990;61(6):931–7.
32. Kürschner G, Zhang Q, Clima R, Xiao Y, Busch JF, Kilic E, Jung K, Berndt N, Bulik S, Holzhütter H-G. Renal oncocytoma characterized by the defective complex I of the respiratory chain boosts the synthesis of the ROS scavenger glutathione. Oncotarget. 2017;8(62):105882.
33. Sazonova MA, Shkurat TP, Demakova NA, Zhelankin AV, Barinova VA, Sobenin IA, Orekhov AN. Mitochondrial genome sequencing in atherosclerosis: what's next? Curr Pharm Des. 2016;22(3):390–6.
34. Księżakowska-Łakoma K, Kulczycka-Wojdala D, Kulig A, Baum M, Wilczyński JR. The presence of A5935G, G5949A, G6081A, G6267A, T9540C mutations in MT-CO1 and MT-CO3 genes and other variants of MT-CO1 and MT-CO3 gene fragments in the study population diagnosed with endometrial cancer. Ginekol Pol. 2017;88(7):343–8.
35. Alsbeih GA, Al-Harbi NM, El-Sebaie MM, Al-Rajhi NM, Al-Hadyan KS, Abu-Amero KK. Involvement of mitochondrial DNA sequence variations and respiratory activity in late complications following radiotherapy. Clin Cancer Res. 2009;15(23):7352–60.
36. Atala A. Re: bone metastasis in prostate cancer: recurring mitochondrial DNA mutation reveals selective pressure exerted by the bone microenvironment. J Urol. 2016;3(196):957–8.
37. Choi S-J, Kim S-H, Kang HY, Lee J, Bhak JH, Sohn I, Jung S-H, Choi YS, Kim HK, Han J. Mutational hotspots in the mitochondrial genome of lung cancer. Biochem Biophys Res Commun. 2011;407(1):23–7.
38. Mohideen AMSH, Dicks E, Parfrey P, Green R, Savas S. Mitochondrial DNA polymorphisms, its copy number change and outcome in colorectal cancer. BMC Res Notes. 2015;8(1):272.
39. Jiang Y, Ellis T, Greenlee AR. Genotyping Parkinson disease-associated mitochondrial polymorphisms. Clin Med Res. 2004;2(2):99–106.
40. Jönsen A, Yu X, Truedsson L, Nived O, Sturfelt G, Ibrahim S, Aa B. Mitochondrial DNA polymorphisms are associated with susceptibility and phenotype of systemic lupus erythematosus. Lupus. 2009;18(4):309–12.
41. Alsbeih GA, Alharbi NM, Elsebaie MM, Alrajhi NM, Alhadyan KS, Abuamero KK. Involvement of mitochondrial DNA sequence variations and respiratory activity in late complications following radiotherapy. Clin Cancer Res. 2009;15(23):7352.

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ORIGINAL ARTICLE

Effects of continuous subcutaneous insulin infusion on clinical parameters in patients with different sociodemographic and clinical characteristics

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Abstract

Aim The aim of this study was to investigate the sociodemographic and clinical characteristics of the patients using continuous subcutaneous insulin infusion (CSII) therapy. We reported the effects of CSII therapy on some clinical parameters.

Materials and methods One hundred twenty-seven patients using CSII therapy were enrolled in the study. A total of 102 patients were included after 25 cases were excluded due to inadequate data. Sociodemographic, clinical, and laboratory characteristics were obtained from the hospital database.

Results In our study, no relationship was found between educational level and the effective use of carbohydrate counting and diabetes complications. After switching to CSII therapy, patients' glycated hemoglobin (HbA1c) levels were reduced to 8.2% (5.3–14) at their last visit ($p=0.005$). Also, total daily insulin dosage with 48 IU/day (7.3–180) was significantly decreased to 40 IU/day (15–276) after CSII therapy ($p=0.001$). CSII therapy was significantly associated with less episodes of diabetic ketoacidosis at the last visit than at the initiation of CSII therapy ($p=0.033$).

Conclusion Switching to CSII therapy can improve glycemic control, reduce insulin dosage requirements, and improve patient quality of life by reducing episodes of diabetic ketoacidosis.

Keywords Continuous subcutaneous insulin infusion · Educational levels · Glycemic control · Insulin dosage · Complications of diabetes mellitus

Introduction

The frequency of using CSII therapy increases in patients with type 1 and type 2 diabetes [1]. Because of the efficacy in achieving target levels of glycated hemoglobin (HbA1c) that has been shown in some studies, it has been used as an alternative to multiple daily insulin (MDI) therapy [2, 3]. In CSII therapy, basal insulin is supplied in a continuous infusion with a rapid (lispro, aspart, glulisine) or short acting (regular) insulin. The patients trigger the boluses before meals and blood glucose excursions outside the target range [4]. By mimicking physiological insulin release, it leads to greater flexibility in meals and activities and improves the quality of life.

Although indications for CSII therapy are more clear for type 1 diabetes mellitus, it is not distinct for patients with type 2 diabetes mellitus. Patients' desire to improvement of life quality, high activity levels that require changes in insulin doses, recurrent hypoglycemic episodes and planning

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a pregnancy, not meeting the glycemic goal, and dawn phenomenon are among the indications for CSII therapy [5]. Furthermore, some devices used with CSII therapy can receive glucose data from continuous glucose monitoring (CGM) devices, and others use that data and adjust basal rate delivery. Continuous glucose monitoring systems can facilitate optimal glycemic control.

The CSII therapy offers flexibility for patients their daily activities, leisure time, and diet than the patients on MDI and enables them to meet their personalized needs in management of diabetes [6]. A review of 74 studies shown that the CSII therapy provided better glycemic control and quality of life in both children and adults as compared to multiple daily injections of insulin [7]. Bayrakdar et al. showed that patients using CSII therapy have shown better quality of life scores than patients with MDI therapy in their study ($p = 0.001$) [8]. Furthermore, many studies have demonstrated that CSII therapy has been more effective in blood glucose regulation [2, 9, 10]. Misso et al. found that switching the therapy to CSII therapy resulted in a 0.3% increase in HbA1c [3]. However, Carlsson et al. found that CSII therapy was associated with 0.4% lower HbA1c levels after 1–2 years [11]. CSII therapy can delay the onset and slow progression of diabetes-associated complications, including retinopathy, nephropathy, and neuropathy by intensive glycemic control [12].

In this study, we aimed to explore the sociodemographic and clinical characteristics of the patients using CSII therapy. We also focused on the effects of the CSII therapy on some clinical-laboratory parameters like HbA1c levels, insulin dose requirements, the experience of diabetic ketoacidosis, lipid parameters, and chronic complications related to diabetes mellitus.

Materials and methods

This retrospective study enrolled 127 patients using CSII therapy and attended the Endocrinology department between January 2008 and December 2020. After a comprehensive review, 25 cases were excluded from the study due to inadequate data. A total of 102 patients were included. Differentiation of two types of diabetes mellitus was based upon a combination of the clinical presentation, family history, age, and body habitus. The diagnosis was often supported by laboratory studies such as antibodies, insülin, and C-peptide levels. The circulating, islet-specific pancreatic autoantibodies against glutamic acid decarboxylase 65 (GAD65) was measured. Also, low levels of C peptide levels which was measured after acute disease was indicator of the type 1 diabetes. Before starting CSII therapy, all of the patients were using at least four injections a day. One patient had type 2 diabetes mellitus with recurrent hypoglycemic episodes while 101 patients had type 1 diabetes mellitus with the following reasons for CSII therapy: poor metabolic control despite intensified insulin treatment, presence of low insulin requirements, the desire to have a more flexible lifestyle, correction of the dawn phenomenon, reduction of hypoglycemic emergencies, and improvement of the quality of life. Sociodemographic characteristics of the patients, total daily insulin doses, incidence of hypoglycemic attacks, episodes of diabetic ketoacidosis, HbA1c levels, lipid profile, usage of carbohydrate counting and glucose monitoring system, and presence of macrovascular and microvascular complications were all reported from data collected throughout clinic visits retrospectively.

Patients in the study were using same older sensor augmented insulin pumps and glucose monitoring systems.

The study protocol was approved by ethics committee of Uludag University Faculty of Medicine (2021–6/67).

Statistics

Statistical analyses were performed using the SPSS software version 15. The variables were investigated using visual and analytical methods to determine whether or not they are normally distributed. Descriptive analysis was presented using medians and interquartile range for non-normally distributed and ordinal variables. The Chi-square test or Fisher's exact test, where appropriate, was used to compare these proportions in different groups. Non-parametric tests were conducted to compare the non-normally distributed and ordinal variables. The Wilcoxon test was used to compare the change in the HbA1c levels, lipid profile, need of total daily insulin dosage, and episodes of diabetic ketoacidosis after switching to CSII therapy.

Results

A total of 102 patients were included in the study. The median age of the patients was 28 (20–44) years. Seventy-one patients (69.4%) were female. Sociodemographic and clinical characteristics of the patients are presented in Table 1. The median age of the patients at the diagnosis of diabetes mellitus was 12 (3–26) years. Cases of educational level with less than high school were 6.9% of the cases, educational level with high school were 53.9%, and educational level with university and above were 39.2% of the cases. Only one patient had type 2 diabetes mellitus, and recurrent hypoglycemic events were the indications for CSII therapy in these patients. Majority of the patients with type 1 diabetes mellitus desired to use CSII therapy because of recurrent hypoglycemic events.

The median HbA1c level was 9% (5.8–16.5) at the beginning of the CSII therapy. (48.1 %) of cases had a HbA1c

Table 1 Sociodemographic and clinical characteristics of the patients

Parameters		Results
Age (years)		28 (20–44)
Gender [female-*n*(%)/male-*n*(%)]		71 (69.4)/31(30.4)
Age at the diagnosis of diabetes mellitus (years)		12 (3–26)
Educational level (*n*-%)	Less than high school	7 (6.9)
	High school	55 (53.9)
	University and above	40 (39.2)
HbA1c levels (%)	≤7.5%	48.1
	7.5–9%	25.9
	≥9%	25.9
Duration of CSII therapy (years)		2 (1–10)
Using glucose monitoring system (*n*-%)		14 (13.7)
Using carbohydrate counting effectively (*n*-%)		13 (12.7)
Problems related to using CSII therapy (*n*-%)	No problems	34 (33.3)
	Problems with air bubbles disturbing insülin delivery	13 (12.7)
	Experience of diabetic ketoacidosis	1 (0.97)
	No improvement of hypoglycemic events	2 (1.94)
	Difficulty in using	2 (1.94)
	Others	9 (8.73)
	Unknown	41 (40.2)

HbA1c, glycated hemoglobin, *CSII*, continuous subcutaneous insulin infusion

level above 9; 25.9% of them had a HbA1c level between 7.5 and 9%, and 25.9% of them had a HbA1c level below 7.5%. The median duration of CSII therapy was 2 (1–10) years (Table 1). CSII therapy was discontinued in 10 cases during the follow-up period: 2 patients due to trouble using the pump, 1 case due to ketoacidosis triggered by air bubbles preventing insülin transmission, and 7 cases due to unknown reasons.

Fourteen cases (13.7%) started to use glucose monitoring system simultaneously with CSII therapy. All the cases included in the study had received education in carbohydrate counting, but 13 (12.7%) of the cases had been using carbohydrate counting effectively. Thirty-four patients (33.3%) did not experience any problems with CSII therapy. The problems encountered by the patients were air bubbles affecting insülin delivery, diabetic ketoacidosis, no improvement of hypoglycemic events, and having difficulty in using CSII therapy (Table 1).

Thirty-three patients (32.4%) had diabetic nephropathy, 24 patients (23.5%) had diabetic neuropathy, and 19 patients (18.6%) had diabetic retinopathy. Also, six patients (5.9%) had macrovascular complications. Patients with microvascular complications had higher levels of HbA1c than without microvascular complications, which was not statistically significant (respectively; HbA1c levels; 9.2 (6.6–16.5), 8.8 (6.10–13.2), $p=0.061$). But, there was no association between the levels of HbA1c and development of macrovascular complications ($p=0.760$).

Patients with higher levels of education were female ($p=0.049$); 62.5% of patients with university and above educational level were female, and 70.9% of patients with high school level were female. Patients who had educational level with university and above used carbohydate counting more efficiently than educational level with less than high school and with high school patients, but this difference was not statistically significant (respectively; 53.8%, 20%, 50%, $p=0.418$). However, there was no association between the usage of glucose monitoring system and educational levels of the patients. When we analyzed the association between educational levels of the patients and age of the diagnosis with diabetes mellitus, duration of the CSII therapy, need of total daily insulin dosage, lipid profile, and episodes of diabetic ketoacidosis at baseline and at the last visit of CSII therapy, we could not find any significant association.

It was seen that there was no association between the educational levels and development of macrovascular complications of diabetes mellitus. But higher rate of diabetic retinopathy, neuropathy, and nephropathy was seen in patients with less than high school education level than in patients with high school and above education level that was statistically insignificant (respectively $p=0.707$, $p=0.390$, $p=0.162$).

After switching to CSII therapy, patients' median HbA1c levels were reduced to 8.2% (5.3–14) at their last visit, which was statistically significant ($p=0.005$). Patients who started to use glucose monitoring system simultaneously with CSII therapy had lower HbA1c levels than patients

without glucose monitoring system at the last visit of CSII therapy, which was not statistically significant [respectively; 8.25% (6.4–12.1), 8.9% (6–16.5), $p=0.152$]. Also, the total daily insulin dosage with 48 IU/day (7.3–180) was significantly decreased to 40 IU/day (15–276) after CSII therapy ($p=0.001$). However, the lipid profile [triglyceride, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL)] measured at the start of CSII therapy and measured at the last visit during CSII therapy did not vary significantly. CSII therapy was associated with less episodes of diabetic ketoacidosis at the last visit than at the initiation of CSII therapy, which was statistically significant ($p=0.033$) (Table 2). Hypoglycemic events were decreased in the majority of patients; however, two patients discontinued CSII therapy due to ongoing hypoglycemic attacks.

Discussion

CSII therapy has been shown to improve general health, quality of life, and treatment satisfaction of patients [13]. CSII is a preferable with lower daily insulin requirements, reduced risk of hypoglycemia, and better patient satisfaction [14]). Continuous subcutaneous insulin infusion therapy has been also known to be effective treatment for improving glycemic control [15]. DeVries et al. found a reduction of hemoglobin A1c at 0.84% at 16 weeks in patients with poor glycemic control after switching to CSII therapy [10]. This study supported that switching to CSII therapy resulted in a statistically significant decrease in patients' median HbA1c levels and similar to the study of DeVries et al., patients with HbA1c levels above 9% constituted the majority of our study's participants. In contrast, Bruttomesso et al. demonstrated no difference on HbA1c levels between CSII therapy and MDI therapy [16]. Weissberg et al. also showed that there has been a significant reduction of HbA1c with the CSII therapy in a meta-analysis. The reduction of HbA1c was found to be consistent with the use of CSII therapy for more than 1 year in this meta-analysis ($p=0.001$) [17]. In our study, the median duration of using CSII therapy was 2 (1–10) years, and 96.6% of the patients had been using CSII therapy for more than 1 year. According to Hoogma et al., hypoglycemic events were on the rise in patients using MDI therapy [9]. In this study, majority of the patients intended to use CSII therapy because of recurrent hypoglycemic events and during CSII therapy only two patients had no improvement in hypoglycemic events.

Many studies demonstrated that requirements of insulin dosage were significantly lower in patients receiving CSII therapy [9, 10, 16, 18]. This study also found that total daily insulin dosage was significantly decreased after CSII therapy ($p=0.001$). Although, DeVries et al. found no difference in ketoacidotic episodes between CSII therapy and MDI therapy, in our study, less episodes of diabetic ketoacidosis were seen after switching to CSII therapy, which was statistically significant ($p=0.033$).

In addition, Almogbel et al. showed a significant decrease in the levels of triglyceride, total cholesterol, and LDL levels with CSII therapy [19]. Moreover, Schreiver et al. reported significant decreases in LDL cholesterol, total cholesterol, triglycerides, and VLDL cholesterol levels within 2–4 weeks of CSII therapy [20]. In this study, we could not find significant change in lipid parameters after switching to CSII therapy. This may be due to the lower percentage of participants who had dyslipidemic LDL, total cholesterol, triglyceride, and HDL cholesterol levels when the CSII therapy was started.

Glycemic control is one of the important predictors of complications related with diabetes mellitus [21–23]. Clinical studies have shown that controlling hyperglycemia lowers the risk of developing microvascular complications [24]. In our study, patients with microvascular complications had higher levels of HbA1c than without microvascular complications as similar in literature ($p=0.061$). Nonetheless, there was no association between the levels of HbA1c and development of macrovascular complications ($p=0.760$). This may be due to the fact

Table 2 Comparison of some clinical and laboratory parameters at the beginning of CSII therapy and at the last visit during CSII therapy

Parameters	At baseline of CSII therapy	At the last visit	*p* value
HbA1c (%)	9 (5.8–16.5)	8.2 (5.3–14)	**0.005**
Total daily insulin dosage (IU/day)	48 (7.3–180)	40 (15–276)	**0.001**
Triglyceride (mg/dL)	88 (13–776)	89 (32–564)	0.122
Total cholesterol (mg/dL)	187 (17–299)	179 (92–303)	0.316
LDL (mg/dL)	109 (58–177)	105 (36–254)	0.284
HDL (median-mg/dL)	55 (30–90)	54 (21–105)	0.296
Episodes of diabetic ketoacidosis (*n*-%)	30 (29.4)	21 (32.3)	**0.033**

CSII, continuous subcutaneous insulin infusion, *HbA1c*, glycated hemoglobin, *LDL*, low density lipoprotein, *HDL*, high density lipoprotein

A *p*-value of less than 0.05 was considered to show a statistically significant result

that macrovascular complications are more multifactorial. The United Kingdom Prospective Diabetes Study (UKPDS) found that intensive blood glucose control alone did not reduce macrovascular complications significantly [25]. Also, Gaede et al. suggested that the risk of macrovascular complications could be reduced when glucose, blood pressure, and lipid levels were targeted simultaneously [26].

The combination of a CSII system with a continuous glucose monitoring (CGM) system is another very promising therapeutic option. Floyd et al. had shown that CGM was associated with lower HbA1c levels than self-monitoring blood glucose [27]. In our study, patients using glucose monitoring system simultaneously with CSII therapy had lower HbA1c levels than patients without glucose monitoring system at the last visit of CSII therapy, which was not statistically significant ($p = 0.152$).

This study also emphasized identifying the patients' sociodemographic characteristics which can be associated with improved glycemic control, complications related to diabetes, and clinical parameters. Patients with educational level university and above, used carbohydate counting more efficiently than patients with lower levels of education which was not statistically significant ($p = 0.418$). Although, Gomez et al. demonstrated that metabolic control was improved with a higher educational level, we found no association between the educational levels of the patients and glycemic control [28]. Interestingly, higher rate of the diabetic retinopathy, neuropathy, and nephropathy was seen in patients with educational level lower than high school than others that was statistically insignificant ($p = 0.162$).

There were some limitations of the study. Firstly, the economic burden on healthcare insurance systems affects wider use of insulin pumps and also use of glucose monitoring system. The type of insulin pump and the generation of glucose sensors decide the accuracy and outcomes. The patients in our study were using the same older sensor augmented insulin pumps, and only 13.7% of the cases were using glucose monitoring system. We could not determine the glucose variability which may be associated with diabetic complications because of socioeconomic factors. Secondly, due to retrospective nature of the study, we could not determine self-monitoring of the blood glucose levels of the patients. Thirdly, we failed to take account of changing in body mass index of the patients, in physical activity after switching to CSII therapy which can affect the complications and laboratory parameters.

Conclusion

Continuous subcutaneous insulin infusion therapy leads to better glycemic control with lower insulin requirements, reduced HbA1c levels, and less episodes of diabetic ketoacidosis than MDI therapy. CSII can thus be considered a valuable therapy option in adult patients with diabetes mellitus.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Yasemin Aydoğan Ünsal, Özen Öz Gül, and Büşra Gürbüz. The first draft of the manuscript was written by Yasemin Aydoğan Ünsal, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability and material (data transparency) Not applicable.

Code availability (software application or custom code) Not applicable.

Declarations

Ethics approval and consent to participate Approval was obtained from the Ethics Committee of Uludag University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Consent for publication Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

References

1. Thompson B, Korytkowski M, Klonoff DC, Cook CB. Consensus statement on use of continuous subcutaneous insulin infusion therapy in the hospital. J Diabetes Sci Technol. 2018;12(4):880–9.
2. Pickup J, Mattock M, Kerry S. Glycaemic control with continuous subcutaneous insulin infusion compared with intensive insulin injections in patients with type 1 diabetes: meta-analysis of randomised controlled trials. BMJ. 2002;324:705.
3. Misso ML, Egbert KJ, Page M, O'Connor D, Shaw J. Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. Cochrane Database Syst Rev. 2010;20(1):CD005103. https://doi.org/10.1002/14651858.CD005103.pub2.
4. Weinstock RS. Continuous subcutaneous insulin infusion (Insulin pump). [Internet]. Uptodate 2021. (Access Feb 2021). https://www.uptodate.com/contents/continuous-subcutaneous-insulin-infusion-insulin-pump?search=continuous%20insulin%20infusion&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1.
5. Yao P, Tadi P. Insulin Pump. [Updated 2020 Jul 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555961/.
6. Linkeschova R, Raoul M, Bott U, et al. Less severe hypoglycaemia, better metabolic control, and improved quality of life in type 1 diabetes mellitus with continuous subcutaneous insulin infusion (CSII) therapy: an observational study of 100 consecutive patients followed for a mean of 2 years. Diabet Med. 2002;19:746–51.
7. Cummins E, Royle P, Snaith A, Greene A, Robertson L, McIntyre L, Waugh N. Clinical effectiveness and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes:

systematic review and economic evaluation. Health Technol Assess. 2010;14(11):iii–iv, xi–xvi, 1–181. https://doi.org/10.3310/hta14110.
8. Bayrakdar A, Noureddine S, Farhood L, Nasrallah MP. Comparison of quality of life in a group of Lebanese type 1 diabetics on insulin pump and those on multiple daily injections. J Med Liban. 2014;62(1):22–6. https://doi.org/10.12816/0002623.
9. Hoogma RP, Hammond PJ, Gomis R, Kerr D, Bruttomesso D, 5-Nations Study Group, et al. Comparison of the effects of continuous subcutaneous insulin infusion (CSII) and NPH-based multiple daily insulin injections (MDI) on glycaemic control and quality of life: results of the 5-nations trial. Diabet Med. 2006;23(2):141–7.
10. DeVries JH, Snoek FJ, Kostense PJ, Masurel N, Heine RJ, Dutch Insulin Pump Study Group. A randomized trial of continuous subcutaneous insulin infusion and intensive injection therapy in type 1 diabetes for patients with long-standing poor glycemic control. Diabetes Care. 2002;25(11):2074–80.
11. Carlsson BM, Attvall S, Clements M, Gumpeny SR, Pivodic A, et al. Insulin pump-long-term effects on glycemic control: an observational study at 10 diabetes clinics in Sweden. Diabetes Technol Ther. 2013;15(4):302–7.
12. Nathan DM, DCCT/EDIC Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care. 2014;37(1):9–16.
13. Abdul-Rasoul M, AlOtaibi F, Abdulla A, et al. Quality of life of children and adolescents with type 1 diabetes in Kuwait. Med Princ Pract. 2013;22:379–84.
14. Pozzilli P, Battelino T, Danne T, Hovorka R, Jarosz-Chobot P, et al. Continuous subcutaneous insulin infusion in diabetes: patient populations, safety, efficacy, and pharmacoeconomics. Diabetes Metab Res Rev. 2016;32(1):21–39. https://doi.org/10.1002/dmrr.2653.
15. Diabetes C, Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977–86.
16. Bruttomesso D, Crazzolara D, Maran A, Costa S, Dal Pos M, et al. In Type 1 diabetic patients with good glycaemic control, blood glucose variability is lower during continuous subcutaneous insulin infusion than during multiple daily injections with insulin glargine. Diabet Med. 2008;25(3):326–32.
17. Weissberg-Benchell J, Antisdel-Lomaglio J, Seshadri R. Insulin pump therapy. A meta-analysis. Diabetes Care. 2003;26:1079–87.
18. Hanaire-Broutin H, Melki V, Bessieres-Lacombe S, Tauber J. Comparison of continuous subcutaneous insulin infusion and multiple daily injection regimens using insulin lispro in type 1 diabetic patients on intensified treatment. Diabetes Care. 2000;23(9):1232–5.
19. Almogbel E. Impact of insulin pump therapy on glycemic control among adult Saudi type-1 diabetic patients. An interview-based case-control study. J Family Med Prim Care. 2020;9(2):1013–9.
20. Schreiver C, Jacoby U, Watzer B, Thomas A, Haffner D, et al. Glycaemic variability in paediatric patients with type 1 diabetes on continuous subcutaneous insulin infusion (CSII) or multiple daily injections (MDI): a cross-sectional cohort study. Clin Endocrinol (Oxf). 2013;79:641–7.
21. Writing Team for the Diabetes C, Complications Trial/Epidemiology of Diabetes I, Complications Research G. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA. 2002;287:2563–9.
22. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, et al. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. N Engl J Med. 2003;348:2294–303.
23. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353:2643–53.
24. Bate KL, Jerums G. 3: Preventing complications of diabetes. Med J Aust. 2003;179(9):498–503. https://doi.org/10.5694/j.1326-5377.2003.tb05655.x.
25. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. BMJ 1998;317:703–13.
26. Gaede P, Vedal P, Larsen N, et al. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med. 2003;348:383–93.
27. Floyd B, Chandra P, Hall S, et al. Comparative analysis of the efficacy of continuous glucose monitoring and self-monitoring of blood glucose in type 1 diabetes mellitus. J Diabetes Sci Technol. 2012;6(5):1094–102.
28. Gómez-Peralta F, Menéndez E, Conde S, Conget I, Novials A, ennombre de la SED y de losinvestigadores del estudio SED1. Clinical characteristics and management of type 1 diabetes in Spain. The SED1 study. Endocrinol Diabetes Nutr. 2021;2:S2530-0164(21)00003-S2533. https://doi.org/10.1016/j.endinu.2020.11.002 English, Spanish.

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ORIGINAL ARTICLE

The effect of 12 weeks of training in water on serum levels of SIRT1 and FGF-21, glycemic index, and lipid profile in patients with type 2 diabetes

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Abstract

Introduction Diabetes is one of metabolic diseases. In the patients with diabetes, glucose uptake is decreased and fibroblast growth factor 21 (FGF-21) plays an important role in glucose uptake. Also, Sirtuin 1 (SIRT1) is a protein regulating glucose and fat metabolism. Therefore, this study was performed to determine the effect of 12 weeks of exercises in water on serum levels of SIRT1 and FGF-21, glycemic index, and lipid profile in women with type 2 diabetes.

Materials and methods For this purpose, 40 women with type 2 diabetes (with mean age of 43 years old and body mass index (BMI) of 31 kg/m^2) were randomly divided into control ($n=20$) and experimental ($n=20$) groups. The exercise program was performed in water for 12 weeks, with an intensity of 45–65% of maximum heart rate. Blood samples were used for pre-test and post-test to evaluate serum levels of SIRT1 and FGF-21, glycemic index, and lipid profile. Then, data were analyzed using paired-samples *t*-test and independent-samples *t*-test at significance level of $p<0.05$.

Results The results of statistical analysis showed that the water exercise program significantly increased serum levels of FGF-21, SIRT1, and high-density lipoprotein cholesterol (HDL-C) in women with type 2 diabetes. In contrast, this type of exercise significantly reduced resistance to insulin, glucose, insulin, total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C).

Conclusion Based on results of the present study, it seems that exercise in water may play an effective role in glucose homeostasis and reduction of body fat mass, by increasing levels of SIRT1 and FGF-21 and it should be considered to prevent from development of type 2 diabetes. It can also be used as a type of exercise recommended by sports and health professionals in these people.

Keywords Training · SIRT1 · FGF-21 · Glycemic index · Diabetes

Introduction

Diabetes is the most common chronic disease and one of the most important health, medical, and economic problems in the world leading to about 4 million deaths annually [1]. It has been estimated that prevalence of diabetes will increase from 9.3% in 2019 to 10.2% in 2030 [1]. Diabetes causes major changes in most systems of the body and causes immediate or late complications of the disease. This disease leads to complications, such as cardiovascular complications, nephropathy, hypertension, and retinopathy [2]. The patients with diabetes have low levels of SIRT1 and FGF-21 [3]. SIRT1 is a nicotinic adenosine-dependent deacetylating protein in the nucleotide (nicotinamide adenine dinucleotide (NAD)/nicotinamide adenine dinucleotide + hydrogen (NADH)) regulating glucose/fat metabolism through its deacetylation activity on many substrates and has a positive effect on pancreatic beta cells. Insulin secretion protects cells from oxidative stress and inflammation and plays an important role in insulin signaling in fat and muscle cells [4]. It is also involved in function and biosynthesis of mitochondria and improvement of aerobic metabolism; thus, suppression of SIRT1 causes systemic inflammation, increases oxidative stress, and decreases aerobic metabolism [5]. Given that

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mitochondrial function is beyond boundaries of cells and regulates communication between cells and tissues, then it influences physiology of the organism; therefore, mitochondrial dysfunction is a major cause of many diseases, such as diabetes and neurodegenerative diseases [2]. Mitochondrial biogenesis causes the body to undergo various physiological conditions including the increase in the ability of skeletal muscles after exercise and normal tissue response to thyroid hormone [6]. Therefore, a decrease in SIRT1 levels may be one of the main mechanisms of disease progression [7]. SIRT1 also positively regulates insulin secretion in beta-pancreatic cells and protects cells from oxidative stress and inflammation [3].

FGF-21 is one of the most important and well-known hepatokines for improving glucose metabolism in the liver, skeletal muscle, and adipose tissue [8] and plays an important regulatory role in glucose and fat metabolism in humans. It also stimulates glucose uptake in adipocytes by inducing glucose transporter 1 (GLUT-1) and inhibits glucagon secretion by pancreatic alpha cells [9]. Laboratory studies on FGF-21 level have reported that FGF-21 signaling cascade requires fibroblast growth factor receptors (FGFR) and β-Klotho cofactors, and that fibroblast growth factor receptors 1–4 (FGFR1-4) and β-Klotho were highly expressed in liver, adipose, and pancreatic tissues [10]. When β-Klotho binds to FGF-21, insulin sensitivity and glucose metabolism are stimulated thus, controlling glucose [10]. Recently, FGF-21 has been shown to act as a hormone regulating glucose tolerance [11] and is increased in metabolic disorders, such as diabetes, obesity, fatty liver, and metabolic syndrome. It is also positively correlated with body mass index (BMI) and insulin resistance and has attracted a lot of attention as a promising molecule for treatment of obesity and type 2 diabetes [8, 12]. Research has shown that FGF-21 increases glucose uptake separately from insulin in adipocytes [13]. This stimulatory effect is mediated by FGF-21 through GLUT 1 expression, modulating the increase in insulin-independent glucose uptake into adipocytes [13]. It seems that FGF-21 can have therapeutic potential and can be used as a useful agent for treatment of metabolic diseases, such as diabetes. The effect of physical activity on beta cell's function and diabetes has been reported in several studies [14, 15]. Activation of SIRT1 during exercise to increase oxidative capacity induces mitochondrial biogenesis [14]. Exercise causes extensive changes in cellular metabolism and NAD + to NADH ratios [15]. Regular exercise restores SIRT1 levels in the kidneys and liver, normalizes cellular metabolism, and reduces severity of disease [15]. In a study, Hong et al., (2016) concluded that performing 40 min of exercise in water for 12 weeks significantly increased SIRT1 levels [16]. SUWA et al. reported a positive correlation between physical activity, SIRT1 activation, and glucose metabolism, and particularly, they showed that low-intensity exercise increased expression of SIRT1 and the glucose transporter 4 (GLUT4) [17]. Sarga et al. also found that endurance training increased SIRT1 levels [18]. The results of studies on the effects of exercise on FGF-21 level are also contradictory. Some studies have reported the elevated serum levels of FGF-21 following physical activity, whereas in the study by Besse-Patin et al., 8 weeks of endurance training had no effect on serum levels of FGF-21 [19]. On the other hand, Yang et al. (2011) reported that serum FGF-21 levels were decreased in obese women after 3 months of physical activity [20]. Since physical activity has significant effects on metabolic disorders caused by obesity, type 2 diabetes, and heart disease, the effect of physical activity on FGF-21 levels has recently been considered [11]. It has been shown that 8 weeks of physical activity leads to the increase in FGF-21 levels [11]. Also, a decrease in physical activity reduces energy expenditure and can play a role in development of overweight. According to the previous research, the reduced physical activity and weight gain both lead to insulin resistance, which is a defining factor in development of type 2 diabetes [21]. Among sports, the effect of water sports on SIRT1 and FGF-21 levels has been less studied so far. Water is an environment that provides the necessary resistance to needs of each person, then it causes muscle activity and involvement of larger muscle groups to overcome resistance, and it can be very important in increasing mechanical pressure on muscles. Also, unlike other sports, water sports cause involvement of both upper and lower limbs and increase metabolism in the body [22].

Given that in the people with diabetes, the liver cannot convert glucose to glycogen or, if needed, releasing it into bloodstream in the form of glucose, this adds to the challenge of controlling blood glucose. On the other hand, the possibility for development of fatty liver due to high insulin resistance and FGF-21 and SIRT1 levels plays an important role in reducing insulin resistance and increasing glucose, but so far, there has been no report on the effect of exercise in water on SIRT1 and FGF-21 levels in the patients with diabetes. Now the question is: Does exercise in water influence SIRT1 and FGF-21 levels, glycemic index, and lipid profile in women with type 2 diabetes? Therefore, this study was done to investigate the effect of exercise in water on serum levels of SIRT1 and FGF-21, glycemic index, and lipid profile in women with type 2 diabetes.

Methodology

The present quasi-experimental pretest–posttest and applied study was performed on the women with type 2 diabetes in Urmia City (West Azerbaijan Province, Iran) aged between 40 and 45 years old. Eighty people announced their willingness to participate in the study through making calls and

referral to the Urmia Diabetes Association, of which 40 eligible subjects who had no history of regular exercise during the previous 2 years voluntarily participated as subjects of the study. Inclusion criteria were having type 2 diabetes, fasting glucose above 126 mg/dL, glycosylated hemoglobin between 6.5 and 9.9%, treatment with oral hyperglycemia drugs and diet (without insulin injection), having no history of any regular exercise, and the ability to exercise in the water. Exclusion criteria also included having any cardiovascular, respiratory, neuropathy, retinopathy, other chronic diseases and musculoskeletal disorder, unwillingness to participate in research, consumption of dietary supplements, and lack of regular attendance at training sessions.

Method of data collection

Level of physical activity of the subjects was determined by a general physical activity questionnaire. Also, before implementation of the research protocol, the necessary examinations of all the subjects were performed under supervision of a specialist and the participants were asked about not taking any dietary supplements and then, they were allowed to enter the research based on the specialist's opinion. Before starting the study, in a briefing session of all the programs, correct way of performing the exercises and the possible risks were explained to the participants. In this form, it was emphasized that participation and withdrawal from the study by the volunteer are completely free and optional and all the information of the volunteer is completely confidential and the results of the research will be published in the form of general and group information, which was a limitation of this study. The subjects did not have strict control over their diet, so they were asked not to change their usual daily diet and to avoid taking any extra food and supplements. Subjects were randomly divided into experimental and control groups ($N=20$) after getting acquainted with the training programs. During the study period, 8 subjects were excluded from the study due to not attending more than 3 consecutive sessions, traveling, and non-participation in the post-test; thus, finally, each group included 20 people.

Aerobic capacity and body composition of the subjects using a treadmill

Aerobic capacity was determined using treadmill (Techno-Gym S.p.A, Class: RUNRACE 1400HC, Italy) and body composition analysis was recorded using a pneumatic composite device made in South Korea (InBody 720). Bruce protocol treadmill was used to measure aerobic capacity using the following equation.

$$\mathrm{VO2max} = 14.76 - 1.379(\mathrm{time}) + 0.451(\mathrm{Time}) - 0.012(\mathrm{time})$$

In this protocol, working pressure is increased by changing speed and slope percentage. In the first stage of the test (0–3 min), people start running with a speed of 1.7 mph.At the beginning of the second stage (4–6 min), the slope is increased by 2% and the speed is increased to 2.5 mph. In the next stage of the test, the slope is elevated by 2% and the speed is increased by 0.8 or 0.9 mph and continues until the subject becomes exhausted [23].The subjects' BMI ranged from 25 to 30 kg/m^2.

Exercise protocol

In the present study, all training steps were performed for the experimental group in the pool and in a shallow water area. The training program in each session lasted 1 h. Each water training session had three stages: step 1: Adapting to the water environment and warming up (15 min) including stretching movements in all joints and major muscle groups, walking forward, backward, sideways, standing on the heel, and jogging in the water. The second stage, the training stage (30 min) included weight transfer from front to back, fast walking in the water, walking sideways, imitating movement of a football shot, imitating throwing ball by hand in front of the body, moving legs closer and closer to the hands, and then, standing and squatting from center of the body. The third stage consisted of stretching, deep breathing, and floating exercises (15 min). These exercises were performed in an indoor pool with a water temperature between 26 and 28 °C [23]. The water training protocol included a gradual increase in training intensity. For this purpose, during 12 weeks, the training period was increased as it approached the end of the study. For controlling training intensity, the maximum heart rate was used and for obtaining the maximum heart rate of the subjects, the 220-age formula was used. During the training period, the heart rate started from 45% of the maximum heart rate and at the end of the training, it was increased to 65% of the maximum heart rate so that, the number of training sessions in the first 4 weeks included 3 sessions per week with an intensity of 45% of the maximum heart rate and in the second 4 weeks, it was increased to 4 sessions with an intensity of 55% of the maximum heart rate and in the third 4 weeks, it was increased to 5 sessions per week with an intensity of 65% of the maximum heart rate [23]. This training protocol was also implemented according to the recommendation by the American college of sports medicine (ACSM) for the elderly [23]. For controlling intensity of exercise, heart rate was measured 3 times per session including before and after the main exercises in water, and once during cooling using a Polar heart rate monitor made in Finland.

Laboratory analysis

For analyzing the research variables, 10 ml of blood was taken from brachial vein of each individual's left hand after 12 h of fasting in the pre-test (beginning of the study) and post-test (48 h after the last training session in order to eliminate the effects of the last training session) in laboratory conditions and the blood sample was immediately poured into anticoagulant tubes containing EDTA (ethylene diamine tetraacetic acid). After collecting the samples, the tubes containing blood samples were centrifuged at 3000 rpm for 20 min and the isolated serum was stored in a − 70 °C freezer to measure blood factors. Blood sampling was performed between 8 and 11 am to observe circadian rhythm of hormone secretion. For measuring the amount of SIRT1, Casabayo kit (China) was used with a sensitivity of less than 0.039 ng/mL and a coefficient of change in the test less than 8, and coefficient of change outside the test of 10%. Serum levels of FGF-21 were measured using enzyme-linked immunosorbent assay (ELISA) method and assay kit ((ZellBio) GmbH, Ulm, Germany) with a sensitivity of 2.5 pg/mL, coefficient of change in the test less than 10%, and coefficient of change outside the test less than 12%. Blood glucose levels were measured using a special glucose kit made by Pars Azmoon Company (Iran) with a sensitivity of 5 mg/dL, coefficient of change within the test of 1.49%, and the coefficient of change outside the test of 0.69%. Insulin levels were measured by the ELISA kit (MERCODIA, Sweden) with a sensitivity of 1 mU/L, coefficient of change within the test of 3.2%, and the coefficient of change outside the test of 2.8%. Insulin resistance was determined by hemostasis model assessment (HOMA) based on fasting blood glucose in mmol/L at fasting insulin concentration in mL/L divided by a fixed number of 22.5 [24].

$$(\text{Plasma insulin (microunits/dL)} * \text{plasma glucose (mmol/L)})/22.5 = \text{insulin resistance}$$

Also, cholesterol was measured using a kit made by Pars Azmoon Company (Iran) with a sensitivity of 5 mg/dL, coefficient of change within the test of 0.64%, and the coefficient of change outside the test of 9.5%. Level of triglycerides was measured using a kit made by Pars Azmoon Company with a sensitivity of 5 mg/dL, coefficient of change within the test of 1.47%, and the coefficient of change outside the test of 1.06%. High-density lipoprotein (HDL) was measured using a Pars Azmoon kit with a sensitivity of 1 mg/dL, coefficient of change within the test of 0.82%, and the coefficient of change outside the test of 1.08%. LDL-C was also measured using Pars Azmoon kit with a sensitivity of 1 mg/dL, coefficient of change within the test of 0.67%, and the coefficient of change outside the test of1.45%.

Statistical methods

Shapiro–Wilk test was used to check normality of data distribution, according to which normal distribution of the data was confirmed. Correlated pairs *t*-test was used to compare the results within the group and independent-samples *t*-test was used to evaluate the results between groups. All the statistical analyses were performed by SPSS software version 23 at a significant level of 5%.

Results

First, the hypothesis of normal distribution of the data was tested and confirmed by Shapiro–Wilk test. Also, for further confirming the assumption that the two groups were identical at the beginning of the training cycle, the independent-samples *t*-test was used. The results of this test showed no significant difference in none of anthropometric variables including age, height, weight, fat percentage, BMI, and maximum oxygen consumption (Table 1). On the other hand, the results of the correlated pairs *t*-test showed that among anthropometric variables, weight, fat percentage, and BMI were significantly reduced in the post-test compared to the pre-test in the experimental group. However, in the control group, a slight increase was observed in these variables in the post-test, but this increase was not statistically significant (Table 1).

The results of the paired-samples *t*-test showed that 12 weeks of training in water significantly increased serum levels of SIRT1, FGF-21, and HDL in women with type 2 diabetes compared to baseline conditions. Also, it significantly reduced weight, BMI, body fat percentage, and levels of triglyceride, total cholesterol, insulin resistance, glucose, insulin, and LDL compared to baseline conditions, while no significant difference was observed in the control group ($p > 0.05$).

Also, the results of independent-samples *t*-test showed a significant difference in mean values of SIRT1 ($p = 0.000$), FGF-21 ($p = 0.000$), body fat percentage ($p = 0.001$), triglyceride ($p = 0.001$), glucose ($p = 0.000$), BMI ($p = 0.001$), ($p = 0.000$) HDL-C, LDL-C ($p = 0.002$), cholesterol ($p = 0.001$), insulin ($p = 0.000$), and insulin resistance ($p = 0.002$) between the experimental and control groups ($p < 0.05$) (Table 2).

Discussion

The present study was conducted to evaluate the effect of 12 weeks of training in water on serum levels of SIRT1, FGF-21 and insulin resistance index, and lipid profile in

Table 1 Mean, standard deviation, and independent *t*-test results

Variables	Groups	Count	Pre-test	Post-test	Levene test *p*-value	Independent *t*-test *p*-value
Age (years)	Control	20	4.4 ± 43.5	-	0.817	0.386
	Experimental	20	8.3 ± 43.7	-		
Height (cm)	Control	20	3.3 ± 168.8	-	0.414	0.276
	Experimental	20	6.2 ± 168.4	-		
Weight (kg)	Control	20	2.42 ± 86.82	0.4 ± 86.79	0.702	0.994
	Experimental	20	0.44 ± 86.83	*0.78 ± 82.4		
Body fat percentage (%)	Control	20	0.34 ± 25.5	0.3 ± 28.3	0.364	0.436
	Experimental	20	2.3 ± 28.62	*2.4 ± 26.8		
BMI (weight/height squared)	Control	20	1.4 ± 30.78	0.4 ± 29.1	0.836	0.969
	Experimental	20	4.3 ± 30.31	*0.3 ± 27.06		

*Significance relative to pre-test values in each group using paired *t*-test ($p < 0.05$)

women with type 2 diabetes. Our results showed that exercise in water increased serum level of SIRT1 in women with type 2 diabetes. A number of studies have investigated the effect of different sports exercises on these indicators in the past, some of which are consistent with the present study [22, 25] and some are inconsistent [26]. Little et al. reported that HIIT training for 2 weeks in 7 young men increased SIRT1 levels [25]. Despite the above results, findings of some previous studies have indicated that SIRT1 levels did not change significantly after exercise. Gurd et al. showed no change in SIRT1 level after 6 weeks of HIIT training with 90% of oxygen intensity [26]. This discrepancy between the findings can be attributed to the type of training, intensity of training, type of subjects, and level of readiness of individuals. In fact, researchers have suggested that exercise increases expression and levels of SIRT1 by depleting

Table 2 Intragroup and intergroup changes in SIRT1, FGF-21, glycemic index, and lipid profile in control and experimental groups

Variables	Groups	Count	Pre-test	Post-test	Intragroup changes *p*-value	Intergroup group changes *p*-value
SIRT1 (ng/mL)	Control	20	7.35 ± 0.8	7.32 ± 0.1	0.221	0.000*
	Experimental	20	7.31 ± 0.11	9.61 ± 1.15#	0.000*	
FFG21 (Pg/mL)	Control	20	249 ± 0.8	249.3 ± 2.1	0.559	0.000*
	Experimental	20	249.4 ± 2.12	269.6 ± 0.8#	0.000*	
Insulin (IU/L)	Control	20	10.32 ± 0.1	10.49 ± 0.31	0.455	0.002*
	Experimental	20	10.3 ± 0.1	9.3 ± 0.14	0.000*	
Glucose (Mmol/L)	Control	20	90.69 ± 0.43	90.7 ± 0.52	0.453	0.000*
	Experimental	20	90.59 ± 0.42	82.4 ± 0.7	0.001*	
HOMA-IR	Control	20	45.2 ± 0.13	45.5 ± 0.48	0.833	0.001*
	Experimental	20	45.2 ± 0.17	34.2 ± 0.51	0.002*	
TG (mg/dL)	Control	20	128.1 ± 0.57	128.01 ± 3.51	0.914	0.000*
	Experimental	20	128.1 ± 3.54	111.1 ± 0.56	0.001*	
TC (mg/dL)	Control	20	172.2 ± 1.5	171.5 ± 1.03	0.392	0.001*
	Experimental	20	171.1 ± 1.15	161.7 ± 1.1	0.001*	
LDL-C (mg/dL)	Control	20	101.96 ± 0.66	102.22 ± 0.6	0.648	0.000*
	Experimental	20	102.06 ± 0.62	91.51 ± 0.95	0.002*	
HDL-C (mg/dL)	Control	20	42.5 ± 0.63	42.54 ± 1.51	0.270	0.002*
	Experimental	20	42.28 ± 0.59	47.85 ± 3.48	0.000*	

*Significance relative to pre-test values

#Significance compared to the water exercise group

cellular charge through phosphate- and calcium-dependent pathways and activity of AMP-activated protein kinase (AMPK)- and calmodulin-dependent kinase enzymes [26]. SIRT1 is involved in reactive oxygen species (ROS) production and inflammation so that, SIRT1 level is decreased by increasing inflammation and ROS production, and its activity is impaired. Another reason for the increase in SIRT1 level observed in the present study is reduction of ROS and inflammation caused by diseases, such as diabetes and obesity, because it has been confirmed that exercise increases the body's antioxidant capacity and this factor reduces inflammation and ROS [22, 27]. Increasing the SIRT1 index in the body increases mitochondrial biogenesis and elevates athletic performance [25]. This factor increases maximum oxygen consumption (VO2MAX), increases capacity and amount of enzymes in beta-oxidation cycle, and adapts the body to using less from body's sugar reserves and instead using more from body's fat reserves [22]. These factors can be considered as reasons for weight loss and reduction of body fat percentage observed in this study.

SIRT1 deacetylates and activates a wide range of metabolic regulators including PGC-1, thereby inducing mitochondrial biogenesis. SIRT1 expression is decreased in the people suffering from the decreased insulin sensitivity. Overexpression of SIRT1 is associated with the improved insulin sensitivity [28]. Therefore, it has been suggested that SIRT1 activation is probably a new method for prevention and treatment of type 2 diabetes that is associated with the improved glucose homeostasis and the decreased insulin resistance [2, 29]. It also has a positive role in pathway of insulin signaling in fat cells and muscle tissues and is involved in function and biosynthesis of mitochondria and improves aerobic metabolism and also its expression is associated with the improved insulin sensitivity [7]. SIRT1 also increases lipolysis, inhibits lipogenesis, and is involved in reverse cholesterol transport [3]. The present study showed significant decrease in LDL-C and triglycerides in the experimental group but no change was observed in the control group. Consistent with the present study, Oberbach et al. reported a significant reduction in triglycerides and LDL-C after 4 weeks of aerobic exercise [30]. Also in the present study, cholesterol was reduced and HDL-C was significantly increased. These results were consistent with the results of Kadglou et al. They reported a significant decrease in LDL-c and cholesterol as well as a significant increase in HDL-C after 16 weeks of aerobic exercise with an intensity of 50–85% VO2max [31]. Also, the results were inconsistent with the results of research by Bello et al. They did not observe any significant change in HDL-C after 8 weeks of aerobic exercise with an intensity of 50–75% of maximum heart rate and suggested a longer duration of training for a significant change in these parameters [32].

Systemic induction of FGF-21 prevents obesity, lowers glucose and insulin resistance, and decreases triglyceride and low-density lipoprotein (LDL) levels [33]. FGF-21 is a metabolic hormone secreted from liver and muscle tissues in response to various physiological and pathological stressors, such as autophagy, mitochondrial dysfunction, drug intoxication, and exercise [24]. In the present study, it was found that 12 weeks of exercise in water significantly increased serum levels of FGF-21 in women with type 2 diabetes compared to baseline conditions. There was also a significant difference between the experimental and control groups. FGF-21 has been shown to be positively correlated with changes in serum free fatty acid (FFA) levels. Various studies have investigated the metabolic effects of exercise and stated that these exercises stimulate oxidation of fatty acids; thus, this type of exercise is able to increase lipolysis of adipose tissues and their transfer to bloodstream and their oxidation in muscle tissue [24, 34]. Therefore, in the present study, an increase in FGF-21 level after performing exercise in water could be associated with an increase in transport of fatty acids in bloodstream (to transfer FFA to muscle tissue for catabolism) [35]. The exact mechanism regarding the effect of exercise in water on FGF-21 level has not yet been determined, but it has been shown that FGF-21 has a significant positive relationship with physical activity so that, after a period of physical activity, its serum level is increased. Regular exercise increases sensitivity to FGF-21 by increasing aerobic capacity [36]. Kim and Song (2017) in their study noted that resistance training increased level of FGF-21 in skeletal muscle of obese mice with diabetes [37]. Also, Fassihi et al. (2020) in their study concluded that intermittent exercise increased expression of FGF-21 in the patients with diabetes [10]. Toloui Azar et al. (2018) also reported that high-intensity intermittent exercise increased FGF-21 level in inactive obese women [24]. It has been stated that the increase in training duration is also one of the factors influencing expression of FGF-21 protein. In a longer study, Dolui et al. (2016) showed that 8 weeks of aerobic exercise with an intensity of 60–75% of the target heart rate for 30–45 min significantly increased serum levels of FGF-21 [38]. This similarity in results of this study to those of the present study can be attributed to training modality, intensity, and duration of training, because duration and intensity of training stimulate homeostasis of muscle tissue and are effective in secretion of myokines [24, 39]. Among effective signaling mechanisms, one can mention activation of the protein kinase B (AKT) signaling pathway due to muscle contraction, which increases FGF-21 level in muscle [40]. But in contrast to the present study, Kong et al. (2016) showed that 5 weeks of exercise did not significantly change serum level of FGF-21 in obese women [41]. The difference in the results of these studies with the present study can be attributed to the differences in the studied groups, number

of subjects, intensity, and type of exercise. Laboratory studies on FGF-21 level have reported that the FGF-21 signaling cascade requires FGFR and β-Klotho cofactors, and that FGFR1-4 and β-Klotho were highly expressed in liver, adipose, and pancreatic tissues [10]. When β-Klotho binds to FGF-21, insulin sensitivity and glucose metabolism are stimulated, thus controlling blood sugar [42].

The β-Klotho is an essential cofactor for FGF-21 activity. Binding of β-Klotho to FGF-21 increases insulin sensitivity and glucose metabolism, thus controlling blood sugar [43]. Therefore, FGF-21 is probably increased after exercise and has a positive effect on improving glucose and insulin resistance by inducing glucose in adipocytes through stimulating GLUT4 expression and inhibiting glucagon secretion from the pancreas [10]. Also in the present study, reduction of fat percentage and weight and improvement of lipid profile and insulin resistance were reported after 12 weeks of training in water. One of improvement mechanisms in these anthropometric indices is the increase in SIRT1 and FGF-21 levels in the training group because these indices have a positive effect on fat metabolism [44], and improve it by changing content and activity of glycolytic and aerobic enzymes, increasing GLUT4 level in skeletal muscle and also, increasing activity of β-hydroxyl coenzyme dehydrogenase (a key catalyst for lipid oxidation) [8]. Like other studies, there were some limitations in the present study including the lack of control over the diet and stress level of the subjects, as well as small sample size. Therefore, according to the results of the present study and the results of other studies, it is suggested to conduct extensive studies with larger sample size and longer duration in this field. It is also recommended to use sports exercises in welfare centers and healthcare centers to improve complications of diabetes in the patients.

Conclusion

According to the results of the present study and considering that in the patients with diabetes, it is difficult to maintain strength and function due to blood sugar-related problems and disturbance of the body's metabolic activity, performing exercise in water can be effective when there is no other option available. Finally, based on the results of this study and similar cases, exercise in water is recommended, as a physiological stimulant, improving factors related to metabolic health, cardiovascular health, aerobic capacity, enhancing strength and performance, and ultimately, reducing inflammatory factors and inflammation in the patients with diabetes.

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Declarations

Ethics approval This article has been approved by the ethics committee of the Iranian Institute of Physical Education and Sport Sciences and have an ethics code number SSRI.REC-2107–1165.

Consent for publication An informed written EC was obtained from all the participants.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. HassanzadehGhahdarijani E, Sabohi F, Shirazi M. Comparison of vacuum-assisted closure therapy and modern dressing on wound healing in patients with diabetic foot ulcers. J Diabetes Nurs. 2020;8(4):1200–11.
2. Mohsenzadeh M, Aghaei F, Nameni F. The effect of high intensity interval training on SIRT1 and PGC1-α gene expression in soleus muscle of type 2 diabetes in male rats. Med J Tabriz Uni Med Sciences Health Services. 2020;42(4):447–55.
3. Vizvari X, Farzanegi P, Abbas ZadeSourati H. Effect of vigorous aerobic exercise on serum levels of SIRT1, FGF21 and Fetuin A in women with type II diabetes. mljgoums. 2018;12(2):1–6. https://doi.org/10.29252/mlj.12.2.1.
4. Colak Y, Yesil A, HuseyinMut H, Caklili O, Ulasogl C, Senates E, et al. A potential treatment of non-alcoholic fatty liver disease with SIRT1 activators. Review J Gastro intestin Liver Dis. 2014;23(3):311–9. https://doi.org/10.15403/jgld.2014.1121.233.yck.
5. Muller G. Microvesicles/exosomes as potential novel biomarkers of metabolic diseases. Diabetes Metab Syndr Obes. 2012;5:247–82. https://doi.org/10.2147/DMSO.S32923.
6. Gibala JM. Physiological adaptations to low-volume, high-intensity interval training in health and disease. J Physiol. 2012;590(5):1077–84. https://doi.org/10.1113/jphysiol.2011.224725\.
7. Saremi A, Shahrjerdi Sh, Kavyani A. The effect of aerobic training on metabolic parameters and serum level of Sirtuin1 in women with type 2 diabetes. Arak Medical University Journal (AMUJ). 2016;19(114):88–97.
8. Alamdari KA, Khalafi M. The effects of high intensity interval training on serum levels of FGF21 and insulin resistance in obese men. Iranian Journal of Diabetes and Metabolism. 2018;18(1):41–8.
9. Iglesias P, Selgas R, Romero S, Díez JJ. Mechanisms in endocrinology: biological role, clinical significance, and therapeutic possibilities of the recently discovered metabolic hormone fibroblastic growth factor 21. Eur J Endocrinol. 2012;167:301–9.
10. FasihiRamandi EL, Khaledi N. High intensity interval training induced changes in the hepatic FGF-21 gene expression and serum TNF-α in diabetic male rats. Bi-Quarterly Journal of Research in Sports Medicine and Technology. 2020;10(19):57–68.
11. Alizadeh L, Tofighi A, Tolouei Azar J. The effect of 8 weeks of high intensity interval training (HIIT) on Serum Irisin, FGF21 and glycemic indices in type 2 diabetic women. J Appl Health Studies in Sport Physiology. 2020;6(2):17–24.
12. Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell metabolism. 2013;18(3):333–40.

13. Abasi A, Hedayatzadeh S, Abdi A, Abaszadeh H. The effect of eight weeks of aerobic exercise on FGF-21 serum, apolipoprotein A-1 and relative levels LDL/ HDL in obese women. Physiological research and management in sports. 2016;8(1):77–87.
14. Pacifici F, Di Cola D, Pastore D, Abete P, Guadagni F, Donadel G. Proposed tandem effect of physical activity and Sirtuin 1 and 3 activation in regulating glucose homeostasis. International Journal of Molecular Science. 2019;20(47):1–14.
15. Radak Z, Suzuki K, Posa Ac, Petrovszky Ac, IstvanBoldogh KE. The systemic role of SIRT1 in exercise mediated adaptation. Redox Biology. 2020;35(4):1–7.
16. Huang CC, Wang T, Tung YT, Lin WT. Effect of exercise training on skeletal muscle SIRT1 and PGC-1alpha expression levels in rats of different age. Int J Med Sci. 2016;13:260–70.
17. Suwa M, Nakano H, Radak Z, Kumagai S. Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor gamma coactivator-1alpha protein expressions in rat skeletal muscle. Metabolism. 2008;57:986–98.
18 Sarga L, Nikolett H, Lauren K. Aerobic endurance capacity affects spatial memory and SIRT1 is a potent modulator of 8-oxoguanine repair. Neuroscience. 2013;252:326–36.
19. Besse-Patin A, Montastier E, Vinel C, Castan-Laurell I, Louche K, Dray C, et al. Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine. Int J obes (Land). 2014;38(5):707–13.
20. Yang SJ, Hong HC, Choi HY, Yoo HJ, Cho GJ, Hwang TG, et al. Effects of a three-month combined exercise programme on fibroblast growth factor 21 and fetuin-A levels and arterial stiffness in obese women. Clin Endocrinol (oxf). 2011;75(4):464–9.
21. Paneni F, Costantino S, Cosentino F. Insulin resistance, diabetes, and cardiovascular risk. Curr Atheroscler Rep. 2014;16(7):419. https://doi.org/10.1007/s11883-014-0419-z.
22. Soltani M, Rashidlamir A, Fathi M, Ghahremanimoghaddam M. The effect of eight weeks of water training on Sirt1, Pgc-1α and body fat percentage in obese men. J Babol Univ Med Sci. 2018;20(9):55–60.
23. Tolueiazar J, Tofighi A, Ghafari Gh. The effect of eight weeks of selected aerobic exercise in water on lipocalin levels, 2 glycemic indexes and lipid profile in middle-aged men with Overweight. Sports Life Sciences. 2019;11(3):299–314. https://doi.org/10.22059/JSB.2018.248017.1229.
24. Tolueiazar J, Tofighi A, Alizadeh R. The effect of high-intensity periodic training on serum levels of fibroblast growth factor, 21 Insulin resistance and lipid profile in inactive obese women. Sports Life Sciences. 2018;10(4):449–64.
25. Little JP, Safdar A, Wilkin GP, Tarnopolsky MA, Gibala MJ. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. J Physiol. 2010;588(6):1011–22.
26. Gurd BJ, Perry CG, Heigenhauser GJ, Spriet LL, Bonen A. High-intensity interval training increases SIRT1 activity in human skeletal muscle. Appl Physiol Nutr Metab. 2010;35(3):350–7.
27. Okoshi MP, Pagan L, Damatto R, Cezar M, Campos D, Lima A, et al. Physical training improves cardiac structure and function, antioxidant capacity, and exercise tolerance in spontaneously hypertensive rats. FASEB J. 2017;31((1Suppl)):838/6.
28. Moynihan KA, Grimm AA, Plueger MM, Bernal Mizrachi E, Ford E, Cras-Méneur C, et al. Increased dosage of mammalian Sir in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. J Cell Metab. 2005;2(2):105–17. https://doi.org/10.1016/j.cmet.2005.07.001.
29. Munehiro K, Daisuke K. SIRT1 in type 2 diabetes: mechanisms and therapeutic potential. Diabetes Metab J. 2013;37(5):315–25. https://doi.org/10.4093/dmj.2013.37.5.315.
30 Oberbach A, To¨njes A, Klo¨ting N, Fasshauer M, Kratzsch Jr, Busse MW, et al. Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. Eur Jof Endocrin. 2006;154(4):577–85.
31 Kadoglou N, perrea d, iliadis f. Exercise reduces resistin and inflammatory cytokines in patients with type 2 diabetes. Diabetes Care. 2007;30(3):719–21.
32. Bello AI, Owusu-Boakye E, Adegoke BO, Adjei DN. Effects of aerobic exercise on selected physiological parameters and quality of life in patients with type 2 diabetes mellitus. Int J General Med. 2011;4(2):723–72.
33. Yoo HJ, Choi KM. Hepatokines as a link between obesity a cardiovascular diseases. Review Diabetes Metab J. 2015;39:10–5. https://doi.org/10.4093/dmj.2015.39.1.10.
34. Talanian JL ,Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of highintensity aerobic interval training increases the capacity for fat oxidation during exercise in women. J Appl Physiol. 2007;102:1439–47.
35. Cuevas-Ramos D, Almeda-Valdes P, Gómez-Pérez FJ, Meza-Arana CE, Cruz-Bautista I, Arellano-Campos O, et al. Daily physical activity, fasting glucose, uric acid, and body mass index are independent factors associated with serum fibroblast growth factor 21 levels. Eur J Endocrinol. 2010;163(3):469–77.
36. Taniguchi H, Tanisawa K, Sun X, Cao Z-B, Oshima S, Ise R, et al. Cardiorespiratory fitness and visceral fat are key determinants of serum fibroblast growth factor 21 concentration in Japanese men. The Journal of Clinical Endocrinology & Metabolism. 2014;99(10):E1877–84.
37. Kim HJ, Song W. Resistance training increases fibroblast growth factor-21 and irisin levels in the skeletal muscle of Zucker diabetic fatty rats. J Exerc Nutr Biochem. 2017;21(3):50–4.
38. Abbasi DA, Hedayatzadeh S, Abdi A, Abbaszadeh SH. The effect of 8 weeks of aerobic exercise on serum levels of FGF21, apolipoprotein A-1and LDL-c to HDL-C ratio in obese women. 2016;8(1):77–87
39. Kim KH, Kim SH, Min Y-K, Yang H-M, Lee J-B, Lee M-S. Acute exercise induces FGF21 expression in mice and in healthy humans. PloS one. 2013;8(5):e63517.
40. Tanimura Y, Aoi W, Takanami Y, Kawai Y, Mizushima K, Naito Y, et al. Acute exercise increases fibroblast growth factor 21 in metabolic organs and circulation. Physiological reports. 2016;4(12):e12828.
41. Kong Z, Sun S, Liu M, Shi Q. Short-term high-intensity interval training on body composition and blood glucose in overweight and obese young women. J Diab Res. 2016;2(16):1–9
42. Diaz-Delfin J, Hondares E, Iglesias R, Giralt M, Caelles C, Villarroya F. TNF-alpha represses betaKlotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway. Endocrinology. 2012;153(9):4238–45.
43. Díaz-Delfín J, Hondares E, Iglesias R, Giralt M, Caelles C, Villarroya F. TNF-a represses –Klotho expression and impairs FGF21 action in adipose cells involvement of JNK1 in the FGF21 pathway. Endocrinology. 2012;153(9):4238–45.
44. Ghanbari-Niaki A, Saeidi A, Ahmadian M, Gharahcholo L, Naghavi N, Fazelzadeh M, et al. The combination of exercise training and Zataria multiflora supplementation increase serum irisin levels in postmenopausal women. Integr Med Res. 2018;7(1):44–52.

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ORIGINAL ARTICLE

Selenoprotein P levels in patients with diabetes mellitus with complications

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Abstract

Aims Increasing evidence has shown that selenoprotein P levels are elevated in type 2 diabetes mellitus and are associated with insulin resistance and release. This study aimed to determine if there was a connection between selenoprotein P levels and metabolic parameters in patients with diabetes with microvascular complications.

Methods Serum selenoprotein P concentrations were measured by ELISA in 44 patients with diabetes with complications and 36 patients with diabetes without complications.

Results There was no statistically significant difference in selenoprotein P levels between the groups [1.9 (0.9–2.6) and 1.9 (0.8–2.4) ng/mL, respectively, $p=0.565$]. Selenoprotein P, glucose, glycosylated hemoglobin, C-reactive protein, triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels were not statistically significantly correlated in patients with complications. However, there was a significant correlation with high-density lipoprotein cholesterol ($r=-0.401$, $p=0.042$).

Conclusions We did not find high selenoprotein P levels in patients with complications, but its inverse association with high-density lipoprotein cholesterol indicates that it may play a role in developing cardiovascular disease in this community of patients.

Keywords Selenoprotein P · Microvascular complications · Diabetic complications · Diabetes mellitus

Introduction

Diabetes mellitus (DM), which has a mortality rate of about 4.2 million people globally, affects over 500 million people aged 20 to 79 years. It is expected to impact more than 750 million people in the next 25 years [1]. Diabetes, which is considered an epidemic, will continue to be a significant health and economic challenges both now and in the future as industrialization and food habits improve. The most common form of diabetes is included in the physiopathology of insulin resistance in the tissues in type 2 diabetes mellitus (T2DM). Hyperglycemia caused by insulin resistance can lead to micro- and macrovascular complications. Microvascular complications, such as nephropathy, neuropathy, retinopathy, and ischemia, account for the majority of complications and are associated with morbidity and mortality [2]. Effective treatments and hyperglycemia-controlling diets can dramatically reduce the risk of complications because the complications are usually permanent. Although such effective treatments can reduce microvascular complications by 50–76% [3], they may increase mortality by causing adverse events such as hypoglycemia [4].

The liver releases a group of proteins called hepatokines into the circulation, which affect carbohydrate and lipid metabolism. There is evidence that hepatokines play a role in T2DM [5]. Selenoprotein P (SeP; encoded by SELENOP) is a hepatokine mainly produced by the liver. SeP, also used as a diagnostic parameter for blood selenium status, functions as a selenium supply protein in the body [5–7]. The high level of SeP in patients with diabetes and the fact that the administration of high concentrations of SeP causes insulin resistance in tissues and a decrease in insulin secretion

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from the pancreas make it a diabetogenic protein [8, 9]. Experimental administration of SeP neutralizing antibodies resulted in improvements in glucose metabolism and insulin resistance and release [9]. It has been reported that initial SeP concentrations are a more reliable test for predicting glucose intolerance that will develop after 4 years compared with diabetes parameters such as HOMA-IR, age, glycosylated hemoglobin (HbA1c), and fasting plasma glucose [10]. It was reported that high SeP levels in patients with DM were also associated with inflammation and atherosclerosis [11].

The fact that SeP causes insulin resistance and reduces pancreatic insulin secretion has made it a possible target for diabetes treatment [8]. If a relationship exists between SeP and diabetes complications, it will contribute to the prevention or treatment of complications. However, we found no studies in the literature indicating a relation between SeP and the complications of diabetes. Therefore, this study aimed to evaluate whether there was a relationship between the microvascular complications of T2DM and SeP.

Methods

Study population

In this cross-sectional study, 44 patients with T2DM with complications, and 36 age- and sex-matched patients with T2DM without complications who presented to the internal diseases outpatient clinic were included. Pregnant women, patients with chronic diseases other than diabetes, and those taking selenium and other supplements for the last 6 months were excluded from the study. All patients were evaluated for diabetic complications through clinical examinations and specific laboratory tests.

Of the patients with complications, 12 had nephropathy, six had neuropathy, seven had retinopathy, and six had ischemia. Twelve patients had more than one complication, six had nephropathy and neuropathy, two had nephropathy and retinopathy, one had retinopathy and ischemia, one had nephropathy, retinopathy and ischemia, one had neuropathy, retinopathy and ischemia, and one person had neuropathy, nephropathy, retinopathy, and ischemia.

Laboratory analysis

Venous blood samples were obtained from all patients from the forearm in the morning after 8–10 h of fasting. Blood was collected in plain tubes and allowed to clot for 30 min before being centrifuged for 10 min at 2000 *g*. Approximately 0.5 mL of the serum was transferred to microcentrifuge tubes and stored at −80 °C until the SeP levels were measured. Routine biochemical tests were immediately performed. HbA1c levels of the blood samples collected in K_2EDTA tubes were studied. Urine albumin, protein, and creatinine tests were performed in the first urine in the morning.

Glucose, creatinine, lipid parameters [low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglycerides], C-reactive protein (CRP) in serum, creatinine, protein, and albumin in urine were studied in an autoanalyzer (AU5840; Beckman Coulter, CA, USA) using routine laboratory methods. HbA1c was measured using high-performance liquid chromatography (HPLC) (Premier Hb9210; Trinity Biotech, Co. Wicklow, Ireland). Urine protein/creatinine and urine albumin/creatinine ratios were obtained by calculation.

Serum SeP concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Beijing, China) using the sandwich-ELISA method. The test was conducted according to the kit instructions. The optical density was measured spectrophotometrically at 450 nm using a microplate reader (SPECTROstar Nano, BMG Labtech).

Statistical analysis

According to the data distribution, results were expressed as mean ± SD or median (25th–75th percentile). The normality of the data was determined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Student's *t* test was used for parametric data, and the Mann–Whitney *U* test was used for non-parametric data to compare the two groups. Chi-square analysis was used to compare categorical results. The Kruskal–Wallis test was conducted to compare the differences between more than two groups. The Mann–Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. The relationship between SeP and other parameters was assessed using Spearman's non-parametric correlation test. All statistical analyses were performed using the SPSS for Windows, version 21.0 statistical package (SPSS, Chicago, IL). p values <0.05 were considered statistically significant.

Results

Comparisons of anthropometric measurements and biochemical parameters between the complication group and the without complication group are summarized in Table 1. There were no statistically significant differences in serum SeP levels between the DM groups

Table 1 Laboratory characteristics and anthropometric data of the groups

Parameters	Patients with complication $n=44$	Patients without complication $n=36$	p value
Age	61.9 ± 9.4	59.4 ± 10.7	0.261
Sex, F/M	20/24	20/16	0.369
Waist circumference (cm)	111.5 ± 11.9	105.7 ± 6.9	**0.009**
Waist to hip ratio	0.99 ± 0.07	0.95 ± 0.07	**0.012**
BMI (kg/m^2)	33.5 (29.5-38.5)	32.7 (29.5-36.8)	0.588
Systolic TA (mmHg)	134.5 ± 18.6	133.5 ± 20.4	0.843
Diastolic TA (mmHg)	81.2 ± 11.2	79.1 ± 11.6	0.465
Duration of diabetes (month)	122 (72-195)	120 (48-180)	0.149
SeP (ng/mL)	1.9 (0.9-2.6)	1.9 (0.8-2.4)	0.565
Glucose (mg/dL)	150 (133-195)	146 (118.5-178)	0.287
HbA1c (%)	8.5 ± 1.5	7.8 ± 1.6	**0.038**
Creatinine (mg/dL)	1.0 ± 0.4	0.8 ± 0.2	**0.008**
CRP (mg/dL)	0.4 (0.2-0.7)	0.2 (0.1-0.6)	0.092
Triglycerides (mg/dL)	145 (107-204)	148 (107-216)	0.829
Total cholesterol (mg/dL)	196 ± 45	193 ± 45	0.992
LDL cholesterol (mg/dL)	115 ± 37	109 ± 34	0.596
HDL cholesterol (mg/dL)	44 (40-56)	46 (42-56)	0.253

The values are presented as mean ± SD or median (25th and 75th percentiles). The values in boldface indicate a statistically significant ($p < 0.05$)

BMI, body mass index; *CRP*, C-reactive protein; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *SeP*, selenoprotein P; *TA*, tension arterial

with and without complications. In patients with complications, waist circumference ($p=0.009$), waist-to-hip ratio ($p=0.012$), HbA1c ($p=0.038$) and serum creatinine ($p=0.008$) values were significantly higher than in patients without complications. However, there were no statistically significant differences in diabetes duration, systolic and diastolic blood pressures, body mass index (BMI), and lipid parameters between patients with and without complications.

The results of correlation analysis between SeP and other parameters in the complicated group are summarized in Table 2. Only HDL cholesterol was negatively correlated with SeP ($r=-0.401$, $p=0.042$) (Fig. 1).

Anthropometric measurements and laboratory parameters according to the type of complications are summarized in Table 3. There was no statistically significant difference in SeP values between the patients with and without complications. As expected, urinary protein/creatinine and urine albumin/creatinine ratios were significantly higher in patients with nephropathy than in patients without complications ($p<0.001$, $p<0.001$, respectively). Only the duration of diabetes was significantly longer in patients with retinopathy than in patients without complications ($p=0.003$). There was no statistically significant difference in any parameter in patients with ischemia and neuropathy. Patients with more than one complication had significantly higher HbA1c levels than patients without complications ($p=0.001$). In addition, these patients had significantly higher urine protein/creatinine and albumin/creatinine ($p<0.001$, $p<0.001$, respectively) than patients without complications.

Table 2 Correlation of SeP with other parameters in the patients with complication

Parameters	Correlation coefficient (r)	p value
Duration of diabetes	0.027	0.869
Waist circumference	− 0.251	0.109
Waist to hip ratio	− 0.075	0.639
BMI	− 0.226	0.149
Glucose	0.062	0.690
HbA1c	0.152	0.326
CRP	− 0.333	0.096
Protein/creatinine ratio	0.093	0.612
Albumin/creatinine ratio	0.136	0.377
Triglycerides	0.257	0.205
Total cholesterol	− 0.080	0.699
LDL cholesterol	− 0.112	0.585
HDL cholesterol	− 0.401	**0.042**

See Table 1 for abbreviations. The value in boldface indicate a statistically significant ($p < 0.05$)

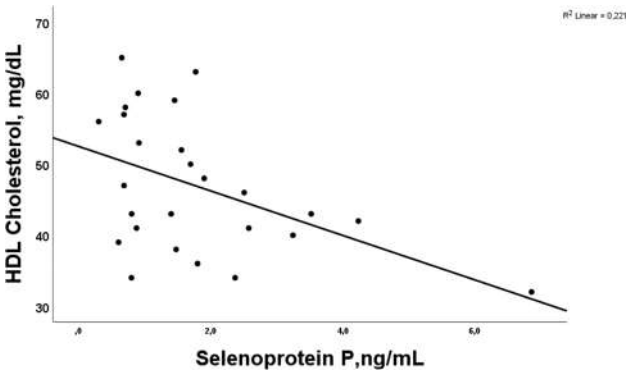

Fig. 1 Scatter plot of HDL cholesterol and SeP in the patients with complication

Discussion

Our aim in the present study was to evaluate whether SeP, which is stated to be diabetogenic, had a relationship with microvascular complications seen in patients with T2DM. Our study revealed that serum SeP concentrations in patients with diabetic with complications were not different from those without complications. As far as we know, it is the first study to examine SeP levels in such a population.

Serum SeP levels are elevated in people with diabetes, and according to both experimental and clinical research, SeP induces insulin resistance [8, 11–13]. Misu et al., in an experimental study, reported that hepatic SeP expression and serum SeP concentrations increased in DM and SeP levels were positively correlated with fasting plasma glucose and HbA1c levels. They also showed that when they gave high doses of SeP to primary hepatocyte cell cultures and mice, SeP increased insulin resistance by disrupting insulin signaling [8]. Zhang et al. showed that serum SeP concentrations were higher in people with uncontrolled diabetes (HbA1c > 7%) in a study of 176 patients with T2DM and 142 healthy people [13]. Unlike this study, serum SeP levels in the complicated group with uncontrolled diabetes were comparable to those in the patients without complications in our study. Yang et al. found that serum SeP concentrations increased in proportion to the disease status in three groups of 100 individuals, including non-DM, prediabetes, and DM groups [11]. However, in their study, SeP concentrations were not different between patients with prediabetes and those who developed diabetes. Our study found no difference in SeP concentrations in the complication phase, the more advanced form of diabetes, which supports this viewpoint.

There are also studies in the literature reporting that serum SeP is not associated with diabetes. Altınova et al. showed that SeP levels in pregnant women with gestational diabetes were not different from healthy and non-pregnant women [14]. Another study found that SeP did not differ between healthy and people with T2DM in a small number of study groups [15].

High HbA1c is a valuable marker for DM diagnosis and monitoring, indicating insufficient glucose control for at least 2-3 months. High HbA1c levels are also associated with micro and macrovascular complications and mortality in DM [16]. The literature on the relationship between HbA1c and SeP is contradictory, with studies finding positive [8] and negative [15] correlations, as well as studies finding no association [14]. In our study, HbA1c levels were higher in the complicated group as expected, but there was no correlation between HbA1c and SeP levels.

Dyslipidemia is common in diabetes. High triglycerides, cholesterol, and low HDL levels play an essential role in developing atherosclerosis in individuals with diabetes [17]. Although the precise cause of low HDL in diabetes is unknown, it is thought to be caused by an increase in HDL catabolism due to insulin resistance [18]. In our study, patients with complications had lower HDL levels, and there was a negative correlation between SeP and HDL. However, there was no correlation between SeP and HDL in patients without complications (data not shown). This inverse relationship between SeP and HDL in the complicated group suggests that elevated SeP levels may be associated with an increased risk of cardiovascular disease, common in people with diabetes. In contrast to our results, in a large prospective study, Schomburg et al. [19] reported that low SeP levels were a predictor of cardiovascular disease and death. They attributed this to factors other than HDL, such as the antioxidant property of SeP. Furthermore, only about 11% of the participants in their study had diabetes.

Our study has several limitations. Apart from the low number of patients in the complications group, the presence of metformin-receiving patients in the study groups could be confusing because metformin inhibits the expression of SeP mRNA in the liver, lowering SeP levels [20]. Thus, SeP levels in patients who received metformin treatment may have been affected. Although it is not well established if feeding low or high in selenium may lead to various pathological conditions, in our study, the dietary habits of the patients were not questionned. Another thing to consider is the lack of detailed validation of the SeP ELISA assay in order to obtain a more accurate level of SeP levels in serum [21].

Our findings indicate that SeP, which has previously been identified as a diabetogenic protein, has no relationship with the microvascular complications of diabetes. However, the inverse correlation between SeP and HDL in patients with diabetes with complications suggests that

Table 3 Anthropometric and laboratory findings of patients by type of complication.

Parameters	Patients without complication ($n=36$)	Patients with nephropathy ($n=13$)	p value	Patients with neuropathy ($n=6$)	p value	Patients with retinopathy ($n=7$)	p value	Patients with ischemia ($n=6$)	p value	Patients with multiple complications ($n=12$)	p value
Waist circumference (cm)	105 ± 6.9	112 ± 14.2	0.150	113 ± 11.4	0.116	111 ± 6.0	0.055	114 ± 13.9	0.152	109 ± 12.0	0.366
Waist to hip ratio	0.95 ± 0.1	0.98 ± 0.1	0.200	1.02 ± 0.1	0.060	0.97 ± 0.1	0.483	0.96 ± 0.1	0.886	1.00 ± 0.1	0.032
BMI (kg/m2)	32.7 (29.5–36.8)	34.7 (26.6–40.0)	0.642	35.0 (33.8–35.7)	0.196	31.0 (29.7–36.7)	0.829	35.2 (30.8–42.7)	0.426	30.3 (29.0–36.7)	0.694
Duration of diabetes (month)	120 (48–180)	60 (18–144)	0.320	120 (60–120)	0.926	240 (240–350)	**0.003**	108 (38–130)	0.796	180 (122–222)	0.010
SeP (ng/mL)	1.9 (0.8–2.4)	2.3 (1.2–2.9)	0.319	1.9 (0.7–2.9)	0.857	2.1 (1.5–2.5)	0.693	1.9 (0.9–2.5)	0.653	1.6 (0.8–2.4)	0.739
Glucose (mg/dL)	146 (119–178)	148 (141–210)	0.217	144 (123–154)	0.999	113 (105–176)	0.366	133 (110–147)	0.235	187 (166–250)	0.006
HbA1c (%)	7.8 ± 1.6	8.3 ± 1.5	0.153	8.3 ± 1.6	0.577	8.1 ± 1.5	0.554	7.9 ± 0.9	0.439	9.3 ± 1.3	**0.001**
Creatinine (mg/dL)	0.8 ± 0.2	1.1 ± 0.4	0.013	1.3 ± 0.9	0.042	0.9 ± 0.2	0.147	0.9 ± 0.1	0.408	0.9 ± 0.3	0.482
CRP (mg/dL)	0.2 (0.1–0.6)	0.3 (0.3–0.4)	0.436	0.7 (0.6–1.3)	0.159	0.4 (0.3–0.6)	0.479	0.3 (0.2–0.4)	0.856	0.7 (0.3-1.6)	0.042
Protein/creatinine ratio (mg/g)	96 (77–117)	216 (158–951)	**< 0.001**	64 (53–96)	0.111	89 (75–109)	0.732	98 (77–117)	0.874	382 (197–460)	**< 0.001**
Albumin/creatinine ratio (mg/g)	8.9 (6.5–15.5)	98.7 (34.2–182.6)	**< 0.001**	3.7 (3.4–13.3)	0.196	13.8 (8.1–28.6)	0.308	13.6 (4.7–17.3)	0.640	152.8 (41.8–224.1)	**< 0.001**

See Table 1 for abbreviations

The values are presented as mean ± SD or median (25th and 75th percentiles)

Data for each type of complication were compared statistically to patients without complications. The values in boldface indicate a statistically significant ($p<0.003$)

SeP may play a role in the development of cardiovascular disease in patients with diabetes, but this relationship should be evaluated with more comprehensive studies.

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Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval Kırsehir Ahi Evran University Faculty of Medicine approved the study of Medicine Ethics Committee (approval date and number: 2020-04/31). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication All of the authors confirm the publication.

Conflict of interest/competing interests The authors declare no competing interests.

References

1. Atlas IDFID. 9th edn.(2019), 2020.
2. Litwak L, Goh S-Y, Hussein Z, Malek R, Prusty V, Khamseh ME. Prevalence of diabetes complications in people with type 2 diabetes mellitus and its association with baseline characteristics in the multinational a 1 chieve study. Diabetology & metabolic syndrome. 2013;5:1–10.
3. Association AD. 6. Glycemic targets: standards of medical care in diabetes—2021. Diabetes Care. 2021;44:S73–84.
4. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the task force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of diabetes (EASD). Eur Heart J. 2019;41:255–323.
5. Stefan N, Haring HU. The role of hepatokines in metabolism. Nat Rev Endocrinol. 2013;9:144–52.
6. Brodin O, Hackler J, Misra S, Wendt S, Sun Q, Laaf E, et al. Selenoprotein P as biomarker of selenium status in clinical trials with therapeutic dosages of selenite. Nutrients. 2020;12:1067.
7. Hill KE, Wu S, Motley AK, Stevenson TD, Winfrey VP, Capecchi MR, et al. Production of selenoprotein P (Sepp1) by hepatocytes is central to selenium homeostasis. J Biol Chem. 2012;287:40414–24.
8. Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, et al. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab. 2010;12:483–95.
9. Mita Y, Nakayama K, Inari S, Nishito Y, Yoshioka Y, Sakai N, et al. Selenoprotein P-neutralizing antibodies improve insulin secretion and glucose sensitivity in type 2 diabetes mouse models. Nat Commun. 2017;8:1–17.
10. Oo SM, Misu H, Saito Y, Tanaka M, Kato S, Kita Y, et al. Serum selenoprotein P, but not selenium, predicts future hyperglycemia in a general Japanese population. Sci Rep. 2018;8:1–10.
11. Yang SJ, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. J Clin Endocrinol Metab. 2011;96:E1325–9.
12. Ali SA, Nassif WM, Abdelaziz DH. Alterations in serum levels of fetuin a and selenoprotein P in chronic hepatitis C patients with concomitant type 2 diabetes: a case-control study. Clinics and research in hepatology and gastroenterology. 2016;40:465–70.
13. Zhang Q, Li W, Wang J, Hu B, Yun H, Guo R, et al. Selenium levels in community dwellers with type 2 diabetes mellitus. Biol Trace Elem Res. 2019;191:354–62.
14. Altinova AE, Iyidir OT, Ozkan C, Ors D, Ozturk M, Gulbahar O, et al. Selenoprotein P is not elevated in gestational diabetes mellitus. Gynecol Endocrinol. 2015;31:874–6.
15. Roman M, Lapolla A, Jitaru P, Sechi A, Cosma C, Cozzi G, et al. Plasma selenoproteins concentrations in type 2 diabetes mellitus—a pilot study. Transl Res. 2010;156:242–50.
16. Laiteerapong N, Ham SA, Gao Y, Moffet HH, Liu JY, Huang ES, et al. The legacy effect in type 2 diabetes: impact of early glycemic control on future complications (the Diabetes & Aging Study). Diabetes Care. 2019;42:416–26.
17. Femlak M, Gluba-Brzózka A, Ciałkowska-Rysz A, Rysz J. The role and function of HDL in patients with diabetes mellitus and the related cardiovascular risk. Lipids Health Dis. 2017;16:1–9.
18. Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia. 2015;58:886–99.
19. Schomburg L, Orho-Melander M, Struck J, Bergmann A, Melander O. Selenoprotein-p deficiency predicts cardiovascular disease and death. Nutrients. 2019;11:1852.
20. Speckmann B, Sies H, Steinbrenner H. Attenuation of hepatic expression and secretion of selenoprotein P by metformin. Biochem Biophys Res Commun. 2009;387:158–63.
21. Saito Y, Misu H, Takayama H, Takashima SI, Usui S, Takamura M, et al. Comparison of human selenoprotein P determinants in serum between our original methods and commercially available kits. Biol Pharm Bull. 2018;41:828–32.

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ORIGINAL ARTICLE

Prediction of diabetic retinopathy in patients with type 2 diabetes mellitus by using monocyte to high-density lipoprotein-cholesterol ratio

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Abstract

Purpose Diabetic retinopathy is the leading cause of vision loss and impairment in developed countries. Inflammation is considered one of the main reasons for the progression of diabetes complications. We investigated the relationship of monocyte/high-density lipoprotein-cholesterol ratio (MHR) with type 2 diabetes mellitus (T2DM) and diabetic retinopathy (DR).

Methods A total of 118 T2DM patients, 60 of whom had DR, and 58 age- and sex-matched healthy controls were included in this cross-sectional study. MHR was calculated by blood sampling after a complete ophthalmologic examination on all subjects.

Results MHR was higher in T2DM patients with DR compared to both the control group and without DR (p=0.018). There was a significant positive correlation between MHR and DR (r=0.256 p=0.004). Additionally, MHR was an independent predictor of DR according to multivariate regression analysis (*OR*=1.197, p=0.009). DR could be predicted with 92% sensitivity and 84% specificity when MHR was 16.05, whereas DR predicted with 100% sensitivity and 98% specificity when MHR was 23 in ROC curve analysis (*AUC*: 0.356, *95% CI* 0.251–0.460, $p = 0.008$).

Conclusion This study showed that patients with T2DM may be more likely to develop DR when they have high MHR values. Based on these results, clinicians can also use MHR as a new laboratory marker to predict DR.

Keywords Diabetes mellitus · Diabetic retinopathy · Inflammation · Oxidative stress · Monocyte to high-density lipoprotein cholesterol ratio

Introduction

Diabetes mellitus (DM) is a systemic disease characterized by vascular and neuropathic complications and is becoming an increasing problem worldwide [1]. Diabetic retinopathy (DR) is the most common complication of DM and is the primary cause of vision loss and impairment in developed countries [2, 3]. The pathogenesis of DR is complex and inflammation plays an essential role in the development and progression of DR, along with many factors such as hyperglycemia and hypertension [4–7].

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Circulating monocytes migrate to tissues and turn into macrophages and dendritic cells and are involved in antimicrobial defense mechanisms. They also produce inflammatory cytokines and contribute to local and systemic inflammation. They play a role in many diseases with inflammatory mechanisms such as atherosclerosis [8].

High-density lipoprotein cholesterol (HDL-c) prevents atherosclerosis by transporting cholesterol from peripheral tissues to the liver [9]. Also, HDL-c protects endothelial functions, has an antioxidant effect, and modulates inflammation [10].

Until now, it has been shown that the monocyte/HDL-c ratio (MHR) is an important marker of inflammation and oxidative stress in many inflammation-related diseases, especially cardiovascular diseases [11–13]. The reason why the MHR shows inflammation strongly is due to the anti-inflammatory and antioxidant effects of HDL-c as well as the pro-inflammatory effects of monocytes. It has been reported that MHR can also be a biomarker for vascular and

neuropathic complications of type 2 DM (T2DM) [14, 15]. However, as far as we know, there are not enough studies on MHR and DR.

This study aims to evaluate the relationship between DR and MHR values in T2DM patients.

Methods

Study population

This cross-sectional study was conducted between February 2020 and June 2020 in Ordu University Training and Research Hospital, Internal Medicine, and Ophthalmology Clinic. A total of 118 patients, 60 of whom had DR and diagnosis of T2DM, were included in the study. The control group consisted of 58 healthy individuals who were matched in terms of age and gender. Participants who accepted to participate in the study were questioned in terms of age, gender, hyperlipidemia, smoking, family history, chronic diseases, and medications used.

Smokers, chronic alcoholics, patients with infection, hematological disease, acute or chronic renal failure, chronic liver disease, cardiovascular disease, solid or malignant tumors, and chronic inflammatory diseases were excluded from the study. Our study was approved by the local ethics committee and was conducted in accordance with the ethical principles defined in the Declaration of Helsinki. A written informed consent form was obtained from all participants before the study.

Clinical examination and biochemical analysis

The diagnosis of T2DM was made according to the American Diabetes Association guidelines [16]. After a complete ophthalmological examination was applied to all participants, the diagnosis of retinopathy was made by fundus photographs, fluorescein angiography, and optical coherence tomography. The Diabetic Eye Care Guideline was used for DR diagnostic criteria [17].

Venous blood samples were taken after an overnight fast. All biochemical analyses were studied freshly in our hospital. ARCHITECT c8000 clinical chemistry analyzer (Abbott, IL, USA) was used to analyze creatinine, HDL-c, low-density lipoprotein cholesterol (LDL-c), total cholesterol, triglyceride, and C-reactive protein (CRP) concentrations. Complete blood count was analyzed using the CELL-DYN Ruby automated hematology analyzer (Abbott, IL, USA). Neutrophil/lymphocyte ratio (NLR), monocyte/lymphocyte ratio (MLR), platelet/lymphocyte ratio (PLR), and MHR were calculated and recorded for each participant.

Statistical analysis

In all statistical analyses, SPSS 26.0 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, USA) was used. The Shapiro-Wilk test was used to evaluate the distribution of data. The Kruskal-Wallis test, one-way ANOVA, and Fisher's exact test were used to compare the groups. The numeric variables as mean± SD and median (minimum-maximum) and the categorical variables as number and percent were expressed. Point biserial was applied to evaluate the correlation between the presence of DR and other parameters. Logistic regression analysis was used to calculate predictors of DR. MHR cut-off value was calculated by receiver operating characteristic (ROC) curve analysis to predict DR. A p-value of <0.05 was accepted as statistically significant.

Results

The study was conducted on 176 subjects, of whom 46 (26.1%) were males and 130 (73.9%) were females. Insulin use, hemoglobin, neutrophil, HbA1c, creatinine, HDL-c, and MHR were statistically different when the groups were compared in terms of age, gender, drug use, hemogram, and biochemical parameters (Table 1). In post hoc analysis, there was a significant difference between group 1 and group 2 in terms of Hba1c, HDL-c. It was found that there were differences in neutrophil, HDL-c, and MHR between group 1 and group 3, and there was a difference between group 2 and group 3 in terms of neutrophil, HbA1c, and creatinine (Table 1).

Considering whether there is a correlation between DR and age, hemogram, biochemical parameters, and MHR, positive correlation with neutrophil, HbA1c, creatinine, NLR, and MHR ($r= 0.251$ $p= 0.005$; $r= 0.423$ $p= 0.001$; $r= 0.256$ $p= 0.005$; $r=0.108$ $p=0.005$, $r= 0.256$ $p= 0.004$, respectively) and a negative correlation with HDL-c ($r = -0.299$ $p = 0.001$) were found (Table 2).

As a result of the regression analysis, HbA1c and MHR were found to be independent predictors of DR (Table 3).

ROC curve analysis for MHR is shown in Figure 1. Accordingly, the area below the ROC value of MHR to distinguish DR was 0.657, and $p = 0.003$ significance value. The best cutoff value was 10.27, with a sensitivity of 65.2% and a specificity of 58.3%.

Discussion

In the present study, there was no difference in MHR between T2DM patients without DR and healthy subjects; however, MHR was higher in patients with T2DM with DR compared to healthy control subjects and without retinopathy patients. Also, MHR was determined to be an

Table 1 Baseline characteristics and laboratory parameters of the groups

	Control group (group 1) n=58	*Without diabetic retinopathy (group 2) n=58*	*With diabetic retinopathy (group 3) n=60*	*p* value
Age, years	63.06±9.41	60.16±8.68	63.39±8.46	0.180
Gender, male, *n* (%)	16 (%27.5)	10 (%17.2)	20 (%33.3)	0.127
Insulin use		20 (%34.4)	36 (%60)	**0.006**
Statin use		12 (%20.6)	20 (%33.3)	0.124
Hemoglobin, g/dl	13.80 (10.6–15.5)[a]	13.1 (8.2–16)	12.9 (10.5–15.8)	0.092
White blood cell, 10^3 µl	6.44 (4.01–11.05)	6.68 (2.89–9.56)	7.03 (4.55–11.9)	0.154
Neutrophil, 10^3 µl	3.40 (1.52–6.94)[a]	3.74 (1.36–6.52)[a]	4.3 (2.23–8.94)[b]	***0.049******
Lymphocyte, 10^3 µl	2.11 (1.01–3.97)	2.1 (1.18–3.44)	2.19 (0.94–4.27)	0.848
Monocyte, 10^3 µl	0.51 (0.31–0.94)	0.48 (0.26–1.42)	0.46 (0.31–0.95)	0.798
Platelet, 10^3 µl	269 (131–449)	262(128–351)	233(139–466)	0.770
HbA1c, %	5.7 (5.4–6.1)[a]	7.5 (5.5–12.9)[b]	8.3 (5.3–14.7)[c]	***0.001******
Creatinine mg/dl	0.77 (0.56–1.30)[a]	0.75 (0.51–1.08)[a]	0.84 (0.47–2.9)[b]	***0.009******
Total cholesterol, mg/dl	199 (134–297)	200 (98–310)	196 (92–306)	0.644
Triglycerides, mg/dl	128 (42–411)	140 (55–670)	147(64–407)	0.202
HDL-c, mg/dl	50 (39–99)[a]	45 (28–75)[b]	44 (29–57)[b]	***0.001******
LDL-c, mg/dl	126.96±35.08	122.91±40.20	116.86±36.84	0.456
CRP, mg/dl	0.17 (0.0–5.36)	0.3 (0.0–9.1)	0.19 (0–3.76)	0.398
NLR	1.62 (0.74–4.24)[a]	1.71 (0.10–3.29)[a]	1.96 (0.94–5.32)[b]	***0.013******
MLR	0.23 (0.1–0.55)	0.22 (0.02–1.20)	0.22 (0.11–0.51)	0.989
PLR	117.3 (38.5–296)	113.8 (66.6–201.5)	115.4 (52.2–272.5)	0.793
MHR	9 (4.8–22.3)[a]	10 (4.7–20.4)[a]	12.9 (6–23.7)[b]	***0.018******

Data are expressed as mean ± standard deviation, median (min-max), number (percentage)

$p<0.05$ significant values are written in bold

*There is no difference between the same letters. There is a difference between the different letters

HDL-c high-density lipoprotein cholesterol, *LDL-c* low density lipoprotein cholesterol, *CRP* C-reactive protein, *NLR* neutrophil-to-lymphocyte ratio, *MLR* monocyte-to-lymphocyte, *PLR* platelet-to-lymphocyte ratio, *MHR* monocyte-to-HDL-c ratio

independent predictor of DR. To the best of our knowledge, this study is the first to show the relationship between DR and MHR.

Many epidemiological studies have reported that DM is associated with chronic inflammation and endothelial dysfunction (ED) [18, 19]. Chronic inflammation and ED are two contributing causes of the development and progression of microangiopathic and macroangiopathic complications seen in DM [6]. DR is the most common microangiopathic complication of DM. Increasing evidence indicates that systemic inflammation plays an essential role in the development and progression of DR in the early and later stages of DR by inducing the formation of new blood vessels and macular edema [20], damaging the glial cross and causing neuronal loss [21]. The relationship between DR and blood inflammatory index has been previously demonstrated [22–24]. In addition, studies have also found that many inflammatory cytokines such as tumor necrosis factor-α and vascular endothelial growth factor are increased in the systemic circulation in patients with DR [25].

Monocytes and macrophages are cells that play a primary key role in the synthesis and release of pro-inflammatory and pro-oxidant cytokines [26]. Macrophages that differentiate from activated monocytes that secrete cytokines, growth factors, and interleukins adhere to the outer surface of the retinal capillaries and disrupt the blood-retinal barrier in DR. Therefore, the retinal pigment epithelium acts as a gateway for monocytes that directly or indirectly damage the retina [27, 28]. Despite these known contributions of monocytes in DR development, studies have shown that there is no direct relationship between the monocyte count in the blood and DR [24, 29]. Our study supports this evidence for monocyte count and DR.

HDL-c carries cholesterol from peripheral tissues to the liver, prevents the harmful effects of LDL-c, and reduces LDL oxidation [9]. Besides, HDL-c inhibits monocyte activities, prevents the differentiation of monocytes to macrophages, and limits the inflammatory response. It prevents ED by removing cholesterol from lipid-loaded macrophages in atherosclerotic lesions. Thanks to these properties, HDL-c

Table 2 Correlation of presence of diabetic retinopathy with other parameters

	r	*p* value
Age, years	0.092	0.294
Hemoglobin, g/dl	−0.168	0.060
White blood cell, 10^3 µl	0.221	0.012
Neutrophil, 10^3 µl	0.251	***0.005***
Lymphocyte, 10^3 µl	−0.053	0.558
Monocyte, 10^3 µl	−0.009	0.924
Platelet, 10^3 µl	−0.040	0.650
HbA1c%	0.423	***0.001***
Creatinine mg/dl	0.256	***0.005***
Total cholesterol, mg/dl	−0.092	0.296
Triglycerides, mg/dl	0.014	0,879
HDL-c, mg/dl	−0.299	***0.001***
LDL-c, mg/dl	−0.103	0.249
CRP, mg/dl	0.032	0.719
NLR	0.108	***0.005***
MLR	0.001	0.994
PLR	−0.061	*0.497*
MHR	0.256	***0.004***

HDL-c high-density lipoprotein cholesterol, *LDL-c* low density lipoprotein cholesterol, *CRP* C-reactive protein, *NLR* neutrophil-to-lymphocyte ratio, *MLR* monocyte-to-lymphocyte, *PLR* platelet-to-lymphocyte ratio, *MHR* monocyte-to-HDL-c ratio

has antiatherosclerotic, antioxidant, anti-inflammatory, and antithrombotic effects [9, 10]. Studies on whether the HDL-c level is a risk factor for the development of DR in DM patients present conflicting results. Some studies state that there is no association between DR and HDL-c [30, 31], while others argue that high HDL-c level is a risk factor for DR [32]. Lyons et al.'s study with 988 people showed that low HDL-c levels are a risk factor for DR [33]. In our study, HDL-c levels were found to be higher in the control group compared to the other two groups, while there was no significant difference between T2DM with DR and without DR groups. These findings suggest that this apparent complexity for the relationship between DR and HDL-c may be influenced by differences in other risk factors such as hypertension and hyperglycemia.

Considering the pro-inflammatory effects of the monocyte-macrophage system and the anti-inflammatory effects of HDL-c, it makes sense to combine these two parameters as an inflammatory marker in a single index (MHR). Previous studies have shown that MHR is associated with many inflammatory diseases, primarily cardiovascular diseases [12–15]. MHR has also been studied in DM and its complications, and an association of high MHR with peripheral neuropathy, a common complication, has been proven [14]. Also, Karatas et al. [15] and Onalan's [34] studies have shown a high MHR level relationship with nephropathy, a microvascular complication in DM, and it has been suggested that MHR may be a biomarker for diabetic nephropathy. This high correlation of MHR with DM complications is due to its being a useful marker of both inflammation and vascular ED. In our study, high MHR rates were observed in T2DM patients with DR, but not in others, suggesting that MHR can be used as a marker of inflammation and ED for the development of DR.

The counts of leukocytes including neutrophils, lymphocytes, monocytes, basophils, and eosinophils are used as classical inflammatory markers, especially in cardiovascular diseases. In recent years, the ratios of these parameters (NLR, MLR, PLR, etc.) have been defined as new inflammatory markers and are frequently studied in inflammation-related diseases [35]. Our study has shown that neutrophil count and NLR value are high in DR, and previous studies on this issue also support our findings [22, 36].

This study has some limitations. First of all, this study had a small sample size. DR grading could not be done because sufficient patients could not be reached. Indicating whether there is a difference between non-proliferative and proliferative DR would have made this study more valuable. Secondly, creatinine levels in the DR group were statistically higher, although within clinically normal limits. While we are not sure whether this affected the results, it might have been better for the health of the study to have creatinine levels similar to other groups.

Table 3 Univariate and multivariate logistic regression analyses to identify possible predictors of diabetic retinopathy

	Univariate analysis			*Multivariate analysis*		
	Odds ratio	*%95 CI*	*p*	Odds ratio	*%95 CI*	*p*
HbA1c	1.991	1.293–3.067	**0.002**	1.614	1.244–2.094	**0.001**
Neutrophil count	5.413	0.912–5.064	0.104			
Creatinine	6.873	0.728–64.878	0.092			
NLR	1.372	0.818–2.303	0.231			
MHR	1.188	1.018–1.386	**0.028**	1.197	1.046–1.368	**0.009**

NLR neutrophil to lymphocyte ratio, *MHR* monocyte to HDL-c ratio

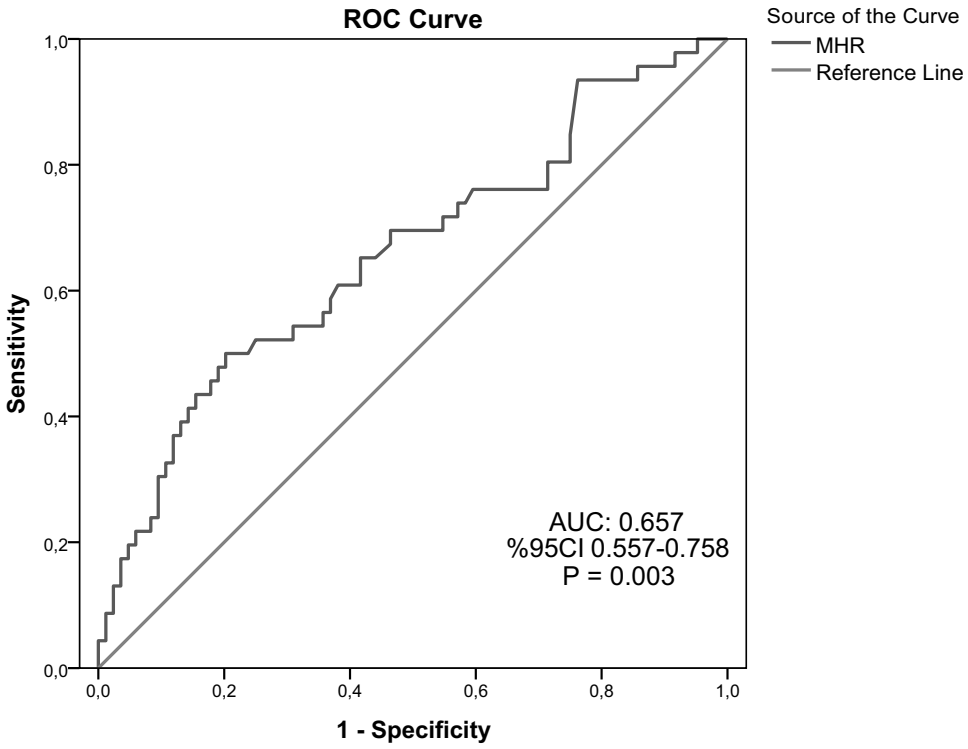

Fig. 1 Receiver operating characteristic (ROC) curve analysis for monocyte to HDL-c ratio as a predictor of diabetic retinopathy

Conclusion

MHR was not higher in T2DM patients without DR than that in the control group. However, T2DM patients with DR had higher MHR values than the other two groups. There is a positive correlation between MHR and DR. Based on these results, MHR could be a biomarker for DR.

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Author contribution Burak Erdem contributed to material preparation and data collection, prepared a study draft, and wrote the manuscript. Yasemin Kaya collected data and performed data analysis. All the authors have read and approved the final version of the manuscript.

Data availability All data are included in this paper.

Declarations

Ethics approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the Ordu University Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

References

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88–98.
2. Wong TY, Cheung N, Tay WT, Wang JJ, Aung T, Saw SM, Lim SC, Tai ES, Mitchell P. Prevalence and risk factors for diabetic retinopathy: the Singapore Malay Eye Study. Ophthalmology. 2008;115(11):1869–75.
3. Varma R, Macias GL, Torres M, Klein R, Peña FY, Azen SP, Los Angeles Latino Eye Study Group. Biologic risk factors associated with diabetic retinopathy: the Los Angeles Latino Eye Study. Ophthalmology. 2007;114(7):1332–40.
4. El-Asrar AM. Role of inflammation in the pathogenesis of diabetic retinopathy. Middle East Afr J Ophthalmol. 2012;19(1):70–4.
5. Tomić M, Ljubić S, Kaštelan S, GverovićAntunica A, Jazbec A, Poljičanin T. Inflammation, haemostatic disturbance, and obesity: possible link to pathogenesis of diabetic retinopathy in type 2 diabetes. Mediators Inflamm. 2013;2013:818671.
6. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD, EURODIAB Prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes–the EURODIAB Prospective Complications Study. Diabetologia. 2005;48(2):370–8.
7. Klein BE, Knudtson MD, Tsai MY, Klein R. The relation of markers of inflammation and endothelial dysfunction to the prevalence and progression of diabetic retinopathy: Wisconsin epidemiologic study of diabetic retinopathy. Arch Ophthalmol. 2009;127(9):1175–82.
8. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol. 2011;11(11):762–74.

9. Assmann G, Gotto AM Jr. HDL cholesterol and protective factors in atherosclerosis. Circulation. 2004;109(23 Suppl 1):III8-14.
10. Connelly MA, Shalaurova I, Otvos JD. High-density lipoprotein and inflammation in cardiovascular disease. Transl Res. 2016;173:7–18.
11. You S, Zhong C, Zheng D, Xu J, Zhang X, Liu H, Zhang Y, Shi J, Huang Z, Cao Y, Liu CF. Monocyte to HDL cholesterol ratio is associated with discharge and 3-month outcome in patients with acute intracerebral hemorrhage. J Neurol Sci. 2017;15(372):157–61.
12. Karataş MB, Çanga Y, Özcan KS, İpek G, Güngör B, Onuk T, Durmuş G, Öz A, Karaca M, Bolca O. Monocyte to high-density lipoprotein ratio as a new prognostic marker in patients with STEMI undergoing primary percutaneous coronary intervention. Am J Emerg Med. 2016;34(2):240–4.
13. Kanbay M, Solak Y, Unal HU, Kurt YG, Gok M, Cetinkaya H, Karaman M, Oguz Y, Eyileten T, Vural A, Covic A, Goldsmith D, Turak O, Yilmaz MI. Monocyte count/HDL cholesterol ratio and cardiovascular events in patients with chronic kidney disease. Int Urol Nephrol. 2014;46(8):1619–25.
14. Gökçay Canpolat A, Emral R, Keskin Ç, Canlar Ş, Şahin M, Çorapçioğlu D. Association of monocyte-to-high density lipoprotein-cholesterol ratio with peripheral neuropathy in patients with type II diabetes mellitus. Biomark Med. 2019;13(11):907–15.
15. Karatas A, Turkmen E, Erdem E, Dugeroglu H, Kaya Y. Monocyte to high-density lipoprotein cholesterol ratio in patients with diabetes mellitus and diabetic nephropathy. Biomark Med. 2018;12(9):953–9.
16. American Diabetes Association. Classification and diagnosis of diabetes. Diabetes Care. 2015;38(Suppl):S8–16.
17. Wong TY, Sun J, Kawasaki R, Ruamviboonsuk P, Gupta N, Lansingh VC, Maia M, Mathenge W, Moreker S, Muqit MMK, Resnikoff S, Verdaguer J, Zhao P, Ferris F, Aiello LP, Taylor HR. Guidelines on Diabetic Eye Care: the International Council of Ophthalmology recommendations for screening, follow-up, referral, and treatment based on resource settings. Ophthalmology. 2018;125(10):1608–22.
18. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet. 1999;353(9165):1649–52.
19. Pitsavos C, Tampourlou M, Panagiotakos DB, Skoumas Y, Chrysohoou C, Nomikos T, Stefanadis C. Association between low-grade systemic inflammation and type 2 diabetes mellitus among men and women from the ATTICA study. Rev Diabet Stud. 2007;4(2):98–104.
20. Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. Exp Diabetes Res. 2007;2007:95103.
21. Lange C, Storkebaum E, de Almodóvar CR, Dewerchin M, Carmeliet P. Vascular endothelial growth factor: a neurovascular target in neurological diseases. Nat Rev Neurol. 2016;12(8):439–54.
22. Luo WJ, Zhang WF. The relationship of blood cell-associated inflammatory indices and diabetic retinopathy: a meta-analysis and systematic review. Int J Ophthalmol. 2019;12(2):312–23.
23. Demirtas L, Degirmenci H, Akbas EM, Ozcicek A, Timuroglu A, Gurel A, Ozcicek F. Association of hematological indices with diabetes, impaired glucose regulation and microvascular complications of diabetes. Int J Clin Exp Med. 2015;8(7):11420–7.
24. Yue S, Zhang J, Wu J, Teng W, Liu L, Chen L. Use of the monocyte-to-lymphocyte ratio to predict diabetic retinopathy. Int J Environ Res Public Health. 2015;12(8):10009–19.
25. Nalini M, Raghavulu BV, Annapurna A, Avinash P, Chandi V, Swathi N, Wasim. Correlation of various serum biomarkers with the severity of diabetic retinopathy. Diabetes Metab Syndr. 2017;11 Suppl 1:S451–4.
26. Ancuta P, Wang J, Gabuzda D. CD16+ monocytes produce IL-6, CCL2, and matrix metalloproteinase-9 upon interaction with CX3CL1-expressing endothelial cells. J Leukoc Biol. 2006;80(5):1156–64.
27. Benhar I, Reemst K, Kalchenko V, Schwartz M. The retinal pigment epithelium as a gateway for monocyte trafficking into the eye. EMBO J. 2016;35(11):1219–35.
28. Rangasamy S, McGuire PG, Franco Nitta C, Monickaraj F, Oruganti SR, Das A. Chemokine mediated monocyte trafficking into the retina: role of inflammation in alteration of the blood-retinal barrier in diabetic retinopathy. PLoS One. 2014;9(10):e108508.
29. Fawwad A, Butt AM, Siddiqui IA, Khalid M, Sabir R, Basit A. Neutrophil-to-lymphocyte ratio and microvascular complications in subjects with type 2 diabetes: Pakistan's perspective. Turk J Med Sci. 2018;48(1):157–61.
30. Davis MD, Fisher MR, Gangnon RE, Barton F, Aiello LM, Chew EY, Ferris FL 3rd, Knatterud GL. Risk factors for high-risk proliferative diabetic retinopathy and severe visual loss: Early Treatment Diabetic Retinopathy Study Report #18. Invest Ophthalmol Vis Sci. 1998;39(2):233–52.
31 Benarous R, Sasongko MB, Qureshi S, Fenwick E, Dirani M, Wong TY, Lamoureux EL. Differential association of serum lipids with diabetic retinopathy and diabetic macular edema. Invest Ophthalmol Vis Sci. 2011;52(10):7464–9.
32. Sasso FC, Pafundi PC, Gelso A, Bono V, Costagliola C, Marfella R, Sardu C, Rinaldi L, Galiero R, Acierno C, de Sio C, Caturano A, Salvatore T, Adinolfi LE, NO BLIND Study Group. High HDL cholesterol: a risk factor for diabetic retinopathy? Findings from NO BLIND study. Diabetes Res Clin Pract. 2019;150:236–44.
33. Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci. 2004;45(3):910–8.
34. Onalan E. The relationship between monocyte to high-density lipoprotein cholesterol ratio and diabetic nephropathy. Pak J Med Sci. 2019;35(4):1081–6.
35. Tamhane UU, Aneja S, Montgomery D, Rogers EK, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. Am J Cardiol. 2008;102(6):653–7.
36. Ulu SM, Dogan M, Ahsen A, Altug A, Demir K, Acartürk G, Inan S. Neutrophil-to-lymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. Diabetes Technol Ther. 2013;15(11):942–7.

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ORIGINAL ARTICLE

Trained nurse–operated teleophthalmology screening approach as a cost-effective tool for diabetic retinopathy

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Abstract

Introduction Teleophthalmology for diabetic retinopathy seems to be a cost-effective, accurate, and reliable method for screening for diabetic retinopathy.

Aims To study the cost-effectiveness of a novel telemedicine-based digital retinal imaging teleophthalmology performed by a locally trained nurse compared to conventional ophthalmologic fundus examination of diabetic patients for early diagnosis of diabetic retinopathy.

Materials and methods We compared the cost of evaluation of diabetic retinopathy in a total of 3090 patients. These were grouped based on the conventional approach of evaluation ($n=1500$) and compared with the teleophthalmology ($n=1590$) approach. The diabetic patients were examined through teleophthalmology by a trained nurse using the Forbes 3nethra fundal camera, and these fundal images were transferred by iCloud to a specialized retina center.

Results In total, 18.2% ($n=562$) patients were diagnosed with diabetic retinopathy (DR). Of these, 8.7% had mild non-proliferative diabetic retinopathy (NPDR), 4.8% had moderate NPDR, 3.8% had severe NPDR, and 0.9% had PDR. The total cost of conventional telemedicine-based digital retinal imaging was approximately INR 550 which in contrast was less than half to the total cost of conventional dilated fundus examination by an ophthalmologist (INR 1400).

Conclusion Our cost analysis indicates that telemedicine-based diabetic retinopathy screening is economical (INR 550 as compared to INR 1400) than conventional retinal examination.

Keywords Teleophthalmology · Diabetic retinopathy · Microvascular complication · Cost-effective tool

Introduction

Telemedicine enables taking health care to remote areas where delivery of specialized expert services was a logistic and infrastructural nightmare. Teleophthalmology relies entirely on streaming visual images from the patient sitting at any geographical location to a qualified ophthalmologist for expert advice for the screening and diagnosis, therapeutic management planning, and follow-up of different eye diseases.

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In the contemporary era, digital innovations that include artificial intelligence (AI), 5th-generation (5G) telecommunication networks, and the Internet of Things (IoT) have the immense potential for creating an inter-dependent ecosystem. These would eventually offer newer opportunities to develop new models of eye care [1].

Diabetic retinopathy (DR) capacity building has been postulated as a suitable model. This has been documented for the Indian scenario which can be performed through trained optometrists for a coordinated screening in India [2]. Ophthalmology has now evolved and rapidly emerged with the adoption of internet by the masses providing numerous economic and health benefits [3, 4]. Diabetic retinopathy is one of the main contributors to blindness in India. Early screening of this complication can prevent blindness and hence most guidelines recommend regular screening for diabetic retinopathy in all individuals with diabetes. There is growing evidence establishing the role of teleophthalmology in screening for diabetic retinopathy and addressing the comorbidities of diabetes [5–7].

Tele-screening for diabetic retinopathy is fast emerging as a cost-effective, accurate, and reliable method for diabetic retinopathy screening. It appears that in an advanced nation like Singapore, artificial intelligence for teleophthalmology-based diabetic retinopathy screening is a cost-effective tool [8].

Among the various field of medicine, telemedicine for the differentiation to transmit medical information electronically to improve patients' health status has been found to be especially beneficial for its cost-effectiveness for the diagnosis of diabetic retinopathy [9]. The Right to Sight is the global initiative program launched by WHO to eliminate avoidable blindness by 2020 (called VISION 2020). Teleophthalmology with smartphones is postulated to be a powerful approach to achieve the aim of VISION 2020 all over the world [10]. The trained nurse staff has immense potential for the delivery of the diabetic retinopathy screening program [11]. Studies demonstrate that patients with diabetes are concerned for it might take a long time to be referred to an ophthalmologist when it is needed. A vast majority of the patients find it easier to have DR screening during routine diabetes follow-up. Also, limited patients doubt the doctor's ability to diagnose DR by evaluating retina photos only [12]. There is a need for the cost-effective tool for the screening for DR. This is even more pertinent now, for the fact that with high rates of sensitivity and specificity with the new devices for teleophthalmology. Therefore, there is an immense opportunity for teleophthalmology [13]. Various studies have demonstrated the cost-effectiveness of using telemedicine for screening for DR [14]. An economic analysis on cost-effectiveness of telemedicine for DR screening by Bjorvig et al. found it to be a much cheaper option for DR screening in places with higher patient workloads [15]. Raman et al. demonstrated a sensitivity of 62.5% and a specificity of 98.7% of the non-mydriatic technique of retinal photography in comparison to indirect ophthalmoscopy. The mydriatic retinal photography technique on the other hand showed a sensitivity of 70% and a specificity of 98% when compared to indirect ophthalmoscopy [16].

Objective

This study aims to evaluate the cost-effectiveness of a novel telemedicine-based digital retinal imaging, performed by a locally trained nurse, compared with the manual conventional ophthalmologic fundus examination of diabetic patients for early diagnosis of diabetic retinopathy.

Materials and methods

We evaluated 3090 diabetic patients at a premier outpatient diabetes center by teleophthalmology. The study was conducted from February 2017 until May 2020. The patients were analyzed across two groups, one with the conventional screening ($n = 1500$) and the second group by the tele-screening ($n = 1590$). We utilized the 3nethra classic digital non-mydriatic fundus camera, which was operated by a locally trained nurse. However, to enhance the sensitivity, we utilized only the mydriatic images. The images were transferred by iCloud to a specialized retina center. These images were evaluated and reported by the qualified ophthalmologist, dedicated only to report the findings for these images. The costs for the standard of care ophthalmic examinations were the mean of the prevalent charges, across the varied class of patients. The cost to the patient in the conventional group included the direct cost of consultation, indirect cost for the repeat visit, and loss of man-hours. The cost to the patient for teleophthalmology included the cost levied by the ophthalmologist and the fixed training cost (Table 2).

The training to the nurse is provided for two sessions, spanning over 2 days for a total of 8 to 10 h with a structured protocol with built-in hands-on experience module. The cost of telemedicine-based digital retinal imaging examination included cost for devices, training, annual maintenance costs, and reporting fee by a retina specialist. These were compared with the costs of the conventional process for the referral to a retina specialist for fundal examination. The patients, who were diagnosed with mild non-proliferative diabetic retinopathy (NPDR), were advised a repeat visit to our center in the subsequent 6 months. The management plan for these patients was customized that necessitated a re-evaluation for the progress of the status of the DR. Categorically, the patients who were diagnosed to have moderate to severe DR were the only ones that were referred for further evaluation by retina specialists. The turnaround time of 24 h was consistently achieved which accounted for the time from sharing of the image for the evaluation to be initiated until the reporting of the findings. The costs were grouped and compared across the conventional and the tele-screening modality.

Results

The mean age was $52.7 \pm$ SD 9.2 years. Of the total 3090 patients, 1350 were females and 1740 were males. The duration of diabetes varied in the range of 1–19 years (mean $9.4 \pm$ SD 6). In total, 52% of the patients had diabetes in the duration that was less than 5 years, 26% of the patients had diabetes in the range of 6–10 years, and 22% of the patients had diabetes with duration more than 10 years.

In the entire cohort, 18.2% ($n = 562$) patients were diagnosed with DR. Of these, 8.7% had mild NPDR, 4.8% had moderate NPDR, 3.8% had severe NPDR, and 0.9% had PDR.

The patients with DR had significantly higher mean HbA1c (9.4 ± 1.3) compared to patients without DR (7.6 ± 1.3, $p = 0.007$) (Table 1).

The primary direct cost of telemedicine-based reporting levied by the ophthalmologist per patient was approximately 300 Indian National Rupee (INR). The total cost of conventional telemedicine-based digital retinal imaging and evaluation for medical assistant, capital cost including the equipment and training maintenance, and reporting fee was approximately INR 550 (Table 2). In contrast, the total cost of conventional dilated fundus examination by an ophthalmologist is approximately INR 1400. There were 9.5% ($n = 293$) of the patients who needed the additional conventional ophthalmologic retinal examination due to the clinically relevant finding on telemedicine evaluation. The additional conventional retinal examination cost calculated per patient for the entire cohort of 1590 patients amounted Rs 57.

Discussion

Our study demonstrates the benefits of virtual tele-consultations for a rapid, cost-effective tool in diabetic retinopathy screening. A cost–benefit analysis indicates that this screening modality is an economical approach. The cost per patient for the tele-retina modality is less than half as compared to the conventional retinal examination (vis-à-vis INR 550 as compared to INR 1400). Apart from being cost-effective, the tele-retina consultative model provides a consistent, a predictable, and a timely quick turnaround time from the evaluation to the report generation. This enhances the efficiency for the DR screening and makes the process effective in our high-volume center. Moreover, the criteria to evaluate the cases for NPDR within 6 months enabled a continuity of care, for a close continuous follow-up.

Our study is in contrast with the studies that have shown no benefit of the computer-aided evaluation for the screening of glaucoma, via tele-consultation in patients with diabetes, through a non-mydriatic approach [16].

Our results add to the evidence-based perspective, as demonstrated in the earlier studies that digital health solutions and telehealth can enable the integration of primary and specialist eye care services for patients with diabetes. As shown in earlier studies, there was an overall improvement of the efficiency, including the cost-effectiveness of diabetic retinopathy management [17–19].

The findings of our study are also in line with the ten emerging trends in the epidemiology of DR, which suggests that photographic screening of DR using teleophthalmology platforms is increasingly recognized as being feasible and cost-effective [20].

Our study is also in concurrence with the recommendations by the current guidelines that suggest that tele-retina screening can help to address the need for timely and effective screening for DR [21]. We utilized only the mydriatic images to enhance the sensitivity of our findings. Our clinical approach has been in the context of the recommendation by the contemporary guidelines from the Canadian Retina Research Network that suggest the preference for mydriatic images for teleophthalmology. However, the manufacturer of the device (3nethra classic digital non-mydriatic fundus camera) does not mandate the need for dilatation of the pupil.

Our study is limited from the perspective of a corroborative evidence as it was conducted at a single center. The larger collaborative work would be more useful to add value to our findings that provide a solution to challenge for the

Table 1 The relationship between retinopathy and its risk factors ($n = 3090$)

Risk factors	With DR	Without DR	*p* value
HbA1c %	9.4 ± 1.3	7.3 ± 1.3	0.007
Fasting blood sugar (mg/dl)	132 ± 23.3	113 ± 19.7	0.001
Body mass index (kg/m^2)	25.3 ± 2.4	23.8 ± 2.2	0.001
Duration of DM (years)	12.8 + 1.8	8.3 + 1.6	0.001

Table 2 Comparison of the cost across the conventional and teleophthalmology groups

Cost component for respective groups at our center	Individual gross cost to patient	INR
Conventional ($n = 1500$)	Direct cost of consultation	600
	Indirect cost for repeat visit	500
	Loss of man-hours (calculated based on travel expense & travel time of pts & working attendants per pt)	300
Total cost to the patient		1400
Teleophthalmology ($n = 1590$)	Cost levied by the ophthalmologist	300
	Fixed device cost, training, annual maintenance costs	193
Additional conventional retinal examination cost (calculated per pt)		57
Total cost to the patient		550

delivery of cost-effective eye care of patients with diabetes in a practical and viable mode. We also did not account for the intangible benefits like the patient satisfaction scores, the cost savings for the man-hours for the time saved in travelling, and enhanced productivity of the patients and the support care providers including the family members. However, we expect to be in a similar line, which needs a further exploration [22, 23].

Conclusion

Teleophthalmology for DR screening is a cost-effective approach which can facilitate higher screening rates. We propose that large-scale adoption of teleophthalmology should be encouraged as a means towards providing low-cost access to DR screening for timely detection as well as management of DR.

References

1. Li JO, Liu H, Ting DSJ, Jeon S, Chan RVP, Kim JE, Sim DA, Thomas PBM, Lin H, Chen Y, Sakomoto T, Loewenstein A, Lam DSC, Pasquale LR, Wong TY, Lam LA, Ting DSW. Digital technology, tele-medicine and artificial intelligence in ophthalmology: a global perspective. Prog Retin Eye Res. 2020;6:100900. https://doi.org/10.1016/j.preteyeres.2020.100900 (**Epub ahead of print**).
2. Rani PK, Peguda HK, Chandrashekher M, Swarna S, Jonnadula GB, James J, Shinde L, Bharadwaj SR. Capacity building for diabetic retinopathy screening by optometrists in India: model description and pilot results. Indian J Ophthalmol. 2021;69(3):655–9. https://doi.org/10.4103/ijo.IJO_1944_20.
3. Carl HP, Ehsan R, Abtin S, Jay LF. Telemedicine in ophtahlmology: numerous factors are driving a transition to remote screening. Retina Today.2017 Apr; 55–58
4. Morse AR. Telemedicine in ophthalmology: promise and pitfalls. American Academy of Ophthalmology. Elsevier Inc., Ophthalmology. 2014 Apr;121(4)
5. Sosale AR. Screening for diabetic retinopathy—is the use of artificial intelligence and cost-effective fundus imaging the answer? Int J Diabetes Dev Ctries. 2019;39:1–3. https://doi.org/10.1007/s13410-019-00729-y.
6. Sosale B, Sosale AR, Mohan AR, Kumar PM, Saboo B, Kandula S. Cardiovascular risk factors, micro and macrovascular complications at diagnosis in patients with young onset T2 diabetes in India: CINDI 2. Indian J Endocrinol Metab. 2016;20:114–8.
7. Viswanathan V. Diabetic kidney disease and diabetic retinopathy: the ominous duo. Int J Diabetes Dev Ctries. 2020;40:313–4. https://doi.org/10.1007/s13410-020-00867-8.
8. Xie Y, Nguyen QD, Hamzah H, Lim G, Bellemo V, Gunasekeran DV, Yip MYT, Qi Lee X, Hsu W, Li Lee M, Tan CS, Tym Wong H, Lamoureux EL, Tan GSW, Wong TY, Finkelstein EA, Ting DSW. Artificial intelligence for teleophthalmology-based diabetic retinopathy screening in a national programme: an economic analysis modelling study. Lancet Digit Health. 2020;2(5):e240–9. https://doi.org/10.1016/S2589-7500(20)30060-1 (**Epub 2020 Apr 23**).
9. Delgoshaei B, Mobinizadeh M, Mojdekar R, Afzal E, Arabloo J, Mohamadi E. Telemedicine: a systematic review of economic evaluations. Med J Islam Repub Iran. 2017;31:113. https://doi.org/10.14196/mjiri.31.113.
10. Mohammadpour M, Heidari Z, Mirghorbani M, Hashemi H. Smartphones, tele-ophthalmology, and VISION 2020. Int J Ophthalmol. 2017;10(12):1909–18. https://doi.org/10.18240/ijo.2017.12.19.
11. Boucher MC, Nguyen MTD, Qian J. Assessment of training outcomes of nurse readers for diabetic retinopathy telescreening: validation study. JMIR Diabetes. 2020;5(2):e17309. https://doi.org/10.2196/17309.
12. Alhumud A, Al Adel F, Alwazae M, Althaqib G, Almutairi A. Patient satisfaction toward a tele-retinal screening program in endocrinology clinics at a tertiary hospital in Riyadh, Saudi Arabia. Cureus. 2020;12(5):e7986. https://doi.org/10.7759/cureus.7986.
13. Kalogeropoulos D, Kalogeropoulos C, Stefaniotou M, Neofytou M. The role of tele-ophthalmology in diabetic retinopathy screening. J Optom. 2020;13(4):262–8. https://doi.org/10.1016/j.optom.2019.12.004 (**Epub 2020 Jan 14**).
14. Javit JC. Cost-savings associated with detection and treatment off diabetic eye disease. Pharmacoeconomics. 1995;8:33–9.
15. Bjorvig S, Johansen MA, Fossen K. An economic analysis of telemedicine to evaluate diabetic retinopathy. J Telemed Telecare. 2002;8:32–5.
16. Raman R, Padmaja RK, Sharma T. The sensitivity and specificity of non-mydriatic digital stereoscopic retinal imaging in detecting diabetic retinopathy. Diabetes Care. 2007;30:1.
17. Gunasekeran DV, Ting DSW, Tan GSW, Wong TY. Artificial intelligence for diabetic retinopathy screening, prediction and management. CurrOpinOphthalmol. 2020;31(5):357–65. https://doi.org/10.1097/ICU.0000000000000693.
18. Kanjee R, Dookeran RI, Mathen MK, Stockl FA, Leicht R. Six-year prevalence and incidence of diabetic retinopathy and cost-effectiveness of tele-ophthalmology in Manitoba. Can J Ophthalmol. 2017;52(Suppl 1):S15–8. https://doi.org/10.1016/j.jcjo.2017.09.022.
19. DeBuc DC. The role of retinal imaging and portable screening devices in tele-ophthalmology applications for diabetic retinopathy management. Curr Diab Rep. 2016;16(12):132. https://doi.org/10.1007/s11892-016-0827-2.
20. Sabanayagam C, Yip W, Ting DS, Tan G, Wong TY. Ten emerging trends in the epidemiology of diabetic retinopathy. Ophthalmic Epidemiol. 2016;23(4):209–22. https://doi.org/10.1080/09286586.2016.1193618 (**Epub 2016 Jun 29**).
21. Dookeran R, Maberley D, Samad A, Chaudhary V, Steering Committee for Tele-Ophthalmology Screening, Canadian Retina Research Network. Evidence-based Canadian guidelines for tele-retina screening for diabetic retinopathy: recommendations from the Canadian Retina Research Network (CR2N) Tele-Retina Steering Committee. Can J Ophthalmol. 2020;55(1 Suppl):14–24. https://doi.org/10.1016/j.jcjo.2020.01.001.
22. Sreelatha OK, Ramesh SV. Teleophthalmology: improving patient outcomes? Clin Ophthalmol. 2016;10(10):285–95. https://doi.org/10.2147/OPTH.S80487.
23. Brady CJ, Villanti AC, Gupta OP, Graham MG, Sergott RC. Teleophthalmology screening for proliferative diabetic retinopathy in urban primary care offices: an economic analysis. Ophthalmic Surg Lasers Imaging Retina. 2014;45(6):556–61. https://doi.org/10.3928/23258160-20141118-11.

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ORIGINAL ARTICLE

Utility of urinary progranulin in patients with type 2 diabetic nephropathy and its correlation with renal function

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Abstract

Introduction Urinary progranulin is an inflammatory marker that may indicate renal damage at an early stage of diabetic nephropathy.

Objectives To determine urinary level of progranulin in patients with type 2 diabetic nephropathy and to ascertain its correlation with renal function.

Methods This was a cross-sectional comparative study that was carried out at Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Pondicherry, India. Thirty-three control subjects and 99 diabetic patients (divided into 3 equal groups based on urinary albumin excretion) were included. Albuminuria was detected by dipstick method in a spot urine sample for all subjects. Urinary albumin level was estimated by nephelometry for diabetic subjects who had trace or no albuminuria by dipstick method. Urinary progranulin was estimated by ELISA technique for all study subjects. eGFR (estimated glomerular filtration rate) was calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) formula for all study subjects.

Results Urinary progranulin levels were found to be significantly elevated in diabetic patients as compared to the control subjects ($p < 0.001$). There was a significant moderately positive correlation between urinary progranulin and urinary albumin values among diabetic study subjects ($p < 0.001$).

Conclusion Urinary progranulin levels are increased in patients with type 2 diabetic nephropathy and it correlated with albuminuria. However, there was no correlation of urinary progranulin with glomerular filtration rate (GFR) in patients with diabetic nephropathy.

Keywords Urinary progranulin · Albuminuria · Glomerular filtration rate

Introduction

Diabetic nephropathy is a common cause of end-stage renal disease (ESRD). Diabetic nephropathy is diagnosed by the detection of an abnormal level of urinary albumin or a decline in the glomerular filtration rate (GFR) in patients with type 2 diabetes mellitus [1]. A twenty-four-h urine protein estimation is the gold standard for the assessment of diabetic nephropathy; however, it is cumbersome. Estimation of microalbuminuria (urinary albumin to creatinine ratio) on a spot urine sample is relatively easier and is commonly used for the diagnosis of diabetic nephropathy, but the test lacks specificity.

Progranulin is an adipokine that is secreted from adipocytes, epithelial cells, neurons, and few cells of the immune system. Progranulin is cleaved by proteolytic enzymes to generate granulin peptides in the body which promote inflammation. Progranulin has been found to be associated with obesity and insulin resistance. Pro-inflammatory actions of progranulin are mediated by the upregulation of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) expression which suggests a possible role of progranulin in the pathogenesis of microvascular complications of diabetes [2]. There is growing evidence to suggest that chronic inflammation plays a crucial role in the pathogenesis of

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diabetic nephropathy [3]. Patients with type 2 diabetes mellitus (T2DM) were found to have higher serum values of progranulin especially those with macroalbuminuria [4]. Serum and urinary levels of progranulin are dependent on renal function. Hence, there is an increase in serum progranulin value and a reduction in urinary progranulin value in patients with ESRD due to reduced excretion of progranulin by the kidneys.

Urinary progranulin levels may reflect an ongoing state of inflammation-mediated renal damage at an early stage of diabetic nephropathy. Till date, there are few Indian studies that have assessed the correlation of urinary progranulin with renal function in patients with T2DM.

The primary objective of this study was to estimate urinary progranulin levels in patients with type 2 diabetes mellitus and to compare them with non-diabetic control subjects. The secondary objective was to find a correlation between urinary progranulin level and renal function in patients with and without diabetic nephropathy.

Material and methods

This was a cross-sectional comparative study that recruited patients attending the medicine out-patient department (OPD) of JIPMER Hospital, Pondicherry from August 2018 to January 2020.

Patients with type 2 diabetes mellitus on oral hypoglycemic drugs or on insulin were included as the study subjects. Patients visiting the OPD for minor ailments, without any comorbidities and no symptoms suggestive of diabetes and with a random blood sugar value less than 140 mg/dL were recruited as control subjects for the study (random blood sugar of less than 140 mg/dL was taken as the cutoff since there is a low likelihood of patients being diabetic below this value). Patients with urinary tract infection, systemic arterial hypertension, and on angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) were excluded from the study. Patients with inflammatory diseases and those on maintenance hemodialysis for chronic kidney disease or patients who had undergone kidney transplantation were also excluded from the study.

Sample size was calculated by using PS calculator version 3.0 Jan 2009. Based on a study done by Nicoletto et al., the difference between mean urinary progranulin levels of diabetic patients and non-diabetic subjects were taken as 1.69 ng/mL with a standard deviation of 0.7 ng/mL. Sample size was calculated to be 33 for the control group and 99 for the study group, which were further sub-divided into 3 groups of 33 each, based on urinary albumin excretion.

Thirty-three non-diabetic subjects were recruited as group 1 and 99 diabetic patients were recruited in the study group. Diabetic patients were further sub-classified based on urinary albumin values into 3 groups, each group having 33 subjects. Subjects without albuminuria were included as group 2; those with microalbuminuria were included as group 3, and those with macroalbuminuria were included as group 4.

The study was approved by the Institute Ethics Committee (Human studies), JIPMER, Puducherry (JIP/IEC/2018/0253). All study subjects received adequate information about the study and gave written, informed consent for participation in the study. Data was obtained from the subjects and their health records regarding the duration of diabetes. Weight, height, body mass index (BMI), and blood pressure (BP) measurements were recorded for all the study subjects.

Spot urine sample was tested for albuminuria for all study subjects using the dipstick method. Urine sample was preserved for the assessment of microalbuminuria in subjects without albuminuria or in subjects with trace albuminuria. Another urine sample was preserved for the assessment of progranulin in all diabetic subjects and controls. All urine samples were stored at −80°C till the time of processing.

Urinary albumin level was estimated by nephelometry for diabetic subjects who had trace or no albuminuria by dipstick method. Subjects with spot urinary albumin level more than 30 mg/L were included in the microalbuminuria group, and those with urinary albumin more than 300 mg/L were included in the macroalbuminuria group. This was based on the correlation of spot urinary albumin in mg/L to urinary albumin excretion in mg/mg of creatinine.

Progranulin estimation in urine samples was done by using quantitative human progranulin ELISA kits having a sensitivity of 5 ng/mL and a detection range of 5–480 ng/mL. The kit was supplied by Shanghai Korain Biotech Co. Ltd. This kit uses enzyme-linked immune sorbent assay (ELISA) based on the biotin double-antibody sandwich technology to assay progranulin.

eGFR (estimated glomerular filtration rate) was calculated using chronic kidney disease epidemiology collaboration (CKD-EPI) formula for control subjects and patients with type 2 diabetes mellitus in this study.

Statistical analysis

Data analysis was done using SPSS version 19.0. The distribution of categorical variables like gender and duration of diabetes in diabetic patients was expressed as frequencies and percentages. Distribution of categorical variables among various groups was analyzed by using chi-square or Fischer exact test.

Continuous data such as age, eGFR, BMI, and urinary progranulin levels showed a non- normal distribution and were expressed as median with inter-quartile range. Comparison of urinary progranulin levels between the study and

control groups was done using Kruskall-Wallis test. Post-hoc analysis between the sub-groups among diabetic patients was done using Mann-Whitney *U* test. Correlation between various parameters was tested using Spearman's correlation test.

Multivariate analysis was done to find out the correlation of urinary progranulin with urinary albumin in the study. Receiver operator characteristic (ROC) curve was plotted to find out the utility of urinary progranulin as a marker for predicting nephropathy in type 2 diabetic patients in relation to microalbuminuria.

All statistical analyses were carried out at 5% level of significance and p value of less than 0.05 was considered as significant.

Results

The baseline characteristics of the study population is shown in Table 1. Seventy-four percent of subjects in the study population had eGFR > 90 mL/min. One study subject had eGFR less than 15 mL/min in the normo-albuminuria group. Fourteen (10%), 16 (12%), and 4 (3%) study subjects were in stages 2, 3, and 4 of chronic kidney disease (CKD) respectively, based on their eGFR values. There was no significant difference in the study groups depending on the distribution of eGFR values.

Among subjects in the normo-albuminuria group, the median urinary albumin value was 11.2 mg/L; and among patients with microalbuminuria, the median urinary albumin value was 73 mg/L.

Forty-three percent of the study subjects with duration of diabetes more than 10 years were in the macroalbuminuria group. This is also shown in Table 1.

The median urinary progranulin levels in non-diabetic controls was 3.24 ng/ml with an inter- quartile range (IQR) of 2.10–5.55 ng/mL while among diabetic study subjects, median urinary progranulin level was 9.19 ng/mL with an IQR of 5.89–12.5 ng/mL. There was a significant difference in the median progranulin value between diabetic subjects and non-diabetic controls ($p < 0.001$).

The median urinary progranulin levels in the diabetic sub-groups are also shown in Table 1. There was a significant difference in the median value of progranulin between non-diabetic subjects and diabetic patients in the various sub-groups ($p < 0.001$). Also, the urinary progranulin value of diabetic patients with micro and macroalbuminuria was higher when compared to those with normoalbuminuria, and the difference was statistically significant ($p < 0.001$).

However, urinary progranulin levels among diabetic patients with microalbuminuria did not differ significantly when compared to those with macroalbuminuria ($p = 0.84$). This is shown in Figure 1.

There was a significant moderately positive correlation between urinary progranulin and urinary albumin values among diabetic study subjects; $r = 0.579$ ($p < 0.001$). This is depicted in Figure 2. In multivariate analysis, urinary albumin showed an association with urinary progranulin (beta = 0.30, $p = 0.017$) after adjustment for age, BMI, and eGFR values.

Receiver operator characteristic (ROC) curve was plotted to assess the utility of urinary progranulin as a marker for predicting diabetic nephropathy in relation to microalbuminuria. A cut-off value of 5.62 ng/mL for urinary progranulin was found to have a sensitivity of 81.8% and specificity of 78.8% (area under curve = 0.894; 95% confidence interval 0.840–0.948; $p < 0.001$). This is shown in Figure 3.

Table 1 Baseline characteristics of the study population

Parameter	Controls	Diabetic with normoalbuminuria	Diabetic with microalbuminuria	Diabetic with macroalbuminuria	*p* value
Median age in years (min–max)	50 (36–68)	54 (32–75)	53 (38–75)	54 (40–70)	-
Male gender (%)	20 (25%)	19 (23.8%)	19 (23.8%)	22 (27.5%)	-
Weight (kg)	67 (39–78)	67 (43–84)	68 (50–80)	65 (48–92)	-
Height (cm)	164 (146–175)	162 (146–174)	164 (142–174)	164 (145–175)	-
BMI in kg/m^2 (min–max)	25 (18.3–30.2)	25.3 (16.8–36.8)	24.9 (18–33.7)	24.4 (19–33.6)	0.81
Duration of diabetes (years)	-	< 10 years–28 (83%) > 10 years–6 (17%)	< 10 years–25 (86%) > 10 years–7 (14%)	< 10 years–19 (57%) > 10 years–14 (43%)	-
Serum creatinine (mg/dL)	0.6 (0.4–1.6)	0.7 (0.3–4.6)	0.7 (0.4–2.5)	0.8 (0.4–2.5)	0.31
eGFR (mL/min/1.73 m^2)	107 (45–137)	102 (10–158)	100 (25–184)	102 (10.6–184)	0.29
Median urinary progranulin (ng/mL)	3.24 (IQR-2.10–5.55)	6.51 (IQR-3.93–8.42)	11.64 (IQR-8.60–14.12)	11.46 (IQR-8.03–14.82)	< 0.001

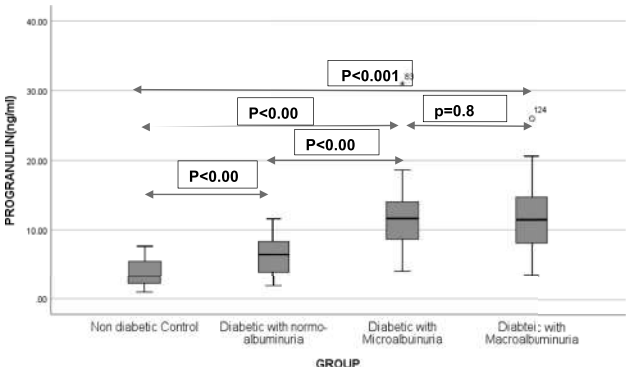

Fig. 1 Box plot showing urinary progranulin according to study groups

There was no significant correlation between urinary progranulin and eGFR in patients with diabetic nephropathy with correlation coefficient, $r = -0.08$ ($p = 0.366$).

Discussion

Patients with type 2 diabetes mellitus have a period of undetected and clinically asymptomatic hyperglycemia, which leads to alterations in metabolic homeostasis. Thus, diabetic patients can have micro and macrovascular complications at the time of diagnosis [5]. There is extensive knowledge regarding the pathogenetic mechanisms involved in the initiation and progression of diabetic nephropathy. Inflammation is one of the important mechanisms that leads to diabetic nephropathy.

Progranulin is an adipokine with both pro and anti-inflammatory actions. It is linked to multiple risk factors which are associated with the development of complications in diabetic patients such as visceral obesity, dyslipidemia, insulin resistance, and elevated levels of cytokines like TNF-α and IL-6 [6–8]. Excretion of progranulin in urine is dependent on renal function [9]. Thus, progranulin is involved in the pathogenesis of microangiopathy as an inflammatory adipokine and is dependent on renal function for its metabolism. It could be a sensitive biomarker for predicting diabetic nephropathy at an early stage and in subsets of patients with diabetic nephropathy where changes in GFR occur in the absence of albuminuria [10–12].

Our study found median urinary progranulin value of non-diabetic control subjects to be lower when compared to the median urinary progranulin value that was observed in controls in a study done by Nicoletto et al. Similarly, lower urinary progranulin values were also seen in diabetic subjects in our study. The reason for this difference is not clear.

The median eGFR values of control subjects in our study are comparable to the median eGFR values in the study done by Nicoletto et al. [12]. As there is no data from Indian studies on baseline urinary or serum progranulin levels in non-diabetic subjects, this needs to be confirmed in a large study population.

There was a significant difference between urinary progranulin values in control subjects as compared to diabetic patients in our study. This is similar to an observation in the study by Nicoletto et al. [12]. Our study also showed a significant difference in the urinary progranulin values between control subjects and each diabetic sub-group. Also, median urinary progranulin values were higher in groups 3 and 4 as compared to group 2. There was no significant difference observed in urinary progranulin values among diabetic patients with microalbuminuria and those with macroalbuminuria. This did not correlate with the finding of decreased

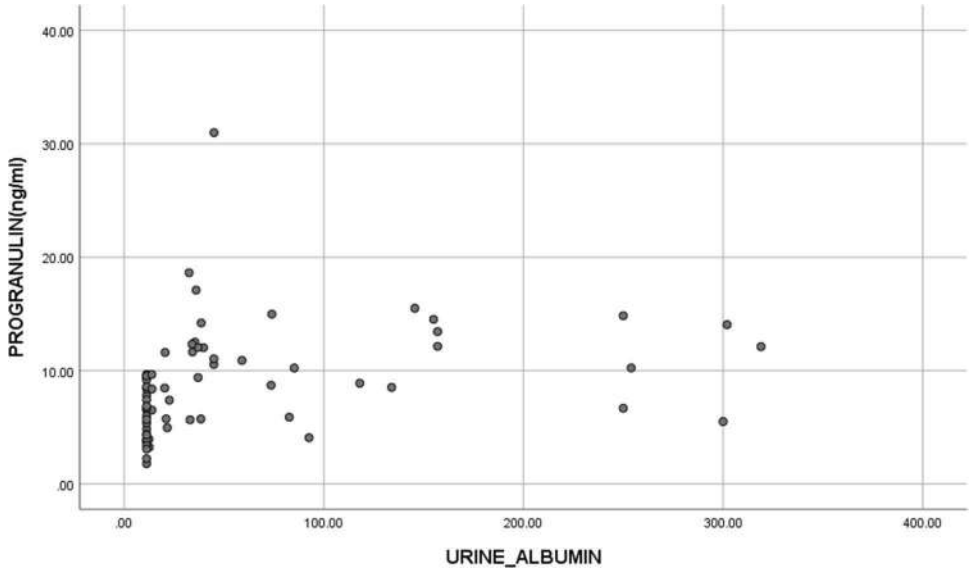

Fig. 2 Scatter diagram showing relationship between urinary albumin and rogranulin

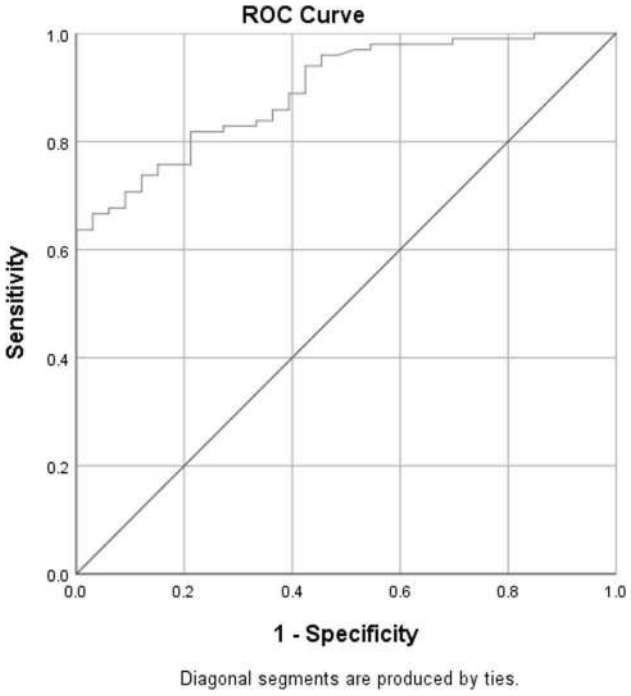

Fig. 3 ROC curve for urinary progranulin

urinary progranulin in advanced cases of diabetic nephropathy as compared to patients with microalbuminuria, which were observed in other studies. The possible reason for this in our study could be that a majority of diabetic subjects with nephropathy (groups 3 and 4) had normal eGFR values. Diabetic subjects with nephropathy having significant reduction in eGFR that could have affected progranulin excretion were not adequately represented in our study population. This could have resulted in no significant difference in urinary progranulin values between groups 3 and 4 in our study.

Our study did not find correlation of urinary progranulin values with GFR in the study subjects. Similar findings were reported by Nicoletto et al. [12] in their study. However, Richter et al. found reduction in urinary progranulin values with decrease in GFR among their study subjects [9]. The reason for these observed trends is not clear.

Correlation analysis of urinary progranulin with albuminuria showed a significant association in our study Nicoletto et al. [12] also reported a similar correlation in their study. This suggests an indirect evidence of graded and incremental relationship between urinary progranulin and renal damage.

Utility of urinary progranulin as a marker of diabetic nephropathy was assessed by the receiver operator characteristic (ROC) curve in our study. Urinary progranulin was found to be fairly sensitive and specific in predicting diabetic nephropathy.

Utility of serum progranulin as a potential predictor of microangiopathy in patients with type 2 diabetes mellitus has been shown in studies done by Xu et al. and Albeltagy et al. [6, 8].

Our study did not show a significant correlation between urinary albumin and duration of diabetes among the study subjects. This stresses on the point that albuminuria is a less sensitive marker of microangiopathy [13]. Pathological changes in kidneys could have already set in by the time albuminuria was detected. However, some studies have shown an association between the duration of diabetes and albuminuria [14].

Limitations of the study

1. Due to the cross-sectional study design, a temporal relationship between elevated urinary progranulin level and the onset of diabetic nephropathy could not be established.
2. Visceral fat, glycosylated hemoglobin, and serum IL-6 were not included which would have provided better results for comparison of various parameters.
3. Diabetic subjects with reduced eGFR were under represented in the study which did not allow us to ascertain a definite relationship of urinary progranulin with eGFR.
4. Spot urinary albumin (instead of 24-h urinary albumin) was used as a marker of diabetic nephropathy in the study subjects.

Conclusion

Urinary progranulin levels are higher in type 2 diabetic patients as compared with non- diabetic subjects, and it has a moderately strong association with albuminuria. However, there is no correlation of urinary progranulin with the glomerular filtration rate (eGFR) in patients with diabetic nephropathy.

Author contribution 1. Abhishek Rai-Data acquisition and analysis.
2. Gandhipuram Periyasamy Senthilkumar-Critical revision of intellectual content
3. Vadivelan Mehalingam-Conception of the work and final approval of the version to be published

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Data availability All data and material in the article comply with field standards.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The study was approved by the Institutional Ethics Committee (Human studies) of JIPMER, Pondicherry vide certificate-JIP/IEC/2018/0253 dated 05/09/2018. All participants gave their informed, written consent to participate in the study.

Consent for publication The authors affirm that study participants provided informed consent for publication of data from the study.

Conflict of interest The authors declare no competing interests.

References

1. Powers AC, Stafford JM, Rickels MR. Diabetes mellitus: complications. In: Jameson JL, Kasper DL, Longo DL, Fauci AS, Hauser SL, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 20th ed. United States of America: McGraw-Hill; 2018. p. 2878.
2. Hossein-Nezhad A, Mirzaei K, Ansar H, Emam-Gholipour S, Tootee A, Keshavarz SA. Obesity, inflammation and resting energy expenditure: possible mechanism of progranulin in this pathway. Minerva Endocrinol. 2012;37:255–66.
3. Matoba K, Takeda Y, Nagai Y, Kawanami D, Utsunomiya K, Nishimura R. Unraveling the role of inflammation in the pathogenesis of diabetic kidney disease. Int J Mol Sci. 2019;10(20):3393.
4. Nicoletto BB, Krolikowski TC, Crispim D, Canani LH. Serum and urinary progranulin in diabetic kidney disease. PLoS One. 2016;11:e0165177.
5. Powers AC, Stafford JM, Rickels MR. Diabetes mellitus: complications. In: Jameson JL, Kasper DL, Longo DL, Fauci AS, Hauser SL, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 20th ed. United States of America: McGraw-Hill; 2018. p. 2875.
6. Xu L, Zhou B, Li H, Liu J, Du J, Zang W, et al. Serum levels of progranulin are closely associated with microvascular complication in type 2 diabetes. Dis Markers. 2015;2015:357279.
7. Qu H, Deng H, Hu Z. Plasma progranulin concentrations are increased in patients with type 2 diabetes and obesity and correlated with insulin resistance. Mediators Inflamm. 2013;2013:360190.
8. Albeltagy ES, Hammour AE, Albeltagy SA. Potential value of serum progranulin as a biomarker for the presence and severity of micro vascular complications among Egyptian patients with type 2 diabetes mellitus. J Diabetes Metab Disord. 2019;18:217–28.
9. Richter J, Focke D, Ebert T, Kovacs P, Bachmann A, Lössner U, et al. Serum levels of the adipokine progranulin depend on renal function. Diabetes Care. 2013;36:410–4.
10. Goldfine AB, Shoelson SE. Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. J Clin Invest. 2017;127:83–93.
11. Ganesh Yerra V, Negi G, Sharma SS, Kumar A. Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-κB pathways in diabetic neuropathy. Redox Biol. 2013;1:394–7.
12. Nicoletto BB, Canani LH. The role of progranulin in diabetes and kidney disease. Diabetol Metab Syndr. 2015;7:117.
13. Papadopoulou-Marketou N, Kanaka-Gantenbein C, Marketos N, Chrousos GP, Papassotiriou I. Biomarkers of diabetic nephropathy: a 2017 update. Crit Rev Clin Lab Sci. 2017;54:326–42.
14. Chowta NK, Pant P, Chowta MN. Microalbuminuria in diabetes mellitus: association with age, sex, weight, and creatinine clearance. Indian J Nephrol. 2009;19:53–6.

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ORIGINAL ARTICLE

Recipient IL28B genotype CT is a predictor of new onset diabetes mellitus in liver transplant patients with chronic hepatitis C

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Abstract

Background New onset diabetes after liver transplantation (NODAT) is increasingly recognized as a complication that affects the quality of life. In addition to well-known risk factors for diabetes in such patients, little is known regarding the genetic predisposition for this condition.

Aims To study the association of IL28B polymorphism with occurrence of new onset diabetes after liver transplantation in HCV patients.

Methods A prospective cohort study. Fifty non-diabetic LDLT recipients were recruited from the Liver Transplantation Unit at El Manial, Cairo University Hospital. FBS was done at 0, 3, and 12 months; HBA1c was done after 6 months. IL28B rs12979860 polymorphism was done to all patients.

Results According to FBS after 3 months and HBA1C, 20 patients developed diabetes, 21 patients prediabetes and 9 remained normal. IL28B CT genotype was 78%, CC 20% and TT 2%. Univariate regression showed that the CT genotype was significantly associated with higher blood glucose at 0 months (coefficient ± SE, 64 ± 4.7; $p < 0.001$), 3 (coefficient ± SE, 58 ± 4.0; $p < 0.001$), and 12 months (coefficient ± SE, 57 ± 3.2; $p < 0.001$), and with the development of diabetes and prediabetes after LDLT (coefficient ± SE, 0.6 ± 0.06; $p < 0.001$).

Conclusions IL28B polymorphism is significantly associated with new onset diabetes after LDLT. CT genotype may represent a marker to identify high risk recipients.

Keywords Interleukin 28 B (IL28B) · Liver donor liver transplantaion (LDLT) · New onset diabetes after liver transplantation (NODAT) · rs12979860

Introduction

New onset diabetes after transplantation (NODAT) is a significant metabolic sequel that impacts both graft and patient survival in addition to their quality of life [1].

Nearly 30–40% of recipients have had diabetes lasting for over 6 months after transplant procedure [2]. However, the actual number of cases with insulin resistance in post-liver transplant patients is yet to be elucidated.

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A significant association between chronic HCV infection and increased insulin resistance, both in the pre- and post-transplant settings has been noted [1, 3]. Although the mechanism behind this is unclear, it has been shown that viral eradication improves insulin sensitivity [4].

It has been established that genetic IL28B polymorphisms which code for IFN-λ have been highly linked with sustained virologic response to peg IFN-a therapy for HCV infection [5]. However, it has been noted that IL28B polymorphisms might also be related to the development of diabetes mellitus in these patients [6]. In fact, previous studies in non-transplant patients have shown higher incidence of insulin resistance in carriers of the T allele of SNP rs12979860 than in CC homozygotes [7].

The aim of this study was to investigate the association of IL28B rs12979860 variant to new onset diabetes after transplantation (NODAT).

Materials and methods

Population of study & disease condition

The study comprises 50 Egyptian patients with ESLD (end-stage liver disease) that had undergone living donor liver transplantation (LDLT) at the Liver Transplantation Unit at El Manial, Cairo University Hospital, during 2017.

Our study included patients with post hepatitis C cirrhosis. Patients were recruited from Kasr Al-Ainy liver transplantation list, which are usually classified as Child's C with MELD score ranging from 13 to 25.

Patients with pre-transplant diabetes mellitus, A1C ≥ 5.7%, IGT, or IFG on previous testing were excluded.

Methodology

This is a cross-sectional study. Subjects who had undergone living donor liver transplantation were enrolled from the Liver Transplantation Unit at El Manial Registry, Cairo University Hospital. Consecutive patients were enrolled at the outpatient clinic, if they had post hepatitis C end-stage liver disease (ESLD) as an indication for transplantation and are still showing up for follow-up visits. Patients with pre-transplant diabetes mellitus were excluded.

Data of patients including baseline fasting blood glucose, lipid profile and other lab tests, immunosuppressive drugs, and doses, including steroids, cyclosporine, and tacrolimus were recorded.

Comprehensive history taking and clinical examination was done to all patients on follow-up visits. Family history of diabetes mellitus, history of gestational diabetes, or delivery of large baby in female patients was noted. Blood pressure was measured and BMI was calculated in kg/m^2.

FBS was done at 0, 3, and 12 months; HBA1c was done after 6 months; CBC, liver function tests, triglycerides, and HDL-C were done to all patients. IL28B genotyping was done using SNP rs12979860-real time PCR technique. Real-time PCR allelic discrimination was performed on Step One™ Real-Time PCR System (Applied Bio systems), using TaqMan SNP Genotyping Assays and TaqMan Universal PCR Master Mix (Applied Bio systems, Foster City, CA, USA). DNA extraction was done using GeneJET Whole Blood Genomic DNA Purification Mini kit, #K0781, #K0782, Thermo Scientific.

Statistical methods

Sample size calculation and statistical analysis were done using MedCalc software (version 17.8 – © 1993–2017 MedCalc Software bvba). According to the correlation coefficient (0.6) correlating genotype with different study groups, with type I error (alpha, significance) = 0.05 and type II error (beta, 1-Power) = 0.20, the minimum required sample was 19 subjects. All data were entered into a Microsoft Excel spreadsheet. Summary statistics included mean, standard error for the mean, minimum, maximum, median, and interquartile range. Chi-squared test was used to compare categorical variables (frequencies). Continuous data was tested for normal distribution using the D'Agostino-Pearson test. (*Sheskin DJ* (*2011*) *Handbook of parametric and nonparametric statistical procedures. 5th ed. Boca Raton: Chapman & Hall /CRC.)* Data was NOT normally distributed; therefore, non-parametric tests were applied. Kruskal–Wallis test was used to compare ordinal data (multiple comparison), and Jonckheere-Terpstra trend test was used to compare medians. Univariate regression analysis was used to test for the association of the genotype as an independent variable against other variables.

Table 1 Summary statistics for fasting blood glucose (FBG) at various intervals and HBA1C at 6 months

	Post-operative FBG (mg/dl)	FBG after 3 months (mg/dl)	FBG after 12 months (mg/dl)	HBA1C (%)
Min	74	10	77	4.3
Max	359	245	208	8.2
Median	113.5	104	99	5.8
IQR	96–132	95–121	93.5–116.5	5.1–6.6
Mean	123.42	112.98	109.17	5.93
SE for mean	6.96	5.45	3.92	0.21

FBG, fasting blood glucose; *IQR*, interquartile range; *Max*, maximum; *Min*, minimum; *SE*, standard error

Results

Fifty LDLT recipient patients with no previous diabetes mellitus were assessed as mentioned for new onset diabetes after transplantation. This was done by fasting blood glucose for all patients at regular intervals and HBA1c as required. Maximum, minimum, mean and standard errors, median and interquartile range (IQR) for fasting blood glucose levels at zero, 3, and 12 months, as well as HBA1C at 6 months are shown in Table 1. Comparison of FBS over time by Kruskal–Wallis test showed a decline in average rank from 0 to 3 months, and a further decline from 3 to 12 months. This decline was shown to be significant by Jonckheere-Terpstra trend test (compares median values trend over time), with *p* value 0.04059, (Fig. 1). Figure 2 shows HBA1c distribution within the study cohort.

According to fasting blood glucose (FBG) after 3, 12 months, and HBA1c criteria, the patients were divided into 3 groups: 20 patients developed diabetes; 21 patients fell into criteria for prediabetes; and 9 remained normal as regards blood glucose level. Percentages for those groups are shown in Fig. 3.

The study group comprised only 6 female subjects out of 50. No significant difference was noted among the three study groups as regards age and gender (Table 2).

Distribution of study sample according to the date of liver transplantation is shown in Fig. 4.

Conventional risk factors for type 2 diabetes were analyzed in the current study. Positive family history of diabetes, history of gestational diabetes, and history of delivery

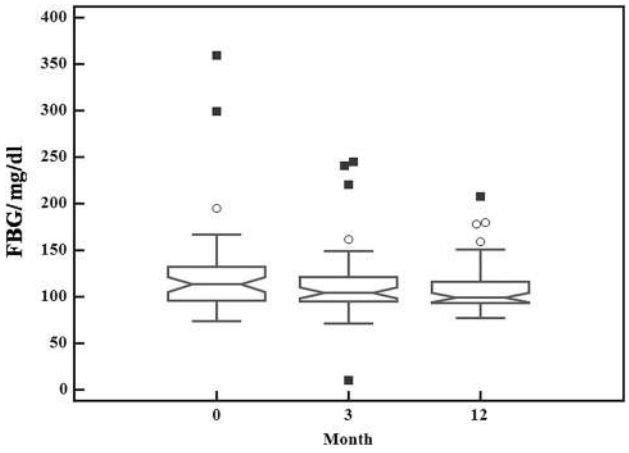

Fig. 1 Box and whisker graphs comparing FBG over time. Boxes represent the interquartile ranges (25–75%); notches mark the medians. Significant decline is evident over time, even in eccentric values (small squares)

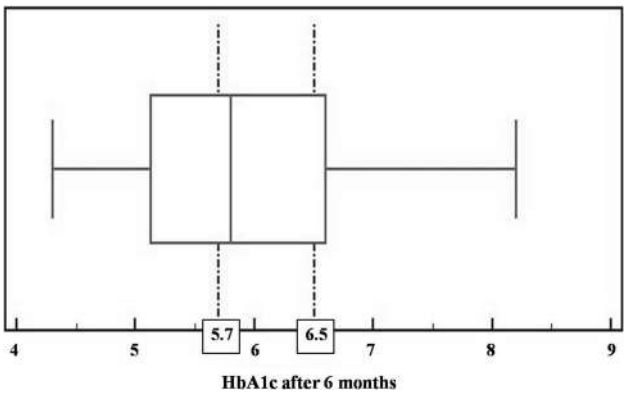

Fig. 2 Box and whisker plot showing the distribution of HBA1c values. The first vertical line at the value 5.7 identifies the threshold for prediabetes, while the second marks the threshold for overt diabetes at 6.5

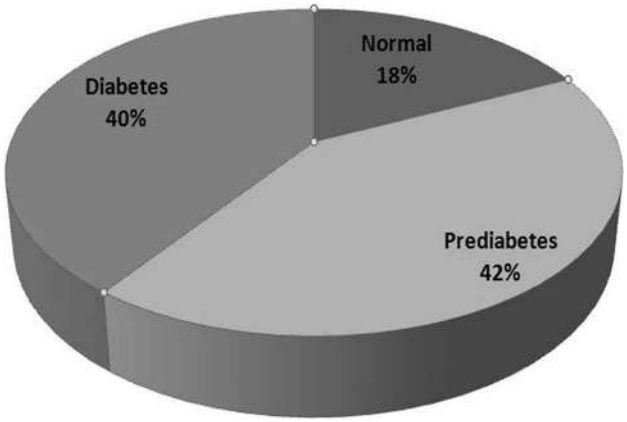

Fig. 3 Percentage of the groups who developed diabetes or prediabetes or remained normal

of a large baby were all non-significant. Two patients who developed overt diabetes had CVD as well as one patient of the prediabetes group and another one from the normal group. Two patients who developed overt diabetes had hypertension as well as three patients of the prediabetes group and another three from the normal group.

Comparison of various laboratory values among different groups revealed a significant increase in total bilirubin in patients with prediabetes than normal subjects. Also, MELD was significantly higher in normal patients than those who developed diabetes (Table 3, Figs. 5 and 6). Comparison of various potential risk factors for diabetes revealed no significant differences among the three groups (Tables 4 and 5). Serum triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were non-significant.

Other potentially confounding factors specific to liver transplant recipients were analyzed. These included metabolic stress due to the major operation, post-operative period, ICU stay, and early post-operative complications. Early complications were encountered in 2 patients from the diabetes group, as well as 4 patients from the prediabetes group, and another 3 from the normal group.

The effect of immunosuppressive agents with variable diabetogenic properties was then addressed. Most patients received steroids, tacrolimus, and MMF. Other drugs such as everolimus, cyclosporine, and mycophenolic acid were used to a lesser extent. None of the study cohort patients received pulse steroids or peg IFN-a treatment. Tacrolimus level and steroid maintenance dose were also included in the analysis.

On analysis of IL28B genotypes, CT was the most prevalent (74%), followed by CC variant (24%). Only one patient had the TT variant (2%), that patient developed prediabetes. The distribution of various IL28B genotypes among the three study groups is shown in Table 6.

Table 2 Distribution of the study sample according to age and gender

	Gender			Age		
	Females	Males	*p* value[1]	Range	Mean ± SD	*p* value[2]
DM	1 (5.0%)	19 (95.0%)	0.3824	43–57	49.9 ± 4.6	0.334761
Normal	1 (11.1%)	8 (88.9%)		33–52	46.1 ± 5.7	
Prediabetes	4 (19.0%)	17 (81.0%)		28–58	46.7 ± 9.0	

[1]Chi-squared test

[2]Kruskal-Wallis test

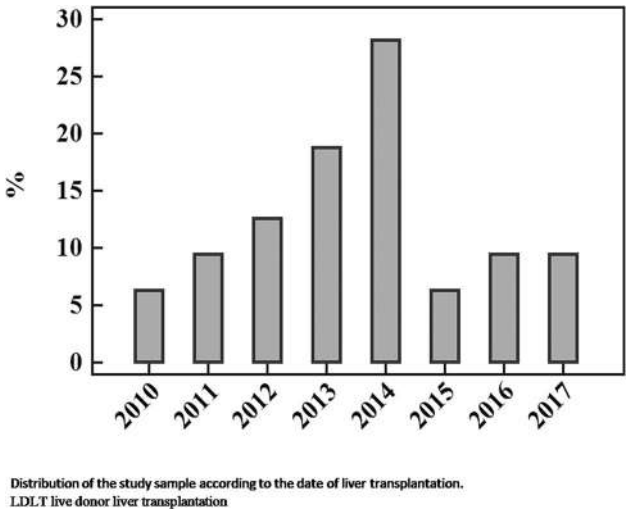

Fig. 4 Distribution of the study sample according to the date of liver transplantation

Univariate regression between genotype variants on one side and each of the following endpoints for assessment of diabetes: (1) fasting blood glucose 0 months, (2) fasting blood glucose 3 months, (3) fasting blood glucose 12 months, and (4) the different diabetes categories (normal, prediabetes, diabetes).

A significant association was found between all above variables and genotype polymorphism. **CT** variant was significantly associated with higher blood glucose at 0, 3, and 12 months, p value < 0.0001. **CC** variant was significantly associated with lower blood glucose at 0, 3, and 12 months, p value < 0.0001. Also, CT variant was significantly associated with development of diabetes and prediabetes, p value < 0.0001. Regression coefficients and p values are shown in Table 7.

Discussion

New onset diabetes after transplantation (NODAT) has become a substantial side effect of liver transplantation [5]. It affects long-term outcomes due to more frequent infections and ischemic heart disease [8].

The aim of this work was to investigate the association of IL28B genotype with the occurrence of NODAT in HCV patients. The study comprised 50 Egyptian patients with post hepatitis C ESLD that had undergone LDLT at the Liver Transplantation Unit at El Manial, Cairo University Hospital. Patients were all classified as Child's C with MELD score ranging from 13 to 25. Patients with pre-transplant diabetes mellitus were excluded.

The study cohort showed a disturbance in FBG done at 0, 3, and 12 months post transplantation, and HBA1c done at 6 months that were higher than expected. The overall outcome of 40% new onset diabetes in addition to 42% prediabetes was striking.

The study results revealed a higher frequency of NODAT in comparison to a study done by Veldt et al. in 2012 on HCV patients who underwent liver transplantation. Out of a total of 221 patients, 69 developed post-transplant DM (31%) [9]. Another study by Duca et al. in 2014 found that 28 out of 99 post-liver transplant patients developed NODAT [10]. The incidence of NODAT according to Xue et al. was 34.6% [11] while it was stated as 28 to 40%, in a review article by Lv C et al. 2015 [12]. In a different cohort of Egyptian living donor liver transplantation recipients, the prevalence of NODAT was 27.43% [13].

Patients may be predisposed to diabetes and insulin resistance due to conventional risk factors for type 2 diabetes in addition to factors specific to liver transplant recipients. Screening for susceptible individuals is essential to recognize high-risk patients. Hence, it may be possible to decrease the number of patients developing diabetes by applying proper lifestyle interventions as well as tailored immunosuppressive therapy [6].

Conventional risk factors for type 2 diabetes were not significant for NODAT in our study cohort, including lipid profile. Therefore, none of those parameters would represent a confounding factor in interpretation of our genetic study.

Antônio et al. had associated NODAT after liver transplantation to male gender, waist circumference, and insulin resistance. They found no correlation with triglyceride levels [14]. On the other hand, Liang et al. demonstrated a significant risk of liver NODAT with higher preoperative

Table 3 Summary and comparison of liver functions and blood picture among different groups

	DM ($n=20$)				Normal ($n=9$)				Prediabetes ($n=21$)				p value[1]
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
ALB (g/dl)	3.9	0.3	3.4	4.5	3.8	0.5	3.1	4.5	3.7	0.5	2.6	4.5	0.661114
ALP (mg/dl)	202.0	202.3	67.0	961.0	119.8	28.0	83.0	178.0	180.8	140.2	51.0	562.0	0.681393
ALT (mg/dl)	42.9	19.9	11.0	75.0	29.4	23.4	8.0	80.0	52.0	41.0	11.0	186.0	0.123099
AST (mg/dl)	48.8	36.8	10.0	163.0	27.8	11.1	15.0	51.0	48.9	32.9	11.0	130.0	0.241079
T.Bil (mg/dl)	1.0	1.1	0.3	5.3	0.7	0.2	0.3	1.0	1.2	0.9	0.4	4.5	0.044231*
GGT (mg/dl)	207.5	328.2	23.0	1440.0	61.3	37.9	18.0	135.0	136.2	137.3	21.0	598.0	0.067061
MELD	17.3	3.3	11.0	25.0	21.1	4.0	12.0	25.0	19.3	4.1	13.0	28.0	0.020420*
HB (g/dl)	12.5	1.9	10.0	15.3	13.3	2.1	11.1	16.5	11.6	1.6	9.2	15.8	0.077603
PLT (10^3/cmm)	145.8	47.0	75.0	231.0	155.3	31.6	105.0	207.0	148.9	41.1	75.0	224.0	0.788730
TLC (10^3/cmm)	5.2	1.4	3.1	8.8	6.3	2.1	3.4	10.1	6.2	2.5	3.3	11.8	0.353564

[1]Kruskal-Wallis test

ALB, albumin; *ALP*, alkaline phosphatase; *ALT*, alanine transaminase; *AST*, aspargine transaminase; *GGT*, gamma glutamyl transferase; *HB*, hemoglobin; *Max*, maximum; *MELD*, model for end-stage liver disease; *Min*, minimum; *PLT*, platelets; *T.BIL*, total bilirubin; *TLC*, total leucocytic count

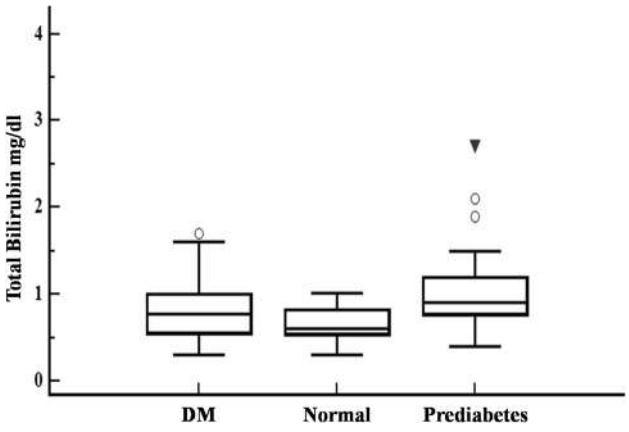

Fig. 5 Box and whisker graphs comparing total bilirubin levels among the study groups. Boxes represent the interquartile ranges (25-75percentiles), transverse lines mark the medians and eccentric values are marked separately. Bilirubin was significantly higher in patients who developed prediabetes

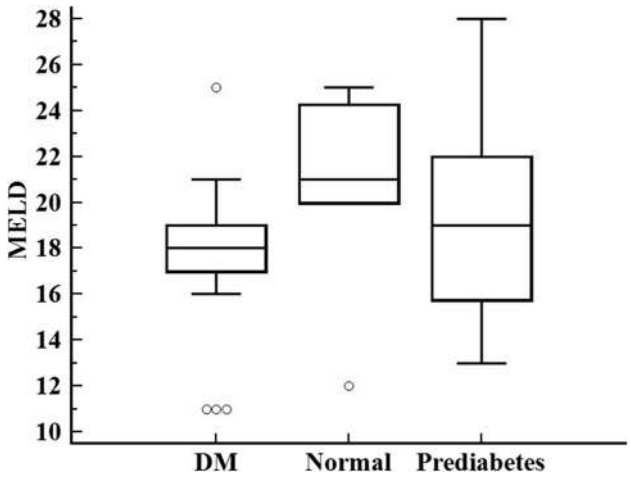

Fig. 6 Box and whisker graphs comparing the MELD score among the study groups. Boxes represent the interquartile ranges (25-75percentiles), transverse lines mark the medians and eccentric values are marked separately. Patients who developed diabetes and prediabetes had a significantly lower MELD than those who did not

triglyceride levels, even within the normal range, among male patients only [15].

Although operative and post-operative risk factors for diabetes including different immunosuppressive agents showed no significant distribution across the study groups, they had a major role in post-operative elevation of FBS. There was a significant decline in FBS over time in the post-operative period by comparing FBS at 0, 3, and 12 months. Zayed et al. have shown that tacrolimus was independently associated with NODAT after living donor liver transplantation [13].

Table 4 Summary and comparison of some potential risk factors for diabetes among different groups (ordinal data)

	DM				Normal				Prediabetes				*p* value[1]
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	
Tacrolimus level	6.1	2.2	2.7	11.5	4.6	2.2	1.7	7.8	5.2	1.8	1.3	9.1	0.312177
Steroids dose	19.0	13.4	0.0	45.0	18.9	8.6	0.0	30.0	12.9	9.6	0.0	30.0	0.103544
BMI	26.5	2.6	21.0	32.0	28.2	3.5	24.0	34.0	27.2	3.2	20.0	33.0	0.491895
HDL	39.9	12.1	28.0	74.0	34.6	7.4	25.0	43.0	50.8	27.7	9.0	86.0	0.158430
TG	128.4	45.6	58.0	204.0	154.0	25.8	114.0	186.0	117.8	36.7	77.0	169.0	0.269808

[1]Kruskal-Wallis test

BMI, body mass index; *LDL*, low density lipoprotein; *Max*, maximum; *Min*, minimum; *TG*, triglycerides

Table 5 Frequency and comparison of some potential risk factors for diabetes among different groups (categorical data)

	DM	Normal	Prediabetes	***p*** value[1]
CVD	2	1	1	0.7687
HTN	2	3	3	0.2734
DRUGS:				
Cellcept	16	4	16	0.1220
Certican	5	1	4	0.6808
Cyclosporine	3	4	6	0.2321
Myfortic	5	6	6	0.0714
Tacrolimus	19	8	19	0.8069
Early complications	9	4	12	0.6908
Late complications	2	3	4	0.3140

[1]Chi-squared test

CVD, coronary vascular disease; *HTN*, hypertension

Table 6 Frequencies of different genotypes among the study groups

	Number	Genotype		
		CC	CT	TT
DM	20	6 (30%)	14 (70%)	0
Normal	9	2 (22%)	7 (88%)	0
Prediabetes	21	4 (19%)	16 (76%)	1 (5%)
Total	50	12 (24%)	37 (74%)	1 (2%)

New onset diabetes after liver transplantation is a particular entity, with a suggested unique acronym (NODALT), as it is directly involved in metabolic mechanisms associated with insulin resistance [16].

It appears that living donor grafts pose additional peculiarity as to the cause of NODALT. Stockmann et al. and Gebhardt et al. found that immunosuppression plays only a minor role in glucose metabolism in such patients [17, 18]. They proposed that the transplanted graft is the culprit for diabetes development; however, other factors include genetic predisposition of the recipient.

Ling et al. have summarized the risk factors for NODALT into 3 categories, namely the liver graft, pancreatic islets, and gut microbiota. The graft category included "donor" genetic polymorphisms only. Donor IL-28 rs12979860 TT was mentioned as a risk factor for post-transplant steatosis but not diabetes [16].

On analysis of recipient IL28B genotypes SNP rs12979860, the current study showed that CT was the most prevalent (78%) in patients who developed diabetes (14 of 20 patients) and prediabetes (16 of 21 patients), followed by CC variant (20%). Only one patient had the TT variant (2%); that patient developed prediabetes.

Veldet et al. in 2012 found that recipient IL28B rs12979860 TT genotype carried more than two-fold the risk to develop NODAT [9] while Duca et al. in 2014 established that IL28B rs12979860 CC genotype was protective against

Table 7 Univariate regression between genotype variants and markers of diabetes

Variable	Coefficient	Standard error	95% CI[1]	*t*	*p* value
FBG 0 months (mg/dl)	64.2781	4.6882	54.8568 to 73.6994	13.7105	< 0.0001
FBG 3 months (mg/dl)	58.4734	4.0110	50.4130 to 66.5337	14.5784	< 0.0001
FBG 12 months (mg/dl)	57.3230	3.2048	50.8757 to 63.7703	17.8864	< 0.0001
Study groups[3]	0.6331	0.06337	0.5058 to 0.7605	9.9911	< 0.0001

[1]Normal, prediabetes, and diabetes groups

CI, confidence interval; *FBG*, fasting blood glucose

NODAT in patients with cirrhosis due to chronic HCV [10]. Watt et al. had shown that IL28B rs12979860 genotype was not associated with post-transplant diabetes or even insulin resistance in 295 liver transplant recipients, 85 of which were chronic HCV patients [19].

In the current study. a univariate regression analysis showed a significant association of CT variant with the development of prediabetes and diabetes to a greater extent. Also, the presence of the "T" allele was significantly associated with higher FBS at all-time intervals. CC variant showed a weaker association with the prediabetic and diabetic groups. Moreover, it was significantly associated with lower FBS levels at all time intervals.

It has been shown that genetic IL28B polymorphisms coding for IFN-λ are highly linked to sustained virologic response (SVR) to pegIFN-a treatment for chronic HCV infection [20]. IFN-λ, together with IFN-a, acts on IFN-stimulated genes (ISGs) via SOCS (suppressors of cytokine signaling) and the JAK-STAT (janus kinase signal transducers and activators of transcription) pathway. However, later studies have linked IL28B polymorphisms to the development of diabetes mellitus in HCV patients [9, 10, 20]. Studies addressing this have shown conflicting results.

It was found that recipient rather than donor IL28B polymorphisms was associated with NODAT. This fact suggests that an immune mechanism maybe the culprit in the development of insulin resistance in NODAT patients [9].

A plausible explanation is that IFN-λ-3, the product of IL28B, was found to be an antiviral cytokine with pro-inflammatory characteristics. Despite these speculations, the actual pathophysiology behind the metabolic effects of IL28B polymorphisms is still unclear. The link between CC IL28B rs12979860 variant and consequent expression of IL28B mRNA in hepatocytes or in in peripheral blood mononuclear cells (PBMC) is also controversial [21–23].

On the other hand, CT and TT IL28B rs12979860 variants were shown in previous literature to be associated with an increased expression of interferon-stimulated genes (ISGs) [23–25]. Suppressors of cytokine signaling 3 and 7 (SOCS-3 and SOCS-7) are two ISGs that were shown to be risk factors for decreased insulin sensitivity in HCV patients [26–28] via disrupting insulin receptor pathway signaling leading to increased insulin resistance and hence NODAT [29, 30]. This work paves the way for research into IL28B rs12979860 polymorphism as a potential risk factor for type 2 diabetes.

Limitation of this study lies in the fact that only 3 glucose readings were analyzed for each patient. Blood glucose levels show variability according to a lot of factors as stressful conditions, infections, and different drug levels. A larger validation cohort is recommended with more frequent blood glucose monitoring and the application of glycosylated hemoglobin.

Conclusion and recommendations

In a cohort of 50 Egyptian LDLT patients with no previous diabetes, 42% developed prediabetes, and 40% developed overt diabetes beyond 3-month postoperative. The CT genotype of IL28B polymorphism can represent a genetic predisposition to diabetes and prediabetes after liver transplantation. It showed a significant association with the development of prediabetes and diabetes. It was also linked to higher FBS at 0, 3, and 12 months. On the other hand, the CC genotype of IL28B seems to be protective against diabetes and prediabetes after liver transplantation. It was linked to lower FBS at 0, 3, and 12 months and showed a significant association with patients who did not develop prediabetes or diabetes.

The authors recommend that IL28B genotype may be used as a marker to identify individuals at high risk to develop diabetes and prediabetes after liver transplantation. Such patients should undergo an individualized approach concerning procedures and choices of immunosuppressive drugs, as well as addressing all known risk factors for diabetes, in order to minimize their risks and hence their outcome.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Research protocols were approved by the medical ethics committee of Kasr Alainy Medical School, Cairo University. The ethical committee certificate reference number: I–030414.

Informed consent A written informed consent was obtained from all participants. In case patients were unable to provide consent, it was taken from designated surrogates.

References

1. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatol. 2008;47(6):1856–62.
2. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. American Journal of Transplantation 2010 May 10;10(6):1420–7. http://www.ncbi.nlm.nih.gov/pubmed/20486907
3. Veldt BJ, Poterucha JJ, Watt KDS, Wiesner RH, Hay JE, Rosen CB, et al. Insulin resistance, serum adipokines and risk of fibrosis progression in patients transplanted for hepatitis C. Am J Transplant. 2009;9(6):1406–13.
4. Romero-Gómez M, Fernández-Rodríguez CM, Andrade RJ, Diago M, Alonso S, Planas R, et al. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. J Hepatol. 2008;48(5):721–7.

5. Khan MS, Shoukat A, Mudassar S, Kawoosa Z, Shah AH, Zargar SA. Impact of IL28B genetic variant's and viral genotype on treatment outcome of hepatitis C infected patients. J Infect Develop Countries. 2018;12(09):762–70. https://doi.org/10.3855/jidc.10175.
6. Krentz AJ, Dmitrewski J, Mayer D, Nattrass M. Effects of immunosuppressive agents on glucose metabolism. Clinical Immunotherapeutics. 1995 Aug;30;4(2):103–23. http://link.springer.com/https://doi.org/10.1007/BF03259076
7. Stättermayer AF, Rutter K, Beinhardt S, Scherzer T-M, Stadlmayr A, Hofer H, et al. Association of the IL28B genotype with insulin resistance in patients with chronic hepatitis C. Journal of hepatology. 2012 Sep;57(3):492–8. http://www.ncbi.nlm.nih.gov/pubmed/22634340
8. Skladaný Ľ, Mesárošová Z, Bachová B, Stančík M, Dedinská I. Alcohol-related liver disease as a new risk factor for posttransplant diabetes after liver transplantation. Transpl Proc. 2019;51(10):3369–74.
9. Veldt BJ, Duarte-Rojo A, Thompson AJ, Watt KD, Heimbach JK, Tillmann HL, et al. Recipient IL28B polymorphism is an important independent predictor of posttransplant diabetes mellitus in liver transplant patients with chronic hepatitis C. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2012 Mar;12(3):737–44. http://www.ncbi.nlm.nih.gov/pubmed/22300408
10. Duca AM, de la Fuente S, Citores MJ, Cuenca AB, Cisneros E, Escamilla N, et al. CC genotype at rs12979860 of IL28B is associated with lower risk of new-onset diabetes after transplantation in adult patients with liver transplantation for hepatitis C cirrhosis. Transplantation proceedings. 2014 Nov;46(9):3114–6. http://www.ncbi.nlm.nih.gov/pubmed/25420838
11. Xue M, Lv C, Chen X, Liang J, Zhao C, Zhang Y, et al. Donor liver steatosis: a risk factor for early new-onset diabetes after liver transplantation. J Diabetes Investig. 2017;8:181–7.
12. Lv C, Zhang Y, Chen X, Huang X, Xue M, Sun Q, et al. New-onset diabetes after liver transplantation and its impact on complications and patient survival. J Diabetes. 2015;7:881–90.
13. Zayed RA, Bahgat MH, Wahab MA, El-Etreby S, Saad RA, Elmorsy FM. Prevalence and risk factors of new onset diabetes after liver transplantation (NODAT): a single Egyptian center experience. Arch Dig Disord. 2017;1(1):7–13.
14. Andrade AR, Bittencourt PL, Codes L, Evangelista MA, Castro AO, Boa SN, et al. New onset diabetes and non-alcoholic fatty liver disease after liver transplantation. Ann Hepatol. 2017;16(6):932–40.
15. Liang J, Yi X, Xue M, Chen X, Huang X, Sun Q, et al. A retrospective cohort study of preoperative lipid indices and their impact on new-onset diabetes after liver transplantation. J Clin Lab Anal. 2020;34:e23192.
16. Ling Qi Xu, Xiao WB, Lanjuan Li, Shusen Z. The origin of new-onset diabetes after liver transplantation. Transpl. 2016;100(4):808–13. https://doi.org/10.1097/TP.0000000000001111.
17. Stockmann M, Konrad T, Nolting S, Hünerbein D, Wernecke KD, Döbling H, et al. Major influence of liver function itself but not of immunosuppression determines glucose tolerance after living-donor liver transplantation. Liver Transpl. 2006;12:535–43.
18. Gebhardt S, Jara M, Malinowski M, Seehofer D, Puhl G, Pratschke J, Stockmann M. Risk factors of metabolic disorders after liver transplantation: an analysis of data from fasted patients. Transpl. 2015;99(6):1243–9.
19. Watt KD, Dierkhising R, Fan C, Heimbach JK, Tillman H, Goldstein D, et al. Investigation of PNPLA3 and IL28B genotypes on diabetes and obesity after liver transplantation: insight into mechanisms of disease. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2013 Sep;13(9):2450–7. http://www.ncbi.nlm.nih.gov/pubmed/23859071
20. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, et al. Interleukin-28B Polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology. 2010 Jul;139(1):120–129.e18. https://linkinghub.elsevier.com/retrieve/pii/S0016508510005743
21. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, et al. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. Cancer biology & therapy. 2008 Jul;7(7):1109–15. http://www.ncbi.nlm.nih.gov/pubmed/18698163
22. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nature genetics. 2009 Oct;41(10):1100–4. http://www.ncbi.nlm.nih.gov/pubmed/19749758
23. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nature genetics. 2009 Oct;41(10):1105–9. http://www.ncbi.nlm.nih.gov/pubmed/19749757
24. Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, Sakai Y, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterol. 2010;139(2):499–509.
25. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology. 2010 Dec;52(6):1888–96. http://www.ncbi.nlm.nih.gov/pubmed/20931559
26. Dill MT, Duong FHT, Vogt JE, Bibert S, Bochud P, Terracciano L, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. Gastroenterology. 2011 Mar;140(3):1021–1031.e10. https://linkinghub.elsevier.com/retrieve/pii/S0016508510017294
27. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. The American journal of pathology. 2004 Nov;165(5):1499–508. http://www.ncbi.nlm.nih.gov/pubmed/15509521
28. Pazienza V, Clément S, Pugnale P, Conzelman S, Foti M, Mangia A, et al. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. Hepatology. 2007 May;45(5):1164–71. http://www.ncbi.nlm.nih.gov/pubmed/17465001
29. Vanni E, Abate ML, Gentilcore E, Hickman I, Gambino R, Cassader M, et al. Sites and mechanisms of insulin resistance in non-obese, nondiabetic patients with chronic hepatitis C. Hepatology. 2009 Sep;50(3):697–706. http://www.ncbi.nlm.nih.gov/pubmed/19582803
30. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nature reviews Molecular cell biology. 2006 Feb;7(2):85–96. http://www.ncbi.nlm.nih.gov/pubmed/16493415

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ORIGINAL ARTICLE

Relationship between nonalcoholic fatty liver disease and bone mineral density in type 2 diabetic patients

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Abstract

Purpose To analyze the relationships between nonalcoholic fatty liver disease (NAFLD) and bone mineral density (BMD) in type 2 diabetes (T2DM) patients.

Methods A total of 446 men aged ≥ 50 years and 306 postmenopausal women with T2DM were recruited. The differences of BMD levels in T2DM patients with or without NAFLD were analyzed. According to the tertile levels of BMD, T2DM patients with NAFLD were divided into three groups (T1, T2, and T3), and the differences in the prevalence of NAFLD were compared among the three groups. The independent influencing factors of NAFLD were analyzed.

Results The right calcaneus and left forearm BMD levels of male and postmenopausal female T2DM patients with NAFLD were lower than those without NAFLD. After grouping according to the tertile levels of BMD of the right calcaneus, the prevalence of NAFLD in the T1 group (56.8%) was higher than that in the T2 or T3 group (42.6% or 40.7%) among male T2DM patients, and the prevalence of NAFLD in the T1 group was higher than that in the T3 group (77.2% vs.53.5%) among postmenopausal female T2DM patients. NAFLD was negatively associated with the right calcaneus and the left forearm BMD, and the right calcaneus BMD was an independent protective factor for NAFLD in male and postmenopausal female T2DM patients (OR = 0.014 and 0.006, $p < 0.05$).

Conclusion For males aged ≥ 50 years or postmenopausal female T2DM patients with NAFLD, the BMD levels were lower, and lower BMD levels were more likely to lead to the development of NAFLD. The BMD was an independent protective factor for T2DM patients.

Keywords Type 2 diabetes · Nonalcoholic fatty liver disease · Bone mineral density · Postmenopausal female · Male

Introduction

In 2019, 463 million people had T2DM worldwide, mostly in developing countries [1]. According to published data by the International Diabetes Federation in 2019, the number of patients with T2DM in China reached 116 million, thus accounting for a quarter of the number of patients with T2DM worldwide. Diabetes has become a chronic disease that seriously affects the health of Chinese people and global populations [2].

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Osteoporosis is a disease characterized by a declining bone mass, microstructural deterioration, and fragility fractures, and it often leads to an increased risk of fractures of the spine, hip, and wrist [3]. In Europe, it is estimated that 22 million women and 5.5 million men suffer from osteoporosis. Osteoporosis is classified as either primary or secondary. Primary osteoporosis is usually associated with advancing age. Postmenopausal osteoporosis is mainly due to the significantly reduced production of estrogen in women after menopause compared with that prior to menopause. The causes of secondary osteoporosis include gastrointestinal disorders, long-term use of glucocorticoids, diabetes, metabolic diseases of the liver, and multiple myeloma [4].

NAFLD is a chronic metabolic disease closely related to insulin resistance (IR) and heredity. It is classified as nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH) cirrhosis, and hepatocellular carcinoma [5]. The prevalence of NAFLD is approximately 25% globally and

in Asian populations. NAFLD is closely related to metabolic syndrome and T2DM, and the prevalence of NAFLD in patients diagnosed with T2DM is 57–80% [6].

A study showed a negative correlation between NAFLD and BMD in postmenopausal women [7]. A meta-analysis suggested that there was no difference in BMD levels between patients with fatty liver and normal controls [8]. Therefore, the relationships between NAFLD and BMD have not been fully defined. The aim of the study was to provide ideas for the early diagnosis and treatment of osteoporosis in T2DM patients with NAFLD by analyzing the relationships between NAFLD and BMD in T2DM patients of different sexes.

Subjects and methods

Subjects

A total of 1213 patients diagnosed with T2DM in the Department of Endocrinology of Lanzhou University First Hospital from April 2016 to December 2020 were selected as the study subjects. According to the inclusion and exclusion criteria, 752 subjects were enrolled in this study, while 101 healthy subjects were enrolled as the control group. This study was approved by the ethics committee of the First Hospital of Lanzhou University, and written informed consent was obtained from all participants. The inclusion and exclusion criteria for this study were as follows.

Inclusion criteria

The inclusion criteria are as follows: (1) male patients with T2DM aged ≥ 50 years and postmenopausal female patients with T2DM; (2) Han nationality; and (3) duration of T2DM ≥ 1 year.

Exclusion criteria

The exclusion criteria are as follows: (1) type 1 diabetes mellitus, gestational diabetes mellitus, and other specific types of diabetes mellitus; (2) diabetic ketoacidosis, diabetic hyperosmolarity, and hyperglycemia; (3) tumor, immune diseases, serious heart, liver, and kidney diseases; (4) chronic and heavy alcohol consumption (over 140 g of ethanol per week for men and over 70 g for women); (5) alcoholic liver disease, drug-induced liver damage, and other diseases that may lead to abnormal liver function; (6) administration of drugs that affect calcium and phosphorus metabolism, such as glucocorticoids, thyroid hormones, vitamin D, etc.; (7) patients with a pathological fracture and history of hyperthyroidism; (8) Cushing's syndrome, anterior pituitary dysfunction, inflammatory bowel disease; (9) history of lipid-lowering drugs and other drugs, parenteral nutrition, and other special conditions that may cause liver fat deposition; (10) a lack of clinical data.

Research methodology

Collection of general characteristics of the study subjects

The medical history, general information (name, sex, age), diabetes duration, medication status, personal history, previous history, and menstrual history of each study subject were collected. The height, weight, and blood pressure were measured as follows: BMI = Weight (kg)/Height (m^2).

Laboratory examinations

After fasting for 8 h, 5 ml of venous blood was extracted from all subjects in the morning, and the serum was separated. The serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid (UA), alanine aminotransaminase (ALT), aspartic acid aminotransaminase (AST), fasting blood glucose (FPG), calcium (CA), and blood phosphorus (P) levels were measured by a BS-220 automatic biochemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). Glycosylated hemoglobin (HbA1c) was determined by high-performance liquid chromatography (Bio-Rad-D10)0.25(OH)D was detected by a RT-6000 ELISA (Shenzhen Redu Bioscience Co., Ltd.). The BMD levels of the right calcaneus bone and the distal left forearm were measured by an Osteosys EXA-3000 dual-energy X-ray absorptiometry system (Beijing Grambo Technology Development Co., Ltd.). The units are expressed as grams per square centimeter.

Diagnostic criteria and grouping

Diagnostic criteria

T2DM is diagnosed according to WHO 1999 criteria. NAFLD was diagnosed according to the criteria published by the American Society of Liver Diseases in 2017. Each subject was examined using abdominal ultrasound by an experienced sonographer. Two of the following three abdominal ultrasound findings suggested diffuse fatty liver: (1) the near-field echo of the liver was a diffuse enhancement, which was stronger than that of the kidney and spleen;(2) the intrahepatic duct structure was not clear; and (3) the far-field echo of the liver gradually weakened.

Study population grouping

According to whether T2DM patients were complicated with NAFLD, they were divided into a NAFLD group and a non-NAFLD group. The differences among the NAFLD group, non-NAFLD group, and healthy control group in the BMD levels of the right calcaneus bone and left forearm for men aged ≥ 50 years and postmenopausal women were analyzed. T2DM Patients of different sexes complicated with NAFLD were divided into groups according to three quartile levels of the right calcaneus bone and the distal left forearm BMD. The prevalence of NAFLD under the different BMD levels was compared.

T2DM patients were grouped according to the BMD level of the right calcaneus bone as follows: total T2DM patients: T1 group: 0.060–0.364 g/cm^2, T2 group: 0.365–0.515 g/cm^2, T3 group: 0.516–0.896 g/cm^2; men with T2DM: T1 group: 0.145–0.470 g/cm^2, T2 group: 0.471–0.580 g/cm^2, T3 group: 0.581–0.896 g/cm^2; postmenopausal women with T2DM: T1 group: 0.060–0.277 g/cm^2, T2 group: 0.278–0.362; T3 group: 0.363–0.690 g/cm^2.

T2DM patients were grouped according to the BMD level of the distal left forearm as follows: total T2DM patients: T1 group: 0.130–0.335 g/cm^2, T2 group: 0.336–0.431 g/cm^2, T3 group: 0.432–0.858 g/cm^2; men with T2DM: T1 group: 0.151–0.402 g/cm^2, T2 group: 0.403–0.472 g/cm^2, T3 group: 0.473–0.858 g/cm^2; postmenopausal women with T2DM: T1 group: 0.103–0.267 g/cm^2, T2 group: 0.268–0.335 g/cm^2, T3 group: 0.336–0.556 g/cm^2.

Statistical methods

Normally distributed measurement data were expressed as the mean ± standard deviation ($\bar{x} \pm s$), and nonnormally distributed measurement data were expressed as the median (p25, p75). The numeration data were expressed as frequencies and percentages (%). One-way ANOVA and nonparametric tests for independent samples were used to analyze the measurement data according to whether they conformed to a normal distribution. Comparisons between the two groups were analyzed based on whether equal variances were satisfied using the LSD-*t* and Tamhane's tests. After adjusting for confounders, an analysis of covariance (ANCOVA) was used to compare differences between multiple groups. The chi-square test was used to compare the differentiation between intergroup rates. Comparisons between the two groups were analyzed using the Bonferroni method under the z-test to adjust *p*-values. Independent influencing factors for NAFLD in T2DM patients were analyzed by binary logistic regression. A p value < 0.05 was considered statistically significant.

Results

Comparison of the BMD levels among the NAFLD, non-NAFLD, and healthy control groups in the total subjects

To the total study subjects, the right calcaneus BMD and left forearm BMD levels of the non-NAFLD group and the NAFLD group were lower than those of the healthy control group (all $p < 0.05$). The right calcaneus BMD and left forearm BMD levels of the NAFLD group were lower than those of the non-NAFLD group (all $p < 0.05$).

The age, SBP, DBP, TG, UA, HbA1c, and FPG levels of the non-NAFLD group and the NAFLD group were higher than those of the healthy control group (all $p < 0.05$), and the 25(OH)D and HDL-C levels of the NAFLD group were lower than those of the healthy control group and the non-NAFLD group (all $p < 0.05$). The age, BMI, SBP, AST, ALT, UA, TC, TG, and FPG levels of the NAFLD were higher than those of the non-NAFLD group (all $p < 0.05$).

After adjusting for age, BMI, SBP, DBP, 25(OH)D, AST, ALT, TC, TG, HDL-C, UA, HbA1c, and FPG, the analysis of covariance showed that the BMDs of the right calcaneus and left forearm were lower in the non-NAFLD and NAFLD groups than the healthy control group (all $p < 0.01$) and the right calcaneus and left forearm BMD levels in the NAFLD group were lower than those in the non-NAFLD group (all $p < 0.01$) (Table 1).

Comparison of the BMD levels among the NAFLD, non-NAFLD, and the healthy control groups in men aged ≥ 50 years

For men aged ≥ 50 years, right calcaneus and left forearm BMD levels of the non-NAFLD group and the NAFLD group were lower than those of the healthy control group (all $p < 0.05$), and right calcaneus BMD levels of the NAFLD group were lower than those of the non-NAFLD group (all $p < 0.05$).

The SBP, TG, UA, FPG, and HbA1c levels of the non-NAFLD group and the NAFLD group were higher than those of the healthy control group (all $p < 0.05$), and the 25(OH)D and HDL-C levels of the NAFLD group were lower than those of the healthy control group (all $p < 0.05$). The BMI, SBP, AST, ALT, UA, TG, and FPG levels of the NAFLD group were higher than those of the non-NAFLD group (all $p < 0.05$), and the HDL-C levels were lower than those of the non-NAFLD group (all $p < 0.05$).

After adjusting for BMI, SBP, DBP, 25(OH)D, AST, ALT, TG, HDL-C, UA, HbA1c, FPG, the analysis of covariance showed that the right calcaneus and left forearm BMD

Table 1 General characteristics of the study population

Index	Healthy control ($N=101$)	Non-NAFLD ($N=340$)	NAFLD ($N=412$)
Gender (men/women)	57/44	238/102	208/204
Age (year)	54.91 ± 6.56	59.66 ± 7.44*	62.09 ± 7.68* #
BMI (kg/m^2)	24.87 ± 3.01	23.14 ± 2.97*	25.49 ± 2.68 #
SBP (mmHg)	124.78 ± 9.43	138.77 ± 22.86*	145.92 ± 22.96* #
DBP (mmHg)	78.10 ± 8.89	81.60 ± 14.80*	83.59 ± 13.97*
25-(OH)D (ng/ml)	19.03 (14.98–25.48)	18.79 (11.74–26.83)	16.94 (8.17–26.28)* #
Ca (mmol/l)	2.25 ± 0.08	2.24 ± 0.18	2.26 ± 0.19
P (mmol/l)	1.17 ± 0.14	1.15 ± 0.19	1.16 ± 0.19
AST (U/l)	19.00 (15.00–24.00)	19.00 (16.00–23.00)	21.00 (17.00–28.00)* #
ALT (U/l)	19.00 (10.00–26.00)	18.00 (14.00–25.00)	23.00 (17.00–34.00)* #
TC (mmol/l)	4.24 ± 0.95	4.19 ± 1.09	4.51 ± 1.13* #
TG (mmol/l)	1.18 (0.99–1.45)	1.33 (0.96–1.91)*	1.74 (1.32–2.41)* #
LDL-C (mmol/l)	2.75 ± 0.57	2.74 ± 0.82	2.79 ± 0.75
HDL-C (mmol/l)	1.45 ± 0.26	1.07 ± 0.28*	1.00 ± 0.22* #
UA (μmol/l)	265.59 ± 72.78	317.10 ± 87.82*	340.90 ± 86.11* #
HbA1c (%)	5.29 ± 0.40	8.48 ± 2.26*	8.56 ± 1.89*
FPG (mmol/l)	5.29 ± 0.49	8.72 ± 2.91*	9.65 ± 2.97* #
Right calcaneus BMD (g/cm^2)	0.573 ± 0.10	0.489 ± 0.15*	0.410 ± 0.15* #
Distal left forearm BMD (g/cm^2)	0.520 ± 0.07	0.407 ± 0.10*	0.363 ± 0.11* #

*$p<0.05$: comparison with healthy controls; #$p<0.05$: comparison with the non-NAFLD group

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *25(OH)D*, 25-hydroxyvitamin D; *Ca*, blood calcium; *P*, blood phosphorus; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *TC*, total cholesterol; *TG*, triglycerides; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *UA*, uric acid; *HbAlc*, hemoglobin A1c; *FPG*, fasting plasma glucose; *BMD*, bone mineral density

Non-normal information is expressed as median (interquartile range) and normal information is expressed as mean ± standard deviation

levels were lower in the non-NAFLD and NAFLD groups than those in the healthy control group (all $p<0.01$). The right calcaneus BMD levels in the NAFLD group were lower than those in the non-NAFLD group ($p<0.01$), while no significant differences were observed in BMD levels of the left forearm between the NAFLD group and non-NAFLD groups ($p>0.05$) (Table 2).

Comparison of the BMD levels among the NAFLD, non-NAFLD, and the healthy control groups in postmenopausal women

For postmenopausal women, right calcaneus and left forearm BMD levels of the non-NAFLD group and the NAFLD group were lower than those of the healthy control group (all $p<0.05$), and right calcaneus BMD levels of the NAFLD group were lower than those of the non-NAFLD group ($p<0.05$).

The age, SBP, UA, FPG, and HbA1c levels of the non-NAFLD group and the NAFLD group were higher than those of the healthy control group (all $p<0.05$), while the HDL-C levels were lower than those of the healthy control group (both $p<0.05$).

The DBP, AST, ALT, TC, and TG levels of the NAFLD group were higher than those of the healthy control group (all $p<0.05$). The age, BMI, SBP, DBP, ALT, UA, TC, TG, and FPG levels of the NAFLD group were higher than those of the non-NAFLD group (all $p<0.05$), and the HDL-C and 25(OH)D levels were lower than those of the non-NAFLD group (both $p<0.05$).

After adjusting for age, BMI, SBP, DBP, 25(OH)D, AST, ALT, TC, TG, HDL-C, UA, HbA1c, and FPG, the analysis of covariance showed that the right calcaneus and left forearm BMD levels were lower in the non-NAFLD and NAFLD groups than those in the healthy control group (all $p<0.01$). The right calcaneus BMD levels in the NAFLD group were lower than those in the non-NAFLD group ($p<0.01$), while no significant differences were observed in the BMD levels of the left forearm between the non-NAFLD and NAFLD groups ($p>0.05$) (Table 3).

Comparison of the prevalence of NAFLD in T2DM patients at different BMD levels

After grouping according to the BMD levels of the right calcaneus, among the total T2DM patients, the prevalence of NAFLD in the T1 group (68.8%) was higher than that in

Table 2 Comparison of the biochemical parameters and BMD levels in males aged ≥ 50 years

Index	NAFLD ($N=208$)	Non-NAFLD ($N=238$)	Healthy control ($N=57$)
Age (year)	60.79 ± 7.70	59.07 ± 7.40	58.63 ± 5.85
BMI (kg/m^2)	25.75 ± 2.57$^{*\#}$	23.51 ± 2.75*	24.84 ± 2.93
SBP (mmHg)	144.65 ± 22.20$^{*\#}$	138.45 ± 23.65*	125.65 ± 8.61
DBP (mmHg)	86.25 ± 14.08*	83.58 ± 15.99	80.04 ± 8.14
25-(OH)D (ng/ml)	19.08 (7.99–27.35)*	20.75 (10.85–28.03)	22.50 (17.14–26.86)
Ca (mmol/l)	2.22 ± 0.23	2.22 ± 0.19	2.21 ± 0.06
P (mmol/l)	1.09 ± 0.17	1.12 ± 0.18	1.10 ± 0.10
AST (U/l)	21.00 (17.00–26.00)$^{*\#}$	19.00 (15.00–23.00)	18.00 (15.00–24.00)
ALT (U/l)	23.00 (18.00–34.00)$^{*\#}$	19.00 (14.00–25.00)	19.00 (10.00–27.00)
TC (mmol/l)	4.29 ± 1.02	4.11 ± 1.11	4.20 ± 0.95
TG (mmol/l)	1.73 (1.28–2.54)$^{*\#}$	1.36 (1.03–1.91)*	1.14 (1.02–1.39)
LDL-C (mmol/l)	2.72 ± 0.68	2.71 ± 0.82	2.81 ± 0.54
HDL-C (mmol/l)	0.93 ± 0.18$^{*\#}$	1.00 ± 0.23*	1.40 ± 0.27
UA (μmol/l)	355.50 ± 82.98$^{*\#}$	330.45 ± 90.22*	296.53 ± 73.40
FPG (mmol/l)	9.67 ± 3.11$^{*\#}$	8.77 ± 2.80*	5.34 ± 0.47
HbA1c (%)	8.45 ± 1.85*	8.63 ± 2.38*	5.34 ± 0.42
Right calcaneus BMD (g/cm^2)	0.507 ± 0.12$^{*\#}$	0.543 ± 0.14*	0.630 ± 0.09
Distal left forearm BMD (g/cm^2)	0.433 ± 0.09*	0.443 ± 0.08*	0.562 ± 0.05

$^{*}p<0.05$: comparison with healthy controls; $^{\#}p<0.05$: comparison with the non-NAFLD group

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *25(OH)D*, 25-hydroxyvitamin D; *Ca*, blood calcium; *P*, blood phosphorus; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *TC*, total cholesterol; *TG*, triglycerides; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *UA*, uric acid; *FPG*, fasting plasma glucose; *HbAlc*, hemoglobin A1c; *BMD*, bone mineral density

Non-normal information is expressed as median (interquartile range) and normal information is expressed as mean ± standard deviation

the T2 and T3 groups (54.6% or 41.0%, $p<0.05$), and the prevalence of NAFLD in the T2 group was higher than that in the T3 group ($p<0.05$).

For the male T2DM patients, the prevalence of NAFLD was higher in the T1 group (56.8%) than in the T2 or T3 groups (42.6% or 40.7%, both $p<0.05$) and no significant difference was shown in the prevalence of NAFLD between the T2 and T3 groups ($p>0.05$).

For postmenopausal women with T2DM, the prevalence of NAFLD in the T1 group (77.2%) was higher than that in the T3 group (53.5%, $p<0.05$) and no significant difference was shown in the prevalence of NAFLD between the other groups (all $p>0.05$) (T2 group, 69.2%, Fig. 1A).

After grouping according to the BMD levels of the left forearm, among the total T2DM patients, the prevalence of NAFLD in the T1 group (66.8%) was higher than that in the T2 and T3 groups (57.3% or 40.6%, $p<0.05$). No significant differences were observed in the prevalence of NAFLD between the T2 and T3 groups ($p>0.05$).

No significant differences were observed in the prevalence of NAFLD among the three groups of male T2DM patients or postmenopausal female T2DM patients (male T2DM patients: 50.7% vs. 50.3% vs. 38.9%; postmenopausal female T2DM patients: 72.3% vs. 67.0% vs. 60.8%, all $p>0.05$) (Fig. 1B).

Correlation analysis between NAFLD and BMD in T2DM patients

In male T2DM patients, NAFLD was significantly negatively correlated with serum P, 25(OH)D, HDL-C, and right calcaneus and left forearm BMD levels ($p<0.05$), and positively correlated with age, BMI, SBP, DBP, AST, ALT, FPG, UA, TC, and TG (all $p<0.05$) (Table 4).

In postmenopausal women with T2DM, NAFLD was significantly negatively correlated with 25(OH)D, HDL-C, and right calcaneus and distal left forearm BMD levels ($p<0.05$) (all $p>0.05$), and positively correlated with age, BMI, SBP, DBP, ALT, HbA1c, FPG, UA, TC, and TG (all $p<0.05$) (Table 4).

Influencing factors analysis of NAFLD in T2DM patients by binary logistic regression analysis

For male T2DM patients, NAFLD was considered the dependent variable, and age, BMI, SBP, DBP, Ca, P, 25(OH) D, AST, TC, HDL-C, FPG, right calcaneus BMD, and left forearm BMD were considered independent variables. The binary logistic regression analysis showed that right calcaneus BMD and HDL-C were independent protective factors

Table 3 Comparison of the biochemical parameters and BMD levels in postmenopausal women

Index	NAFLD ($N=204$)	Non-NAFLD ($N=102$)	Healthy control ($N=44$)
Age (year)	$63.43 \pm 7.44^{*\#}$	$61.02 \pm 7.40^{*}$	50.09 ± 3.68
BMI (kg/m^2)	$25.23 \pm 2.77^{\#}$	$22.27 \pm 3.28^{*}$	24.91 ± 3.15
SBP (mmHg)	$147.21 \pm 23.70^{*\#}$	$139.53 \pm 20.99^{*}$	123.66 ± 10.39
DBP (mmHg)	$80.86 \pm 13.35^{*\#}$	76.99 ± 10.27	75.59 ± 9.28
25(OH)D (ng/ml)	15.58 (8.22–25.28)$^{\#}$	16.86 (13.55–26.41)	16.14 (12.88–19.63)
Ca (mmol/l)	2.30 ± 0.14	2.29 ± 0.14	2.29 ± 0.07
P (mmol/l)	1.22 ± 0.20	1.23 ± 0.18	1.26 ± 0.13
AST (U/l)	22.00 (17.00–31.75)*	21.00 (16.75–28.00)	19.5 (15.0–24.0)
ALT (U/l)	22.00 (16.00–35.00)$^{*\#}$	17.00 (14.00–29.25)	17.00 (8.50–23.75)
TC (mmol/l)	$4.73 \pm 1.21^{*\#}$	4.39 ± 1.04	4.29 ± 0.96
TG (mmol/l)	1.76 (1.38–2.37)$^{*\#}$	1.27 (0.87–1.87)	1.22 (0.91–1.53)
LDL-C (mmol/l)	2.86 ± 0.81	2.79 ± 0.83	2.66 ± 0.61
HDL-C (mmol/l)	$1.07 \pm 0.23^{*\#}$	$1.24 \pm 0.33^{*}$	1.50 ± 0.23
UA (μmol/l)	$326.00 \pm 86.90^{*\#}$	$285.95 \pm 73.41^{*}$	225.52 ± 48.90
FPG (mmol/l)	$9.62 \pm 2.81^{*\#}$	$8.61 \pm 3.16^{*}$	5.22 ± 0.51
HbA1c (%)	$8.67 \pm 1.94^{*}$	$8.14 \pm 1.91^{*}$	5.23 ± 0.36
Right calcaneus BMD (g/cm^2)	$0.312 \pm 0.11^{*\#}$	$0.363 \pm 0.11^{*}$	0.499 ± 0.06
Distal left forearm BMD (g/cm^2)	$0.293 \pm 0.08^{*}$	$0.319 \pm 0.11^{*}$	0.466 ± 0.04

$^{*}p<0.05$: comparison with healthy controls; $^{\#}p<0.05$: comparison with the non-NAFLD group

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *25(OH)D*, 25-hydroxyvitamin D; *Ca*, blood calcium; *P*, blood phosphorus; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *TC*, total cholesterol; *TG*, triglycerides; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *UA*, uric acid; *FPG*, fasting plasma glucose; *HbAlc*, hemoglobin A1c; *BMD*, bone mineral density

Non-normal information is expressed as median (interquartile range) and normal information is expressed as mean ± standard deviation

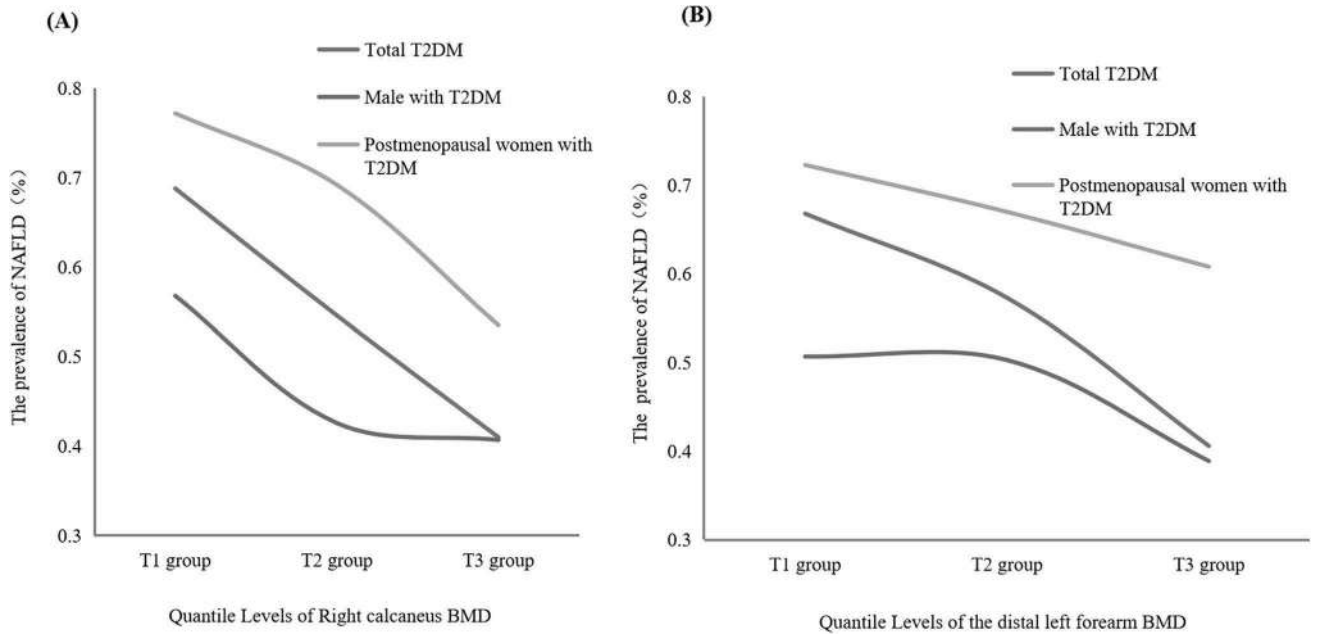

Fig. 1 Comparison of the prevalence of NAFLD at different BMD levels. **A** Quantile levels of right calcaneus BMD. **B** Quantile levels of the distal left forearm BMD

Table 4 Correlations between NAFLD and various indicators in patients with type 2 diabetes

Index	Men aged ≥ 50 years		Postmenopausal women	
	R value	*p* value	*R* value	*p* value
Age (year)	0.103**	0.009	0.129**	0.007
SBP (mmHg)	0.120**	0.002	0.133**	0.005
DBP (mmHg)	0.102**	0.010	0.097*	0.042
BMI (kg/m^2)	0.317**	< 0.001	0.353**	< 0.001
25-(OH)D (ng/ml)	− 0.092*	0.018	− 0.152**	0.001
Ca (mmol/l)	0.056	0.153	0.055	0.245
P (mmol/l)	− 0.077*	0.048	− 0.050	0.292
AST (U/l)	0.153**	< 0.001	0.073	0.127
ALT (U/l)	0.222**	< 0.001	0.134**	0.005
LDL-C (mmol/l)	0.008	0.832	0.041	0.379
HDL-C (mmol/l)	− 0.116**	0.003	− 0.214**	< 0.001
HbA1c (%)	0.008	0.835	0.114*	0.016
FPG (mmol/l)	0.130**	0.001	0.159**	0.001
UA (μmol/l)	0.127**	0.001	0.164**	< 0.001
TC (mmol/l)	0.085*	0.028	0.104*	0.026
TG (mmol/l)	0.215**	< 0.001	0.248**	< 0.001
Right calcaneus BMD (g/cm^2)	− 0.109**	0.005	− 0.179**	< 0.001
Distal left forearm BMD (g/cm^2)	− 0.081*	0.037	− 0.102*	0.03

** indicates a significant correlation at the 0.01 level (two-sided). * indicates a significant correlation at the 0.05 level (two-sided);

SBP, systolic blood pressure; *DBP*, diastolic blood pressure; *BMI*, body mass index; *Ca*, blood calcium; *P*, blood phosphorus; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *HbAlc*, hemoglobin A1c; *FPG*, fasting plasma glucose; *UA*, uric acid; *TC*, total cholesterol; *TG*, triglycerides; *BMD*, bone mineral density

for NAFLD ($p < 0.05$), and age, BMI, AST, and TC were independent risk factors for NAFLD (all $p < 0.05$) (Table 5).

Among the postmenopausal women with T2DM, NAFLD was considered a dependent variable; and SBP, DBP, Ca, P, 25-(OH)D, AST, TC, TG, HDL-C, UA, HbA1c, FPG, right calcaneus BMD, and left forearm BMD were considered independent variables. The binary logistic regression analysis showed that 25-(OH)D, right calcaneus BMD, and HDL-C were independent protective factors for NAFLD ($p < 0.05$), and TC and UA were independent risk factors for NAFLD (all $p < 0.05$) (Table 5).

Discussion

Compared with non-NAFLD patients, T2DM patients with NAFLD have worse blood glucose control, and diabetic retinopathy, nephropathy, and other microvascular and macrovascular complications are more common [9]. NAFLD is a chronic liver disease related to insulin resistance and metabolic disorders. The interaction mechanisms between NAFLD and bone tissue are complex. Chronic inflammation, vitamin D, growth hormone, insulin-like growth factor 1 (IGF-1), osteocalcin (OC), tumor necrosis factor-α (TNF-α), osteoclastogenesis inhibitory factor, and insulin resistance may accelerate the decline in BMD levels in patients with NAFLD [10].

In this study, the level of BMD in the right calcaneus of male T2DM patients aged ≥ 50 years in the NAFLD group was significantly lower than that in the non-NAFLD group. BMD levels in the right calcaneus and the left forearm of postmenopausal T2DM patients in the NAFLD group were significantly lower than those in the non-NAFLD group, suggesting that T2DM patients with NAFLD had lower BMD levels and were more prone to osteoporosis. NAFLD may be a potential risk factor for reducing BMD [7, 11]

Zhu et al. reported that NAFLD-associated liver fibrosis was inversely associated with reduced BMD in postmenopausal women with T2DM or impaired glucose regulation (IGR), after adjusting for age and BMI, the femoral BMD value in the advanced liver fibrosis group was significantly lower than that in the non-advanced liver fibrosis group, suggesting that the risk of osteoporosis in postmenopausal women with T2DM or IGR increased with the severity of liver fibrosis [12]. A case-controlled study from China showed that the BMD levels of the left femoral neck, left forearm, and Ward's triangle in T2DM patients with NAFLD were significantly lower than those in T2DM patients without NAFLD [13]. Therefore, T2DM patients with NAFLD may have an increased risk of osteoporosis.

Table 5 Influencing factor analysis of NAFLD in patients with type 2 diabetes

Index	*B*	*p* value	OR	95% CI
Male				
Age (year)	0.045	0.007	1.046	1.012–1.080
BMI (kg/m^2)	0.347	< 0.001	1.415	1.286–1.556
SBP (mmHg)	− 0.003	0.727	0.997	0.983–1.012
DBP (mmHg)	0.008	0.484	1.008	0.986–1.031
Ca (mmol/l)	− 0.233	0.654	0.792	0.285–2.199
P (mmol/l)	0.200	0.768	1.221	0.323–4.610
25-(OH)D (ng/ml)	− 0.017	0.056	0.984	0.967–1.000
AST (U/l)	0.050	< 0.001	1.051	1.023–1.080
TC (mmol/l)	0.248	0.047	1.281	1.004–1.635
HDL-C (mmol/l)	− 2.175	0.002	0.114	0.030–0.436
FPG (mmol/l)	0.072	0.067	1.075	0.995–1.161
Right calcaneus BMD (g/cm^2)	− 4.267	< 0.001	0.014	0.002–0.123
Distal left forearm BMD (g/cm^2)	1.849	0.237	6.353	0.297–135.975
Female				
SBP (mmHg)	0.007	0.454	1.007	0.989–1.024
DBP (mmHg)	0.015	0.356	1.015	0.984–1.047
Ca (mmol/l)	0.959	0.395	2.608	0.287–23.724
P (mmol/l)	0.724	0.369	2.063	0.426–9.995
25-(OH)D (ng/ml)	− 0.033	0.011	0.968	0.944–0.993
AST (U/l)	0.002	0.738	1.002	0.991–1.013
TC (mmol/l)	0.388	0.025	1.474	1.049–2.070
TG (mmol/l)	0.071	0.738	1.073	0.709–1.624
HDL-C (mmol/l)	− 3.296	< 0.001	0.037	0.009–0.147
UA (μmol/l)	0.006	0.007	1.006	1.002–1.010
HbA1c (%)	0.038	0.695	1.039	0.858–1.258
FPG (mmol/l)	0.098	0.132	1.103	0.971–1.253
Right calcaneus BMD (g/cm^2)	− 5.093	0.003	0.006	0.001–0.185
Distal left forearm BMD (g/cm^2)	− 0.419	0.836	0.658	0.012–35.110

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *Ca*, blood calcium; *P*, blood phosphorus;*25(OH)D*, 25-hydroxyvitamin D; *AST*, aspartate aminotransferase; *TC*, total cholesterol; *TG*, triglycerides; *HDL-C*, high-density lipoprotein cholesterol; *UA*, uric acid; *HbAlc*, hemoglobin A1c; *FPG*, fasting plasma glucose; *BMD*, bone mineral density

In addition, the present study found that with decreasing BMD levels in the right calcaneus, the prevalence of NAFLD in T2DM patients increased significantly. NAFLD was negatively correlated with the BMD of the right calcaneus and the left forearm in male T2DM patients aged ≥ 50 years and postmenopausal female T2DM patients. Until now, the results of changes in BMD and the prevalence of osteoporosis in patients with NAFLD stratified by sex have been inconsistent.

A cohort study with 1720 subjects showed that NAFLD was independently associated with an increased risk of low BMD in both men and women [14]. However, a clinical study from Korea found a significant negative association between BMD and NAFLD in men and a positive association between lumbar BMD and NAFLD in postmenopausal women [15]. However, in three subsequent cohort studies, a significant negative association between BMD levels and NAFLD was found, although these results were only observed in men and not in postmenopausal women [16]. Xia et al. reported that liver fat content and hepatotoxicity biomarkers were negatively correlated with BMD in middle-aged and elderly Chinese men, suggesting that male patients with NAFLD had a high risk of osteoporosis and the potential mechanism may be related to the decreased activity of osteoblasts; however, this difference disappeared in women [17].

It is not clear why the results of NAFLD and BMD are different in different sexes and at different ages, although these discrepancies may be related to the different levels of hormones in the body. Studies have shown that increased release of ROS in patients with T2DM and NAFLD can affect the production and survival of osteoclasts, osteoblasts, and osteocytes [13]. When estrogen or androgen levels are reduced, the ability of the bone to fight oxidative stress is

reduced, the number of osteoblasts decreased and the incidence of osteoporosis in the body increased [18].

Chronic inflammation is a major pathological mechanism that links NAFLD with osteoporosis. Lipid overload leading to cellular lipid toxicity is one of the recognized triggers of the inflammatory cascade of hepatocytes [10]. Circulating TNF-α levels are increased in patients with NAFLD. TNF-α may strengthen osteoclast formation and inhibit bone formation, thereby stimulating osteoclast formation genes, such as interleukin-6, and macrophage colony-stimulating factor expression, while inhibiting bone formation-related genes, such as the expression of alkaline phosphatase, vitamin D receptor, and parathyroid hormone receptor. TNF-α is negatively correlated with vitamin D levels, thus leading to a decrease in BMD [19]. In patients with liver fibrosis, osteoclasts and osteoblasts affect bone metabolism through bone resorption and bone formation, respectively. TNF-α, interleukin-6, and other factors can lead to dysfunction of the abovementioned cells, and these inflammatory cytokines can enhance the activity of osteoclasts, inhibit the apoptosis of osteoclasts, and stimulate the formation of osteoclasts.

In addition, insulin resistance is also one of the major risk factors for osteoporosis in NAFLD patients. The accumulation of lipids in the liver is associated with decreased insulin sensitivity in liver, bone, muscle, and fat tissues [20]. Experimental studies have shown that in rats fed a high-fat diet, the development of insulin resistance may reduce the proliferation and differentiation of osteoblasts and increase the apoptosis of osteoblasts, thus leading to decreased BMD of jaw bone [21].

Studies have shown that NAFLD patients tend to show damage to the hepatic GH/IGF-1 axis and that bone health is associated with the severity and duration of NAFLD. In a study of a liver-specific GH receptor gene deletion mouse model, chronic hepatic steatosis, local inflammation and reduced bone mineral density were found in mice with damage to the GH/IGF-1 axis [22]. Hepatogenic IGF-1 promotes long bone growth and is an important factor in bone strength. Low levels of serum IGF-1 lead to a reduction in bone growth, resulting in elongated and mechanically inferior bone phenotypes. A continuous decrease in IGF-1 levels leads to impaired subperiosteal dilation and poor endosteum adhesion. A long-term decrease in serum IGF-1 indirectly affects bone strength through its effect on the number of myeloid progenitor cells [23].

The current study has some limitations. First, liver biopsy is currently the gold standard for the diagnosis of NAFLD. Due to the great harm to the human body, we used liver ultrasound to determine whether patients had NAFLD, this procedure may have had a certain diagnostic error. Second, this study was a cross-sectional study, and the temporal relationships between BMD, T2DM, and NAFLD could not be determined. Finally, we only screened the BMD of the distal left forearm and the right calcaneus, which might have resulted in biased results.

Conclusion

For male or postmenopausal female T2DM patients with NAFLD, the BMD levels of the right calcaneus bone and left forearm were lower, and the lower BMD levels were more likely to lead to the development of NAFLD. Right calcaneus BMD was an independent protective factor for the development of NAFLD in male or postmenopausal female T2DM patients.

Abbreviations T2DM: Type 2 diabetes mellitus; NAFLD: Nonalcoholic fatty liver disease; BMD: Bone mineral density; FPG: Fasting blood glucose; HbA1c: Glycosylated hemoglobin; TC: Total cholesterol; TG: Serum triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; UA: Uric acid; BMI: Body mass index; CA: Calcium; P: Phosphorus; 25(OH) D: 25-Hydroxy vitamin D

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Miss Binjing Pan, Miss Junxin Yan, and Mr. Pingping Zhao. The first draft of the manuscript was written by Miss Binjing Pan and Dr. Jingfang Liu. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data of this study is not publicly available due to data protection by the First Hospital of Lanzhou University. However, these are available from the corresponding author on reasonable request.

Code availability All data were analyzed by IBM SPSS23.0 software.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to participate Written and oral information of the protocol was explained to them before screening, and informed consent was obtained from each eligible participant.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Lee HS, Hwang JS. Impact of type 2 diabetes mellitus and antidiabetic medications on bone metabolism. Curr DiabRep. 2020;20(12):78. https://doi.org/10.1007/s11892-020-01361-5.
2. Shi RF, Guo XH, Zhang Q. Current status and prospects of self-management education and support for adult type 2 diabetes in my country. Chin J Diabetes Mellitus. 2021;13(02):121–4.
3. Lane JM, Russell L, Khan SN. Osteoporosis. Clin Orthop Relat Res. 2000;372:139–50. https://doi.org/10.1097/00003086-200003000-00016.
4. Marcucci G, Brandi ML. Rare causes of osteoporosis. Clinical cases in mineral and bone metabolism: the official journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases. 2015;12(2):151–56. https://doi.org/10.11138/ccmbm/2015.12.2.151.
5. VanWagner LB, Rinella ME. Extrahepatic manifestations of nonalcoholic fatty liver disease. Current hepatology reports. 2016;15(2):75–85. https://doi.org/10.1007/s11901-016-0295-9.
6. Chinese Society of Endocrinology. Consensus for diagnosis and treatment of nonalcoholic fatty liver diseases and metabolic disorders (2nd Edition). J Clin Hepatol. 2018;34(10):2103–2108. https://doi.org/10.3969/j.issn.1001-5256.2018.10.010.
7. Lee DY, Park JK, Hur KY, Um SH. Association between nonalcoholic fatty liver disease and bone mineral density in postmenopausal women. Climacteric. 2018;21(5), 498–501. https://doi.org/10.1080/13697137.2018.1481380
8. Upala S, Jaruvongvanich V, Wijarnpreecha K, Sanguankeo A. Nonalcoholic fatty liver disease and osteoporosis: a systematic review and meta-analysis. J Bone Miner Metab. 2017;35(6):685–93. https://doi.org/10.1007/s00774-016-0807-2.
9. Rosato V, Masarone M, Dallio M, Federico A, Aglitti A, Persico M. NAFLD and extra-hepatic comorbidities: current evidence on a multi-organ metabolic syndrome. Int J Environ Res Public Health. 2019;16(18):3415. https://doi.org/10.3390/ijerph16183415.
10 Filip R, Radzki RP, Bieńko M. Novel insights into the relationship between nonalcoholic fatty liver disease and osteoporosis. Clin Interv Aging. 2018;13:1879–91. https://doi.org/10.2147/CIA.S170533.
11. Handzlik-Orlik G, Holecki M, Wilczyński K, Duława J. Osteoporosis in liver disease: pathogenesis and management. Ther Adv Endocrinol Metab. 2016;7(3):128–35. https://doi.org/10.1177/2042018816641351.
12. Zhu X, Yan H, Chang X, Xia M, Zhang L, Wang L, Sun X, Yang X, Gao X, Bian H. Association between non-alcoholic fatty liver disease-associated hepatic fibrosis and bone mineral density in postmenopausal women with type 2 diabetes or impaired glucose regulation. BMJ Open Diabetes Res Care. 2020;8(1):e000999. https://doi.org/10.1136/bmjdrc-2019-000999.
13. Zhang YL, Ma WQ, Lu F, Wang GJ, Hu GP, Si W, Liu Y. Study of the relationship between bone mineral density and type 2 diabetes with nonalcoholic fatty liver disease. Chin J Osteopor Bone Miner Res. 2014;03:233–9.
14. Shen Z, Cen L, Chen X, Pan J, Li Y, Chen W, Yu C. Increased risk of low bone mineral density in patients with non-alcoholic fatty liver disease: a cohort study. Eur J Endocrinol. 2020;182(2):157–64. https://doi.org/10.1530/EJE-19-0699.
15. Lee SH, Yun JM, Kim SH, Seo YG, Min H, Chung E, Bae YS, Ryou IS, Cho B. Association between bone mineral density and nonalcoholic fatty liver disease in Korean adults. J Endocrinol Invest. 2016;39(11):1329–36. https://doi.org/10.1007/s40618-016-0528-3.
16. Adams LA, Anstee QM, Tilg H, Argher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017;66(6):1138–53. https://doi.org/10.1136/gutjnl-2017-313884.
17. Xia MF, Lin HD, Yan HM, Bian H, Chang XX, Zhang LS, He WY, Gao X. The association of liver fat content and serum alanine aminotransferase with bone mineral density in middle-aged and elderly Chinese men and postmenopausal women. J Transl Med. 2016;14:11. https://doi.org/10.1186/s12967-016-0766-3.
18. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31(3):266–300. https://doi.org/10.1210/er.2009-0024.
19. Yilmaz Y. Review article: non-alcoholic fatty liver disease and osteoporosis–clinical and molecular crosstalk. Aliment Pharmacol Ther. 2012;36(4):345–52. https://doi.org/10.1111/j.1365-2036.2012.05196.x.
20. Wang Y, Wen G, Zhou R, Zhong W, Lu S, Hu C, Chai Y. Association of nonalcoholic fatty liver disease with osteoporotic fractures: a cross-sectional retrospective study of Chinese individuals. Front Endocrinol. 2018;9:408. https://doi.org/10.3389/fendo.2018.00408.
21. Pramojanee SN, Phimphilai M, Kumphune S, Chattipakorn N, Chattipakorn SC. Decreased jaw bone density and osteoblastic insulin signaling in a model of obesity. J Dent Res. 2013;92(6):560–5. https://doi.org/10.1177/0022034513485600.
22. Liu Z, Han T, Werner H, Rosen CJ, Schaffler MB, Yakar S. Reduced serum IGF-1 associated with hepatic osteodystrophy is a main determinant of low cortical but not trabecular bone mass. J Bone Miner Res. 2018;33(1):123–36. https://doi.org/10.1002/jbmr.3290.
23. Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nasser P, Courtland HW, Williams V, Bouxsein M, Rosen C, Jepsen KJ. Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. J Bone Miner Res. 2009;24(8):1481–92. https://doi.org/10.1359/jbmr.090226.

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ORIGINAL ARTICLE

Effects of 8-week high-intensity interval training and continuous aerobic training on asprosin secretion and fibrillin-1 gene expression levels in diabetic male rats

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Abstract

Background Diabetes has become a big concern for a man.

Objective The present study investigated the effects of an 8-week high-intensity interval training (HIIT) and continuous aerobic training (CAT) on asprosin serum, fibrillin-1 (FBN1) gene expression, insulin, and fasting glucose levels as well as insulin resistance index in diabetic male rats.

Methods A total number of 48 Wistar male rats were randomly assigned to six groups, i.e., non-diabetic control group, diabetic control group, non-diabetic CAT group, non-diabetic HIIT group, diabetic CAT group, and diabetic HIIT group. HIIT and CAT groups performed their own 8-week training protocols, and the rats were anesthetized and killed 48 h after the cessation of the last training session.

Results The relevant variables in the rats were measured. The Kruskal-Wallis test at a significance level of $p < 0.05$ was used for statistical data analysis. It was found that there were significant differences among the levels of asprosin serum ($p < 0.001$), FBN1 gene expression ($p < 0.001$), insulin ($p < 0.002$), and fasting glucose ($p < 0.001$) as well as insulin resistance indexes ($p < 0.001$) of the non-diabetic control group, diabetic control group, non-diabetic HIIT group, non-diabetic CAT group, diabetic HIIT group, and diabetic CAT group.

Conclusions It seems that diabetes has significant effects on increasing asprosin and FBN1 gene expression levels, while HIIT and CAT workouts have significant effects on decreasing asprosin and FBN1 gene expression levels. Therefore, both HIIT and CAT protocols can be utilized as significantly effective strategies for decreasing the levels of asprosin and its upstream gene, i.e., FBN1; thus, a combination of both strategies can be regarded as a useful intervention method for treating diabetes.

Keywords Asprosin · Continuous aerobic training (CAT) · Diabetes · Fibrillin-1 (FBN1) · High-intensity interval training (HIIT) · Insulin resistance

Introduction

Over the past two decades, the world pandemic of diabetes has grown remarkably. Although both type 1 diabetes and type 2 diabetes are becoming alarmingly common worldwide, it is expected that the prevalence of type 2 diabetes will rise more dramatically because of increasing rates of obesity and decreased levels of activity among people from all walks of life across the world [1]. Diabetes mellitus is a group of metabolic disorders commonly characterized by hyperglycemia [2, 3, 4]. Adipokines are hormones secreted from adipose tissues, and a number of them, due to altering homeostasis of glucose and insulin secretion levels, have been established as risk factors for diabetes [5]. Evidently, decreased levels of adipokines serve a major role in developing insulin resistance, type 2 diabetes, and metabolic diseases [6]. The presence of insulin is necessary for the extracellular transmission of glucose into muscular and adipose cells. Timely synthesis and secretion of insulin, connection of insulin to its receptors in the cells, and, finally, permitting glucose to enter into the cells are all

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required so that one remains healthy. In people with diabetes, however, this mechanism might be disrupted, and, consequently, insulin cannot permit glucose to enter into the cells. In the wake of such a disruption, the levels of glucose in the blood rise and, in turn, lead to an increased level of insulin in the blood [7–9]. Manipulating the secretion of a certain adipokine, having been discovered recently, can help treat type 2 diabetes. This hormone is called "asprosin," which is synthesized and enters the bloodstream through FBN1 gene expression. Asprosin can move toward liver cells through the blood circulation system and make the liver release its glucose into the blood. In view of the foregoing, it is critical that asprosin secretion be controlled in people with diabetes, because the less such a hormone is synthesized and secreted, the less it will affect the release of glucose from the liver cells [10]. Thus, investigating effective interventions in the secretion of adipokines and, thereby, homeostasis of glucose is very important. Two major influential interventions are sports activities and diet [11]. Diabetes, being the most common disease worldwide, poses ever-growing threats to global public health security, so that the WHO urges all countries across the world to fight the disease. Diabetes is a metabolic disease where either enough insulin is not produced or body cells do not properly respond to insulin [12]. Aerobic, anaerobic, and/or resistance training programs can help control glucose levels in people with diabetes by increasing the amount of glucose used by muscles as an energy source. Some studies revealed that sports activities, especially tough workouts, could help reduce hypertension. Therefore, the metabolic stress resulting from exercising led to the oxidation of carbohydrates during workouts and increased levels of oxygen consumption after workouts. As a result, the oxidation of fatty acids was increased both during and after workouts—during the rest time—and it could enhance insulin tolerance, insulin sensitivity, and reduced levels of glucose within 2 to 72 h. However, the question regarding what training protocol has the greatest effect on reducing levels of glucose, secretion of insulin, and improvement of insulin sensitivity is yet to be investigated. In view of the foregoing and relative dearth of studies on asprosin secretion and the gene responsible for the same (FBN1) in non-diabetic and diabetic people, the need for conducting studies in this regard is direly felt [13]. In addition, owing to the positive effects of sports activities on treating type 2 diabetes, the study of asprosin is of high importance. To the best of the authors' knowledge, no studies have already been conducted on the effects of high-intensity interval training (HIIT) and continuous aerobic training (CAT) on FBN1 gene expression and secretion of asprosin. Hence, due to the high prevalence of type 2 diabetes and its complications, the present study seeks to find answers to these questions: Does using a CAT protocol have any effects on FBN1 gene expression and asprosin secretion levels? Does using a HIIT protocol have any effects on factors causing type 2 diabetes, including FBN1 gene and asprosin?

Methodology

This field/laboratory study was conducted with the purpose of investigating the effects of HIIT and CAT protocols on asprosin and FBN1 gene expression levels in diabetic male rats. To this end, a total number of 48 2-month Wistar male rats (with an average weight of 7.59 ± 180.23 g) were purchased from the Center for Breeding Laboratory Animals in Mashhad University of Medical Sciences (MUMS), Mashhad, Iran, and they were transferred to the Animal Laboratory of School of Sport Science in Ferdowsi University of Mashhad, Mashhad, Iran. Transference of laboratory animals from one environment to another both makes them distressed and changes their physiological conditions; hence, they need some time to adapt to the new conditions. All of the 48 rats used in the current study were kept under standard conditions (light/dark cycle: 12 h; humidity: 40–50%; and temperature: 20–24°C). In order to induce diabetes in rats, streptozotocin (STZ) (made in Germany) was used. Streptozotocin is very sensitive, so an overly high dosage of the same would kill the rats and an overly low dosage would not induce diabetes in them. Therefore, first, each individual rat was weighed; then, 0.5 g of streptozotocin was dissolved in 10 cc of sterile citrate buffer so that a 50 Mg/Pj solution was prepared. After that, the solution was kept in an ice bath. Then, an insulin syringe was prepared for each individual rat based on its weight (0.1 cc per 100 g of weight). In the next stage, the rats were injected intraperitoneally with streptozotocin. In order for them not to die, we used a dextrose injection of 5% instead of water, and in order to confirm diabetes development in the rats, 4 days after the said injection, the blood was collected from their tails and their fasting glucose levels were measured with a glucometer. It must be noted that the level of 250 mg/100 ml was considered as the indicator of diabetes development. One week after diabetes induction to diabetic groups, the animals were randomly assigned to six groups, i.e., non-diabetic control group (8 rats), diabetic control group (8 rats), non-diabetic CAT group (8 rats), diabetic CAT group (8 rats), non-diabetic HIIT group (8 rats), and diabetic HIIT group (8 rats). Once they had developed diabetes, the treatment protocol was started.

Research protocol implementation method

Familiarization of the rats with HIIT and CAT protocols was started with 10 training sessions in 2 weeks (each group followed its own protocol). On the first day of the training program, the rats were placed on the treadmill with extreme care and calmness, and they started the training program at a low and steady pace. During the following sessions, after the

rats had gotten used to the training program and could perform it quite well, the speed of the treadmill was increased so that the rats could become familiar with the protocol. Within 2 weeks, the time of the training was also increased in order that the rats could reach the actual time predicted for the main part of the training. After 2 weeks, when we faced no problems in the training protocols and the familiarization process, the main part of the training, lasting 8 weeks, was completed.

The 8-week training programs of the HIIT protocol, meant to maximize the performance of the aerobic system (both oxygen absorption and oxidative capacity), were performed at an intensity close to VO2max on a treadmill without incline. Such programs were performed 5 sessions a week, each session lasting 2 to 4 min with an active recovery time of 1 to 2 min. In the first week, the training was started with 35% Vo2max and a speed of 14 m per minute at a low intensity and 85% Vo2max and a speed of 34 m per minute and the speed was increased gradually by 1 m per 2 weeks so that it added up to 18 m per minute and 38 m per minute, respectively, at the end of the 8 weeks.

Method of measuring dependent variables 72 h after the last training session, the rats were anesthetized by intraperitoneal injection of ketamine 90 mg/kg body weight and xylazine 90 mg/kg body weight immediately. It was done with a heparin syringe to prevent clotting, the blood was placed in sodium citrate and centrifuged at 3000 rpm for 10 min to separate plasma. After blood sampling, tissue sampling was performed and the tissues were washed with physiological serum and frozen in liquid nitrogen and stored in a plasma freezer until − 80 plasma and tissue samples were tested.

1. The Asprosin test was performed using an ELISA kit.
2. For each sample, the threshold cycle (Ct) was determined in real-time PCR. GAP.DH gene expression was used as the reference gene expression. The $\Delta\Delta CT^{-2}$ method was used to measure the expression of the FBN1 gene.
3. Glucose concentration was measured by the enzymatic colorimetric method with glucose oxidase technology and using the glucose kit of Pars Azmoun Company in Tehran. The coefficients of changes in glucose inside and outside the test were 1.74 and 1.19%, respectively, and the measurement sensitivity was 5 mg/dL.
4. Serum insulin was measured by the ELISA method and according to the standards of the commercial kit (Demeditec Diagnostic insulin ELIZA, made in Germany).
5. To evaluate insulin resistance, using fasting insulin and glucose levels and placing it in the following formula, the insulin resistance index was measured.

$$\text{HOMA-R} = \frac{\text{Fasting Insulin}\left(\mu\frac{\text{U}}{\text{ml}}\right) x \; \textit{Fasting Glucose}\left(\frac{mmol}{l}\right)}{22.5}$$

Finally, the collected data were categorized and described based on mean and standard deviation. Due to the lack of assumptions for the parametric test, the Kruskal–Wallis nonparametric test was used for comparing FBN1 gene expression, asprosin, insulin, and fasting glucose levels as well as insulin resistance index meant to compare the groups. Finally, SPSS statistical software was used for calculations at an alpha level of 0.05.

Findings

Figure 1 shows the mean body weights of the rats at pretest and posttest measurements.

The results of Fig. 1 show that the mean weight of rats in healthy and diabetic control groups increased after 8 h and the mean weight of trained rats in healthy periodic control groups, healthy continuum, diabetic periodicity, and diabetic persistence decreased.

According to Table 1, the results of the Kruskal–Wallis test revealed that the study groups were significantly different in terms of the variable FBN1. The Mann–Whitney *U*-test was used to compare each pair of groups. The findings of the said test showed that diabetes led to increased FBN1 gene expression ($p < 0.001$). This is while the CAT and HIIT protocols resulted in decreased FBN1 gene expression ($p < 0.001$) in both non-diabetic and diabetic rats. Therefore, it was observed that FBN1 gene expression levels were higher in diabetic rats, and the CAT and HIIT protocols, both, decreased FBN1 gene expression levels. On the other hand, the HIIT protocol had more reducing effects on FBN1 gene expression levels in diabetic rats as compared to the CAT protocol.

The results of the Kruskal–Wallis test revealed that the study groups were significantly different in terms of the variable asprosin. The Mann–Whitney *U*-test was used to compare each pair of groups. The findings of the said test showed that diabetes led to increased asprosin secretion ($p < 0.001$). This is while the CAT and HIIT protocols resulted in decreased asprosin secretion ($p < 0.001$) in both non-diabetic and diabetic rats. Therefore, it was observed that asprosin secretion levels were higher in diabetic rats, and the CAT and HIIT protocols, both, decreased asprosin secretion levels. On the other hand, the HIIT protocol had more reducing effects on FBN1 asprosin secretion levels in diabetic rats as compared to the CAT protocol.

In the light of Kruskal–Wallis test results, presented in Table 1, it was observed that insulin levels ($p < 0.002$) were higher in diabetic rats than in non-diabetic rats, translating into a reduction of insulin secretion levels as a result of the CAT and HIIT protocols. On the other hand, the HIIT protocol had more reducing effects on insulin secretion levels in diabetic rats as compared to the CAT protocol.

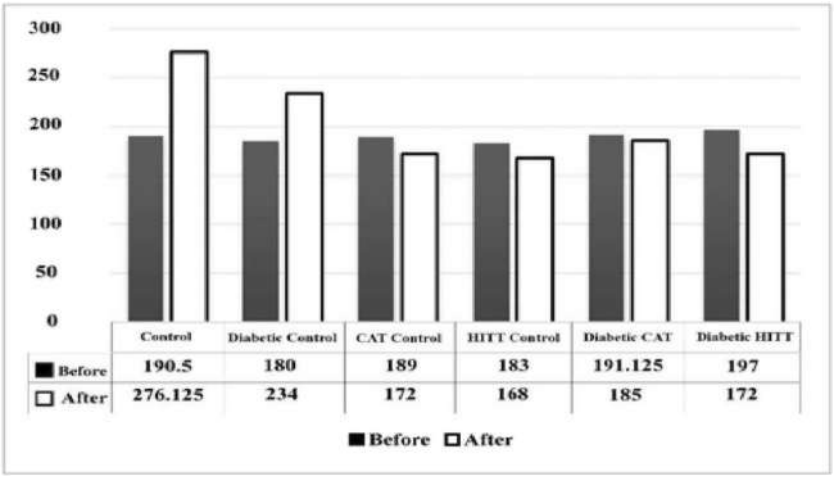

Fig. 1 Mean body weights of the rats used in the present study

Furthermore, it was observed that fasting glucose levels ($p < 0.001$) were higher in diabetic rats than in non-diabetic rats and that the CAT and HIIT protocols, both, resulted in the reduction of fasting glucose levels in both non-diabetic and diabetic rats. On the other hand, the HIIT protocol had more reducing effects on fasting glucose levels in both non-diabetic and diabetic rats as compared to the CAT protocol.

In the present study, the insulin resistance index ($p < 0.001$) was higher in diabetic rats than in non-diabetic rats, and the CAT and HIIT protocols resulted in the reduction of insulin resistance index in diabetic rats. Besides, the HIIT protocol had more reducing effects on insulin resistance index in both non-diabetic and diabetic rats as compared to the CAT protocol. Based on the statistical analysis of the research hypotheses, it was found that significant differences existed among study groups; hence, all research hypotheses were rejected.

Discussion and conclusion

Lack of balance among factors secreted from adipose tissues contributes to the development of metabolic diseases. In the present study, asprosin secretion, insulin secretion, and fasting glucose levels as well as insulin resistance indexes were significantly different in the non-diabetic control group, diabetic control group, non-diabetic training group, and diabetic training group of rats.

Diabetes is a metabolic disease which increases blood glucose levels. The disease is caused by many factors, of which one is glucose release during fasting states. In diabetic patients (type 2 diabetes), due to impaired glucose gain from the blood, the levels of glucose increase, which may in turn lead to physiological and metabolic disorders [14].

Asprosin, being secreted from the adipose cell during fasting states, is directed toward liver cells through the bloodstream and makes the liver release glucose into the blood with

Table 1 A comparison of the mean and standard deviation of fibrillin-1 (FBN1) gene expression, asprosin, insulin, fasting glucose levels, and insulin resistance index in the study groups

Variable	Study Groups						Kruskal–Wallis test results
	Control group Mean ± SD	Control diabetic group Mean ± SD	CAT group Mean ± SD	CAT diabetic group Mean ± SD	HITT group Mean ± SD	HITT diabetic group Mean ± SD	
FBN1	0.00 ± 1.00	0.50 ± 2.95	0.26 ± 1.16	0.41 ± 1.99	0.97 ± 0.97	0.41 ± 1.76	$X^2 = 38.01$,df = 5, $p < 0.001$
Asprosin	2.53 ± 36.45	7.77 ± 97.51	3.73 ± 29.05	5.28 ± 68.50	1.61 ± 21.67	2.53 ± 59.50	$X^2 = 45.24$ df = 5, $p < 0.001$
Insulin	0.27 ± 1.96	0.77 ± 2.49	0.19 ± 2.04	0.92 ± 2.30	0.20 ± 2.10	1.92 ± 1.99	$X^2 = 19.55$ df= 5, $p < 0.002$
Fasting glucose level	10.05 ± 198.62	28.22 ± 426.50	10.14 ± 179.3	38.12 ± 335.1	9.77 ± 170.62	43.34 ± 319.3	$X^2 = 42.27$, df = 5, $p < 0.001$
Insulin resistance index	2.55 ± 17.32	9.63 ± 47.19	1.80 ± 16.24	6.28 ± 34.26	2.05 ± 15.95	6.10 ± 28.25	$X^2 = 36$, df = 5, $p < 0.001$

the cAMP signaling pathway. At this time, insulin is secreted as a reaction to increased glucose levels in the blood. Therefore, in the present study, we first investigated whether the rats' affliction with diabetes could change asprosin levels.

The study found that inducing diabetes in the rats increased asprosin secretion levels significantly. This finding is very important, because it is endeavored that blood glucose levels in fasting states are kept stable and balanced in people with diabetes. However, asprosin secretion levels were higher in diabetic rats than in non-diabetic rats. Therefore, controlling asprosin secretion, or better to say its reduction, is vital for people with diabetes. In addition, the study showed that the levels of insulin secretion and fasting glucose were also higher in diabetic rats than in non-diabetic rats, for which the reason can, to some extent, be ascribed to higher levels of asprosin in diabetic rats as compared to non-diabetic ones.

Thus, based on the findings of the study, it can be concluded that oversecretion of asprosin leads to increased levels of blood glucose and, consequently, increased levels of insulin secretion in people with diabetes, because inappropriately high synthesis and secretion of asprosin in diabetic people makes the liver release more glucose into the bloodstream. As a result, the blood glucose level elevates, in response to which more insulin is secreted. On the other hand, due to the dysfunction of special glucose transporters on the surface of muscle cells (GLUT4), glucose cannot enter into muscle cells. Therefore, physiological and metabolic adjustments, made by means of exercise and nutrition interventions, are used to help decrease the synthesis and secretion of asprosin. In line with this, the effects of HIIT and CAT protocols on asprosin serum levels, and consequently on insulin and blood glucose levels, were investigated in the current study. The results indicated that both HIIT and CAT workouts have significant effects on decreasing asprosin and blood glucose levels both in non-diabetic and in diabetic rats. Hence, people with diabetes can prevent from increased levels of asprosin secretion by performing HIIT and CAT workouts. This will reduce the amount of glucose entering into their bloodstream during fasting states. Now, having regards to the positive effects of both protocols of the study (HIIT and CAT) on the reduction of asprosin secretion and plasma glucose levels, we may ask which one of the two protocols are more effective in reducing asprosin secretion levels. In view of the results of the present study, the HIIT protocol had more significant effects on reducing both asprosin secretion and plasma glucose levels as compared to the CAT protocol. Fibrillin-1 is a large glycoprotein encoded by the FBN1 gene in humans, and it plays a remarkable role in adipogenesis. Researchers observed in inbred rats that FBN1 gene expression levels are associated with changes in adipose cell levels. They discovered that FBN1 gene expression is higher in overweight diabetic rats than in non-diabetic rats with normal weight. Thus, it might be said that the relation between asprosin and diabetes is to a large extent related to the upstream gene, i.e., FBN1. Accordingly, the present study investigated the effects of HIIT and CAT workouts on FBN1 gene expression in diabetic male rats. The results revealed that the rats' affliction with diabetes led to increased FBN1 gene expression. Consequently, synthesis and secretion of asprosin increase in response to increase in this gene expression. In the light of the foregoing, it should be noted that the main purpose of controlling asprosin secretion and plasma glucose levels is to decrease FBN1 gene expression levels. In the present study, it was observed that both HIIT and CAT workouts can decrease FBN1 gene expression levels.

Perspective

Since asprosin and FBN1 gene have been recently discovered, not many studies have been conducted on them. In fact, the present study is considered the first in the field of sports science which examines the effects of two different exercise methods on asprosin and FBN1. Still, owing to the fact that there are some other adipokines which function almost similarly to asprosin, further studies are required to compare the findings of the present study with those of similar ones having been conducted on hormones secreted from adipose tissues. It could be said that HIIT workouts have more positive effects on asprosin and FBN1gene levels in diabetic rats than CAT workouts. Based on the results of the present study, it seems that 8-week HIIT and CAT workouts have positive effects on FBN1 gene expression, asprosin, glucose, and insulin levels as well as insulin resistance index. Therefore, HIIT and CAT workouts could be recommended as important strategies for controlling asprosin and insulin secretion levels, especially during fasting states. In fact, the use of such strategies needs to be taken into account as an effective intervention method. Besides, since both HIIT and CAT protocols proved effective and positive, it is advisable that both types of workouts be included in workout plans for people with diabetes. It is suggested that future studies investigate the effects of other training protocols of varying intensity as well as high-fat and low-fat diets on asprosin secretion levels and its upstream gene (i.e., FBN1) in diabetic and non-diabetic rats. In addition, it is proposed that the effects of sports activities on asprosin receptors be fully investigated in the course of future studies.

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Declarations All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that

the submission is original work and is not under review at any other publication.

Ethics approval Throughout the course of the present study, the fundamental ethical principles of working with animals, as approved by the Research Ethics Committee of Ferdowsi University of Mashhad, were duly respected.

Conflict of interest The authors declare no competing interests.

References

1. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. Diabetes. 2006;55(5):1463–9.
2. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. J Clin Invest. 1995;95(5):2281–90.
3. American College of Sports Medicine, American Dietetic Association. Joint Position Statement: nutrition and athletic performance. American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada. Medicine and Science in Sports and Exercise. 2000;32(12):2130.
4. Wagenmakers AJ, Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays Biochem. 2006;42:105–17.
5. Wagenmakers AJ, Hu G, Rico-Sanz J, Lakka TA, Tuomilehto J. Exercise, genetics and prevention of type 2 diabetes. Essays Biochem. 2006;42:177–92.
6. Colberg SR, Sigal RJ. Prescribing exercise for individuals with type 2 diabetes: recommendations and precautions. Phys Sportsmed. 2011;39(2):13–26.
7. Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, van de Weijer T, Sels JP, Schrauwen P, Hesselink MK. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes. 2010;59(3):572–9.
8. Adeva M, González-Lucán M, Seco M, Donapetry C. Enzymes involved in l-lactate metabolism in humans. Mitochondrion. 2013;13(6):615–29.
9. Sigal RJ, Armstrong MJ, Colby P, Kenny GP, Plotnikoff RC, Reichert SM, Riddell MC. Canadian Diabetes Association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada: physical activity and diabetes. Can J Diabetes. 2013;37(Suppl 1):S40–4.
10. Chudyk A, Petrella RJ. Effects of exercise on cardiovascular risk factors in type 2 diabetes: a meta-analysis. Diabetes Care. 2011;34(5):1228–37.
11. Smitka K, Marešová D. Adipose tissue as an endocrine organ: an update on pro-inflammatory and anti-inflammatory microenvironment. Prague Med Rep. 2015;116(2):87–111.
12. Proença AR, Sertié RA, Oliveira AC, Campaaa AB, Caminhotto RO, Chimin P, Lima FB. New concepts in white adipose tissue physiology. Braz J Med Biol Res. 2014;47(3):192–205.
13. Yamauchi T, Iwabu M, Okada-Iwabu M, Kadowaki T. Adiponectin receptors: a review of their structure, function and how they work. Best Pract Res Clin Endocrinol Metab. 2014;28(1):15–23.
14. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med. 2017;23(7):804–14.

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SHORT ARTICLE

Proportion of natural killer cells in peripheral blood lymphocytes is correlated with cytokine levels in patients with type 2 diabetes mellitus and prediabetes: a preliminary report

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Abstract

Aims Natural killer (NK) cells are important innate cytotoxic lymphocytes and can contribute to the immune response through the synthesis and secrete proinflammatory cytokines. In the present study, we assessed and compared the proportion of NK cells in peripheral blood lymphocytes, various cytokine levels and metabolic parameters in type 2 diabetes mellitus (T2DM), prediabetes and normal glucose tolerance (NGT).

Methods Individuals with 57 T2DM, 58 prediabetes and 60 NGT were enrolled. Fasting plasma glucose, 2-h postload glucose, fructosamine, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein and triglyceride levels were assessed on an automatic biochemical analyzer. Various cytokine levels in the serum including IL-2, IL-5, IL-6, IL-10, IL-12p70, and IFN-γ were assessed using ELISA kits. Proportion of NK cells in peripheral blood lymphocytes was assessed by flow cytometry.

Results The proportion of NK in lymphocytes from prediabetes was significantly increased compared with that obtained from T2DM, while the data was significantly decreased compared with that obtained from NGT. Significant increases in fasting plasma glucose, postprandial plasma glucose and triglycerides were observed in prediabetes compared with NGT.IL-12p70 level was significantly lower in prediabetes and T2DM compared with NGT. IL-6 and IFN-γ levels in prediabetes were significantly lower than those in NGT and T2DM patients, in addition IL-6 level in T2DM patients was significantly higher compared to NGT. The trend of IL-10 level was opposite to that of IL-6.

Conclusions The decreased proportion of NK cells in peripheral blood lymphocytes following some cytokine levels (IL-12p70, IL-6,IL-10 and IFN-γ) was likely involved in the development of T2DM and prediabetes.

Keywords Natural killer cells · Cytokines · Prediabetes · Type 2 diabetes mellitus

Introduction

With the rapid economic development and the improvement of living standards, the incidence of type 2 diabetes mellitus (T2DM) has also increased rapidly [1]. Recent studies have shown that low-grade inflammation is involved in the pathogenesis of patients with T2DM [2, 3]. The chronic inflammatory state in diabetes patients might be related to the impaired immune function [4]. Therefore, the relationship between the immune system and T2DM pathogenesis has attracted extensive attention.

Natural killer (NK) cells are important innate cytotoxic lymphocytes, showing rapid and efficient cytolytic activity to recognize and kill infected cells [5, 6]. The activation of NK cells can exert effector functions without the need for prior exposure to the antigen [6]. They also can contribute to the immune response through the synthesis and secrete a wide range of proinflammatory cytokines and directly killing target cells [7, 8]. Cytokines such as IL-2, IL-6, IL-12, TNF-α and leukoregulin (LR) have a positive regulatory effect on the activation and differentiation of NK cells [9]. At the same time, the levels of IL-5, IL-10, and IFN-γ are related to the synthesis and secretion of NK cells [7–9].

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At present, contradictory findings on the role of NK cells in T2DM still exist [10–14]. Some research showed that patients with T2DM had decreased peripheral NK cells compared to controls [10, 11]. However, some research have different views that the levels of NK cells were similar or raised levels in T2DM compared to controls [12–14]. Thus, the role of NK cells in modulating T2DM and its associated complications remains elusive.

In the present study, we assessed and compared the proportion of NK cells in peripheral blood lymphocytes, various cytokine levels and metabolic parameters in T2DM, prediabetes and normal glucose tolerance (NGT).

Materials and methods

Individuals

The individuals with 57 T2DM, 58 prediabetes and 60 NGT were enrolled from the First Affiliated Hospital of Nanchang University from September 2020 to May 2021. T2DM was defined as fulfilling the American Diabetes Association (ADA) diagnostic criteria. Prediabetic patients were defined as those with FPG (fasting plasma glucose) 6.1–7.0 mM, 2hPG (2-h postload glucose) 7.8–11.1 mM [15]. The NGT group was defined as individuals with FPG <6.1 mM, 2hPG <7.8 mM.

Following local ethics committee approval, written informed consent was obtained from each individual. Subjects showing severe microvascular complications, hypertension, human immunodeficiency virus, splenectomy, immunosuppressive therapy after organ transplantation, rheumatic diseases, severe liver or kidney disease or pregnancy were excluded from the study.

Fasting plasma glucose, 2-h postload glucose, fructosamine, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were assessed with an automatic biochemical analyzer (Hitachi 7180, Japan).

Enzyme linked immunosorbent assay

Various cytokine levels in the serum including IL-2 (cat:EK0397), IL-5 (cat:EK0407), IL-6 (cat:EK0410), IL-10 (cat:EK0416), IL-12p70 (cat:EK0421), and IFN-γ (cat:EK0373) were assessed using specific enzyme linked immunosorbent assay (ELISA) kits (Boster, China) according to the manufacturer's instructions. Blood samples were centrifuged at 1000×*g* for 15 min at 4°C and supernatants (serum samples) were stored at −80 °C for later use. All detection procedures were performed in triplicate wells to guarantee the accuracy of the results.

Analysis of the proportion of NK cells in peripheral blood lymphocytes

The peripheral blood was extracted with EDTA anticoagulant tube on an empty stomach. An antibody cocktail was developed consisting of anti-CD45 PC7 (cat:200241), anti-CD3 ECD (cat: 200115) and anti-CD56 PE (cat:200067) provided by Becton, Dickinson and Company (USA) according to the ratio of 1:1:1. 32 μl blood sample and 6 μl antibody cocktail were added and incubated in dark for 5 min after mixing evenly. After erythrocyte lysis, we added phosphate buffer saline (PBS) to execute the cleaning operation. After three washing steps, the cells were resuspended in 400 μl PBS and tested them on DxFLEX cytometer (Beckman Coulter, USA). For each assay, at least 100,000 events were analyzed. To screen for cross-reactivity, all data were corrected for autofluorescence using isotype-matched negative controls. Data were analyzed with the Cytomics CXP software (Beckman Coulter, USA).

Lymphocytes were determined by light forward (FSC) and side (SSC) scatter labeled with CD45. Lymphocytes were gated based on low side scatter and bright CD45. These gating strategies were based on flow cytometric analysis guidelines by the Centers for Disease Control and Prevention [16]. NK cells were the type of lymphocytes labeled with CD3 negative and CD56 positive. Results are reported as percentage of cells that stained positively for the antibodies of interest.

Statistical analysis

All data are shown as mean ± standard deviation (SD). Statistical analyses were carried out using the SPSS software 11.5 software. Statistical significance was determined by two-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

Results

Anthropometric data and serum biochemistry parameters of the study participants

The baseline characteristics obtained from the three groups are summarized in Table 1. Significant increases in fasting plasma glucose, postprandial plasma glucose and triglycerides were observed in prediabetes compared with NGT ($p < 0.05$). In addition, fructosamine and high-density lipoprotein cholesterol contents in T2DM patients were significantly changed compared with those in prediabetes ($p < 0.05$) (Table 1).

Serum cytokine levels in our study participants

There were no significant differences in the concentrations of IL-2 and IL-5 among the three groups. IL-12p70 level was

Table 1 Anthropometric data and serum biochemistry parameters of study participants

Parameter	NGT	Prediabetes	T2DM	x^2/F value	p value
Number (F/M)	60(29/31)	58(29/29)	57(28/29)	0.065	0.912
Weight (kg)	67.23 ± 8.56	66.59 ± 6.38	67.82 ± 8.63	0.053	0.959
Age (years)	46.22 ± 5.67	47.31 ± 5.71	47.23 ± 6.34	0.035	0.948
BMI (kg/m^2)	25.07 ± 2.61	25.88 ± 2.93	25.74 ± 2.53	0.061	0.953
WBC (×10^9/L)	6.48 ± 1.86	6.62 ± 1.93	6.83 ± 2.12	0.381	0.527
NLR	1.58 ± 0.38	1.63 ± 0.42	1.71 ± 0.46	2.468	0.246
FPG (mmol/L)	5.11 ± 0.54	6.53 ± 0.47*	8.29 ± 1.62*#	56.273	0.000
2hPG (mmol/L)	6.67 ± 0.63	9.79 ± 0.86*	13.72 ± 1.41*#	227.293	0.000
FMN (mmol/L)	2.25 ± 0.24	2.50 ± 0.33	2.75 ± 0.49#	9.836	0.000
TG (mmol/L)	1.59 ± 0.19	1.87 ± 0.22*	1.89 ± 0.34*	30.147	0.000
TC (mmol/L)	4.41 ± 0.55	4.47 ± 0.58	4.51 ± 0.61	0.278	0.623
HDL (mmol/L)	1.49 ± 0.17	1.63 ± 0.24	1.33 ± 0.21#	16.273	0.000
LDL (mmol/L)	2.48 ± 0.28	2.52 ± 0.27	2.59 ± 0.31	1.123	0.354

NGT normal glucose tolerance, Prediabetes impaired glucose tolerance and impaired fasting glucose, T2DM type 2 diabetes mellitus

BMI Body Mass Index, *NLR* Neutrophil to lymphocyte ratio, *FPG* fasting plasma glucose, *2hPG* 2-h postload glucose, *FMN* fructosamine, *TG* triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol

* $p < 0.05$ compared with NGT; # $p < 0.05$ compared with Prediabetes

significantly lower in prediabetes and T2DM compared with NGT. IL-6 and IFN-γ levels in prediabetes were significantly lower than those in NGT and T2DM patients, in addition IL-6 level in T2DM patients was significantly higher compared to NGT. The trend of IL-10 level was opposite to that of IL-6. The specific results were shown in Table 2.

Proportion of NK cells in peripheral blood lymphocytes of study participants

Proportion of NK cells in peripheral blood lymphocytes was assessed by flow cytometry. The proportion of NK in lymphocytes from prediabetes (16.89% ± 3.95%) was significantly increased compared with that obtained from T2DM (11.21% ± 3.06%), while the data from prediabetes was significantly decreased compared with that obtained from NGT(20.81% ± 4.57%) ($p < 0.05$) (Fig. 1).

Discussion

Recent research found that T2DM was associated with low-level inflammatory response, with abnormal activation of multiple immune cells [3, 4]. Natural killer (NK) cells are important innate cytotoxic lymphocytes [5, 6]. NK cells as one of the main members of innate immunity, were closely related to T2DM [14, 17]. On the one hand, the number and function of NK cells in T2DM patients showed abnormal [14]. The damage of NK cells in T2DM patients may be involved in the pathogenesis of obesity, insulin resistance [18, 19]. On the other hand, NK

Table 2 Levels of serum cytokines in study participants

Type of cytokines (pg/mL)	NGT	Prediabetes	T2DM	F value	*p* value
IL-2	2.23 ± 0.31	2.28 ± 0.36	2.27 ± 0.37	0.483	0.614
IL-5	2.48 ± 0.37	2.54 ± 0.44	2.59 ± 0.43	0.388	0.639
IL-6	3.42 ± 0.47	2.54 ± 0.51*	4.53 ± 0.72*#	66.237	0.000
IL-10	0.47 ± 0.12	1.91 ± 0.23*	0.68 ± 0.23*#	311.226	0.000
IL-12p70	1.74 ± 0.32	1.61 ± 0.26*	1.52 ± 0.25*	4.274	0.028
IFN-γ	19.86 ± 2.61	14.39 ± 2.23*	18.91 ± 2.72#	29.235	0.000

NGT, normal glucose tolerance; Prediabetes impaired glucose tolerance and impaired fasting glucose, *T2DM*, type 2 diabetes mellitus

* $p < 0.05$ compared with NGT; # $p < 0.05$ compared with Prediabetes

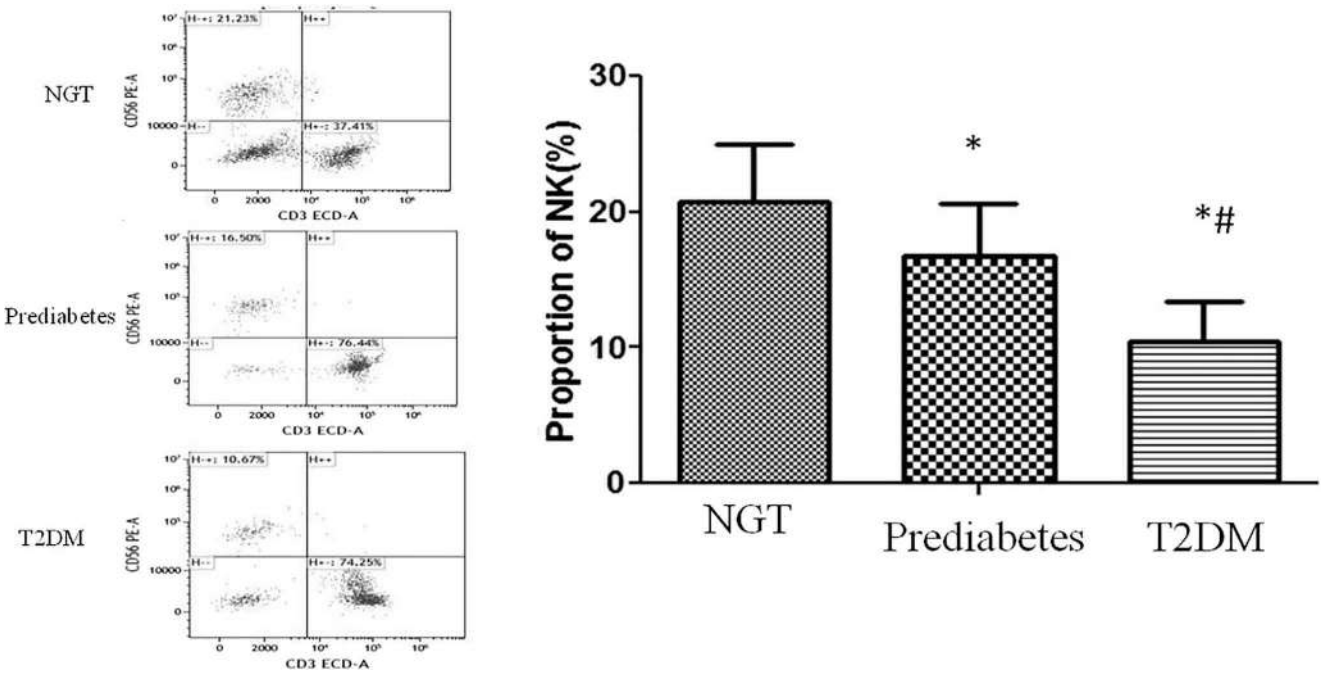

Fig. 1 Proportion of NK cells in peripheral blood lymphocytes. The left picture was the original image of the NK cells' proportion in peripheral blood lymphocytes by the flow cytometer. The right picture was the data of the NK cells' proportion in peripheral blood lymphocytes. The results showed that the proportion value of NK in lymphocytes from prediabetes was significantly increased compared with that obtained from T2DM, while the data from prediabetes was significantly decreased compared with that obtained from NGT. $^{*}p < 0.05$ vs. the NGT group; $^{\#}p < 0.05$ vs. the prediabetes group; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus

cells also can secrete inflammatory cytokines for immunoregulation to become as a bridge between innate and adaptive immunity [7, 8], and thereby to participate in the regulation of low-level inflammatory response. However, at present, contradictory findings on the role of NK cells in T2DM still exist [10–14]. These results showed that the role of NK cells in modulating T2DM and its associated complications remains elusive. The present study results demonstrated that a reduced proportion of NK cells in peripheral blood lymphocytes in T2DM patients compared with the prediabetes who showed its reduced proportion compared with NGT participants. There are two main reasons for the inconsistency of research reports. First of all, the number of people participating in existing research on the association between diabetes and NK cells was small (most research cases were less than 100) [10–14]. Secondly, the selection and reporting bias were mainly due to differences (diabetes duration, complications and immune status) in the population choosing to participate in the survey [10–14].

Previous reports also showed that abnormal lipid metabolism may contribute to impaired pancreatic cells function [20, 21]. Based on these reports, we investigated the correlations of lipid metabolism parameters among NGT, prediabetes and T2DM. The results of this study revealed that the triglyceride concentration was significantly higher in prediabetes and T2DM patients than in NGT participants, while the concentration of high-density lipoprotein cholesterol in T2DM patients was significantly lower than the other two groups, while the concentrations of total cholesterol and low-density lipoprotein cholesterol were not significantly different among above groups. The results have similarities and differences with previous results [20–22] and the bias might be caused by a small number of study participants.

T2DM is a chronic inflammatory state that may be mediated by cytokines. Meanwhile, activated NK cells can synthesize and secrete a variety of cytokines [7, 8]. Therefore, this study also investigated the changes in the concentration of multiple cytokines in three groups of study participants. The results showed that the concentrations of IL-12p70, IL-6, IFN-γ, and IL-10 were significantly different between the above groups. IL-12p70 level was significantly lower in prediabetes and T2DM compared with NGT people. IL-6 and IFN-γ levels in prediabetes were significantly lower than those in NGT and T2DM patients, in addition IL-6 level in T2DM patients was significantly higher compared to NGT. The trend of IL-10 level was opposite to that of IL-6. There might be two main reasons for the changes in a variety of cytokines. First of all, the concentrations of cytokines were related to the immune status to prediabetes and T2DM [23, 24]. From the results of cytokines, the prediabetes was in the immunosuppressive state of increased IL-6 level combined with decreased IL-10 level. T2DM stage

was the state of chronic inflammation where IL-6 was elevated and IL-10 was reduced. Secondly, the concentrations of cytokines were also connected to the numbers and activation levels of NK cells [7, 8]. The decreased proportion of NK cells in prediabetes may lead to lower the concentrations of IFN-γ and IL-12p70. Although the proportion of NK cells in T2DM patients also reduced, it might be due to the activation of NK cells that would promote the secretion of IFN-γ and IL-12p70, which would result in their raise. At the same time, this study found that IL-2 and IL-5 might not be involved in the pathogenesis of prediabetes and T2DM because their concentrations were not significantly different among the three groups. The enhanced knowledge of the present study was that NK cells may be associated with T2DM and some cytokines can play different roles in the pathogenesis of T2DM and prediabetes. Therefore, decreased proportion of NK cells in peripheral blood lymphocytes following some cytokine levels (IL-12p70, IL-6, IL-10, and IFN-γ) was likely involved in the development of T2DM and prediabetes. The study may suggest that it is possible to curb the onset of prediabetes and T2DM by increasing the activation of NK cells.

The present study had two limitations. First of all, there was a risk of bias in the study caused by the limitation of small number at a single hospital. Next, the test item for NK cells was relatively single and NK cell activity or function tests should be added to prove the association between NK cells and T2DM. Multi-site study in the future with more participants should be done to investigate the relationship. It is also necessary to determine the relevance between NK cells and T2DM through changes in NK cell activity or function.

In summary, in this study, our results showed that proportion of NK cells in peripheral blood lymphocytes from T2DM patients was significantly decreased and these cells might affect some cytokine levels (IL-12p70,IL-6,IL-10 and IFN-γ), thereby participating in the pathogenesis of diabetes.

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Author contribution YN and HW were responsible for the conception and design of the study. YN, HW, acquired the data. HW drafted the manuscript and YN revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate All procedures were approved by the Committee of the First Affiliated Hospital,Medical College of Nanchang University (approval no. 2018 16).

Conflict of interest The authors declare no competing interests.

References

1. Amanat S, Ghahri S, Dianatinasab A, Fararouei M, Dianatinasab M. Exercise and type 2 diabetes. Adv Exp Med Biol. 2020;1228:91–105. https://doi.org/10.1007/978-981-15-1792-1_6.
2. Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 Diabetes and its impact on the immune system. Curr Diabetes Rev. 2020;16(5):442–9. https://doi.org/10.2174/1573399815666191024085838.
3. Bonnet F, Scheen AJ. Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: the potential contribution to diabetes complications and cardiovascular disease. Diabetes Metab. 2018 Dec;44(6):457–64. https://doi.org/10.1016/j.diabet.2018.09.005.
4. Lauterbach MA, Wunderlich FT. Macrophage function in obesity-induced inflammation and insulin resistance. Pflugers Arch. 2017 Apr;469(3-4):385–96. https://doi.org/10.1007/s00424-017-1955-5.
5. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol. 2018 Nov;18(11):671–88. https://doi.org/10.1038/s41577-018-0061-z.
6. Pockley AG, Vaupel P, Multhoff G. NK cell-based therapeutics for lung cancer. Expert Opin Biol Ther. 2020 Jan;20(1):23–33. https://doi.org/10.1080/14712598.2020.1688298.
7. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. Front Immunol. 2018 Aug 13;9:1869. https://doi.org/10.3389/fimmu.2018.01869.
8. Zhou J, Tian Z, Peng H. Tissue-resident NK cells and other innate lymphoid cells. Adv Immunol. 2020;145:37–53. https://doi.org/10.1016/bs.ai.2019.11.002.
9. Konjević GM, Vuletić AM, Mirjačić Martinović KM, Larsen AK, Jurišić VB. The role of cytokines in the regulation of NK cells in the tumor environment. Cytokine. 2019;117(5):30–40. https://doi.org/10.1016/j.cyto.2019.02.001.
10. Akesson C, Uvebrant K, Oderup C, Lynch K, Harris RA, Lernmark A, Agardh CD, Cilio CM. Altered natural killer (NK) cell frequency and phenotype in latent autoimmune diabetes in adults (LADA) prior to insulin deficiency. Clin Exp Immunol. 2010 Jul 1;161(1):48–56. https://doi.org/10.1111/j.1365-2249.2010.04114.x.
11. Kim JH, Park K, Lee SB, Kang S, Park JS, Ahn CW, Nam JS. Relationship between natural killer cell activity and glucose control in patients with type 2 diabetes and prediabetes. J Diabetes Investig. 2019 Sep;10(5):1223–8. https://doi.org/10.1111/jdi.13002.
12. Medellín-Garibay SE, Cortez-Espinosa N, Milán-Segovia RC, Magaña-Aquino M, Vargas-Morales JM, González-Amaro R, Portales-Pérez DP, Romano-Moreno S. Clinical pharmacokinetics of rifampin in patients with tuberculosis and type 2 diabetes mellitus: association with biochemical and immunological parameters. Antimicrob Agents Chemother. 2015 Dec;59(12):7707–14. https://doi.org/10.1128/AAC.01067-15.
13. Nam HW, Cho YJ, Lim JA, Kim SJ, Kim H, Sim SY, Lim DG. Functional status of immune cells in patients with long-lasting type 2 diabetes mellitus. Clin Exp Immunol. 2018 Oct;194(1):125–36. https://doi.org/10.1111/cei.13187.
14. Mxinwa V, Dludla PV, Nyambuya TM, Mokgalaboni K, Mazibuko-Mbeje SE, Nkambule BB. Natural killer cell levels in adults living with type 2 diabetes: a systematic review and meta-

analysis of clinical studies. BMC Immunol. 2020 Sep 9;21(1):51. https://doi.org/10.1186/s12865-020-00378-5.
15. Carris NW, Magness RR, Labovitz AJ. Prevention of diabetes mellitus in patients with prediabetes. Am J Cardiol. 2019 Feb 1;123(3):507–12. https://doi.org/10.1016/j.amjcard.2018.10.032.
16. Pinto LA, Trivett MT, Wallace D, Higgins J, Baseler M, Terabe M, Belyakov IM, Berzofsky JA, Hildesheim A. Fixation and cryopreservation of whole blood and isolated mononuclear cells: influence of different procedures on lymphocyte subset analysis by flow cytometry. Cytometry B Clin Cytom. 2005;63(1):47–55. https://doi.org/10.1002/cyto.b.20038.
17. Zhou T, Hu Z, Yang S, Sun L, Yu Z, Wang G. Role of adaptive and innate immunity in type 2 diabetes mellitus. J Diabetes Res. 2018 Nov 8;2018:7457269. https://doi.org/10.1155/2018/7457269.
18. Lee BC, Kim MS, Pae M, Yamamoto Y, Eberlé D, Shimada T, Kamei N, Park HS, Sasorith S, Woo JR, You J, Mosher W, Brady HJ, Shoelson SE, Lee J. Adipose natural killer cells regulate adipose tissue macrophages to promote insulin resistance in obesity. Cell Metab. 2016 Apr 12;23(4):685–98. https://doi.org/10.1016/j.cmet.2016.03.002.
19. Satoh M, Iwabuchi K. Role of natural killer t cells in the development of obesity and insulin resistance: insights from recent progress. Front Immunol. 2018 Jun 11;9:1314. https://doi.org/10.3389/fimmu.2018.01314.
20. Biczo G, Vegh ET, Shalbueva N, Mareninova OA, Elperin J, Lotshaw E, Gretler S, Lugea A, Malla SR, Dawson D, Ruchala P, Whitelegge J, French SW, Wen L, Husain SZ, Gorelick FS, Hegyi P, Rakonczay Z Jr, Gukovsky I, Gukovskaya AS. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. Gastroenterology. 2018 Feb;154(3):689–703. https://doi.org/10.1053/j.gastro.2017.10.012.
21. Imai Y, Cousins RS, Liu S, Phelps BM, Promes JA. Connecting pancreatic islet lipid metabolism with insulin secretion and the development of type 2 diabetes. Ann N Y Acad Sci. 2020 Feb;1461(1):53–72. https://doi.org/10.1111/nyas.14037.
22. Wu H, Nie Y, Xiong H, Liu S, Li G, Huang A, Guo L, Wang S, Xue Y, Wu B, Peng L, Song M, Li G, Liang S. P2X7 Receptor expression in peripheral blood monocytes is correlated with plasma C-reactive protein and cytokine levels in patients with type 2 diabetes mellitus: a preliminary report. Inflammation. 2015 Dec;38(6):2076–81. https://doi.org/10.1007/s10753-015-0189-y.
23. Wang Z, Shen XH, Feng WM, Ye GF, Qiu W, Li B. Analysis of inflammatory mediators in prediabetes and newly diagnosed type 2 diabetes patients. J Diabetes Res. 2016;2016:7965317. https://doi.org/10.1155/2016/7965317.
24. Cronkite DA, Strutt TM. The regulation of inflammation by innate and adaptive lymphocytes. J Immunol Res. 2018 Jun 11;2018:1467538. https://doi.org/10.1155/2018/1467538.

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CASE REPORT

Efficacy of empagliflozin for weight and glycemic control of a patient with Prader-Willi syndrome, systemic lymphedema and extreme obesity: a case report

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Abstract

Case Report A patient with Prader-Willi syndrome (PWS), extreme obesity and hyperglycemia had her body weight increased considerably for 6 months, even with exercise and diet programs. Treatment with metformin and empagliflozin (12.5 mg/day) induced a weight loss of 14 kg (−10.3%) for 6 months and the reduction of glycated hemoglobin A_{1c}.

Keywords Prader-Willi syndrome · Obesity, Morbid · Exercise · SGLT-2 inhibitors · Case report

Introduction

Characteristics of Prader-Willi syndrome (PWS) include hyperphagia, deficiency of anabolic hormones, small hands and feet, short stature, diminished lean mass and bone mineral density, hypogonadism and mild intellectual disabilities [1, 2]. Empagliflozin, a sodium-glucose cotransporter 2 (SGLT-2) inhibitor, may benefit glycemic control and weight loss [3]. Moreover, regular exercise has been linked to increased maximum oxygen uptake and muscle strength in PWS patients [4].

Special attention needs to be paid regarding lymphedema, which may be masked by the prevailing increase of body fat linked to obesity in PWS patients. Lymphedema is characterized by excessive accumulation of interstitial protein-rich fluid due to an impaired transport or drainage of lymph [1]. Also, the abnormal swelling of tissues is connected with chronic inflammation, infections, and even angiosarcoma in some cases [5]. In this case study, we report a patient with PWS, systemic lymphedema and extreme obesity whose weight and glycemic control were significantly improved following empagliflozin therapy.

Case report

At the age of 23 years and 9 months, a patient was referred by the physician nutrition specialist to a physical exercise–based outpatient service due to progressive weight gain, even in the presence of a controlled diet. Her gradually increasing and persistent feeling of heaviness and swelling in both legs had started approximately 2 years earlier (Fig. 1).

The Brazilian patient was delivered by cesarean section associated with acute fetal distress at 32nd week, with a birth weight of 2,140 g. Shortly after birth, hypotonia was observed. She had a global development delay and walked independently at 4 years of age, when PWS was confirmed

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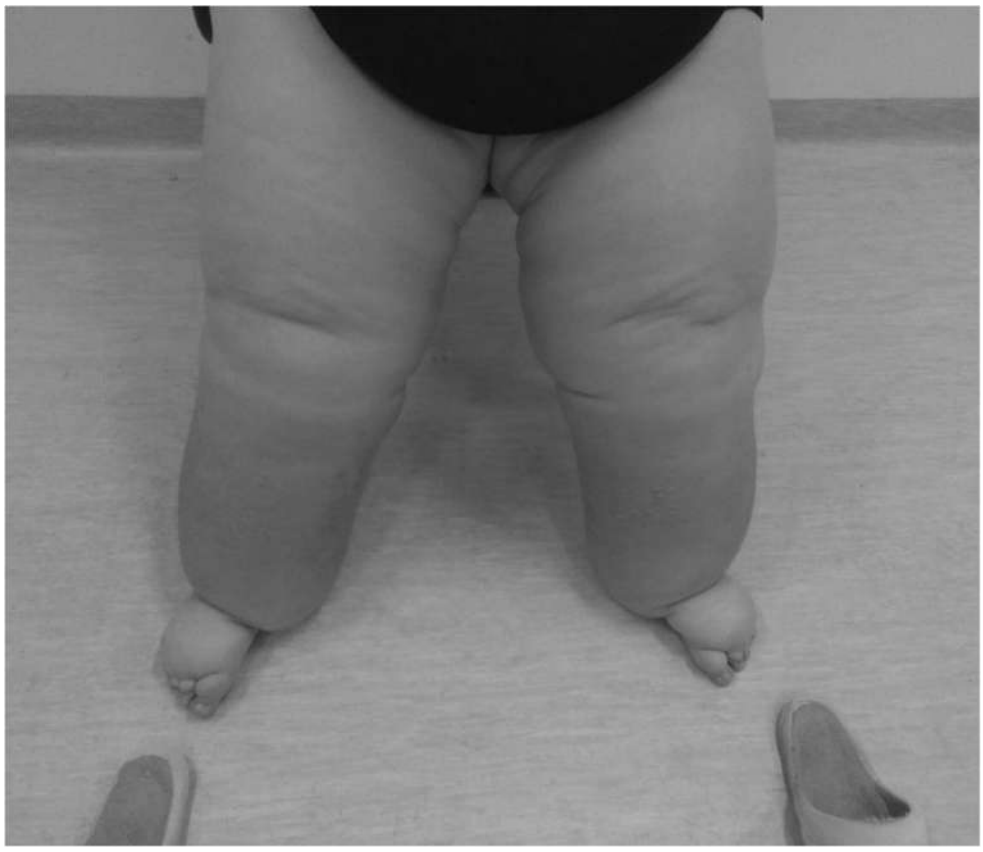

Fig. 1 Front view of the legs in a patient with Prader-Willi syndrome and lymphedema seated in a chair

through the fluorescence in situ hybridization technique. Her further medical history revealed short stature, small hands and feet, mild intellectual disability, severe kyphoscoliosis, skin picking and menarche at age 22. There was no previous history of filariasis, cancer, trauma, leg surgery, underlying systemic diseases or erysipelas. Family history includes cancer and cardiovascular problems, but not skin disease or lymphedema. Manual lymphatic drainage and the use of elastic compression stockings were initiated when patient was 21 years old and did not improve lower limb edema. Last year, due to severe worsening of the edema, the stockings did not fit anymore. Regarding physical activities history, she used to swim during childhood, and during the following years up to 5 years ago she worked out at the gym. In an attempt to reduce edema, the patient reported sleeping with feet up.

The patient presented an extreme obesity status (BMI: 62 kg/m^2) with lymphedema (nonpitting) mainly of the lower limbs (extensive soft tissue induration), but also of belly and arms. The patient was taking the following drugs: metformin 1,000 mg/day, levothyroxine sodium 50 mcg/day, cholecalciferol 14,000 IU/week, paroxetine 20 mg/day.

An individual and supervised exercise program was performed, twice a week. The program consisted of free-weight and exercises with elastic bands, up and down stairs, and exercises with balls of different sizes. Each exercise session lasted 45–50 min, and heart rate and oxygen saturation were measured before and after each exercise session. No adverse events were reported; the patient presented high adherence and was excited with the exercises program. Two months after starting exercises, liraglutide (1.2 mg/day), a glucagon-like peptide-1 analog (GLP-1), was prescribed. This drug caused a transient and relatively small weight reduction (Fig. 2), but was discontinued after 2 months due to the relatively high cost and little efficacy on glucose levels and weight loss in this patient.

Exercise program was interrupted with 6 months of duration due to elective hospitalization for a hospital-based supervised intervention to reduce weight gain, control glycemic levels and evaluate for obstructive sleep apnea syndrome (OSAS). So, at the age of 24 years and 3 months, she was hospitalized. Upon admission, empagliflozin (12.5 mg/day) was initiated. She received a restricted diet of 1,000 kcal/day + 140 g protein and lost a total of 2.8 kg during 5 days of hospitalization. She was discharged due to the outbreak of coronavirus disease 2019. At hospital discharge, a hypocaloric and hyperproteic diet (1,300 kcal + 113 g protein/day) was prescribed.

When administered only with metformin (1,000 mg/day) for glycemic control, empagliflozin (12.5 mg/day) induced a weight loss of 14 kg (−10.3%) for 6 months (Fig. 2). Moreover, glycated hemoglobin A_{1c} (HbA_{1c}) levels changed from 7.9 to 6.5% some months after

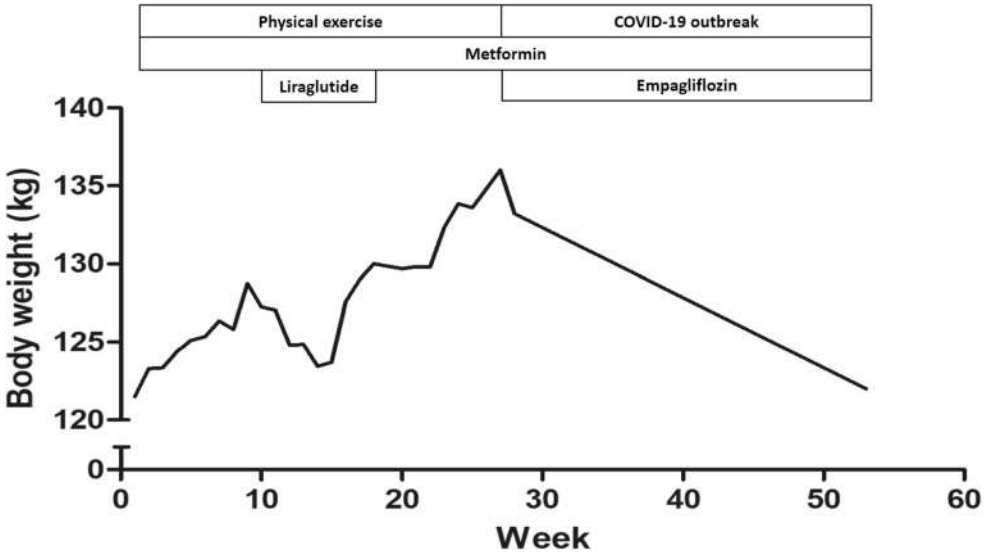

Fig. 2 Course of treatment graph showing the corresponding effects on body weight. The duration of interventions/conditions and pharmacological agents are represented by the length of each box relative to the time period on the *x*-axis

utilizing empagliflozin. Polysomnography (PSG) was performed and confirmed severe OSAS. A second PSG for continuous positive airway pressure titration was performed and CPAP therapy was prescribed, although she is not yet using the device.

Discussion

The progression of obesity is associated with impaired cutaneous lymphatic collecting vessel pumping rate, lymphatic leakiness and macromolecule clearance. The prevalence of lymphedema ranges from 49 to 63% in PWS [6, 7]. Recently, liraglutide treatment (0.9 mg/day) did not reduce body weight [3],[3] while this GLP-1 analog (0.6→1.2 mg/day) stimulated weight reduction in another PWS patient [8]. In our study, liraglutide (1.2 mg/day) provided a transient weight reduction. However, when added to metformin treatment (up to 1,750 mg/day), empagliflozin (10 mg/day) caused a weight loss of approximately 5.5 kg (−7.4%) for 5 months (average monthly loss of 1.1 kg) in a recent study [3]. In our case report, when administered only with metformin (1,000 mg/day), empagliflozin (12.5 mg/day) induced a weight loss of 14 kg (−10.3%) for 6 months (average monthly loss of 2.3 kg). Noteworthy, the absolute body weight of our patient before empagliflozin therapy was 136 kg, much greater than those observed in similar case reports with PWS patients [3, 9]. In this regard, empagliflozin presents protector role against cardiovascular and renal events [10].

SGLT-2 inhibitors reduce glycemia and body weight by inhibiting glucose absorption and transportation in the kidney [3]. We believe that the diuretic effects of this drug could, at least in part, have helped in weight reduction by lymphedema improvement. Although liraglutide induced a transient weight reduction, we concluded that the later observed weight loss was specifically due to empagliflozin administration. Taken together, these findings indicate empagliflozin drug as a suitable approach is an add-on drug to metformin for treating diabetic patients with PWS, although the results of our single case report should be extrapolated with caution.

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Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Ethics Committee (Permit number 40430520.6.0000.5317).

Consent to participate and publication The volunteer and her guardian provided written informed consent about study participation and publication.

References

1. Heitink MV, Sinnema M, van Steensel MAM, Schrander-Stumpel CTRM, Frank J, Curfs LMG. Lymphedema in Prader-Willi syndrome. Int J Dermatol. 2008;47(1):42–4. https://doi.org/10.1111/j.1365-4632.2008.03959.x.
2. Manzardo AM, Heinemann J, McManus B, Loker C, Loker J, Butler MG. Venous Thromboembolism in Prader-Willi Syndrome: a questionnaire survey. Genes (Basel). 2019;10(7). https://doi.org/10.3390/genes10070550.

3. Sano H, Kudo E, Yamazaki T, Ito T, Hatakeyama K, Kawamura N. Efficacy of sodium-glucose cotransporter 2 inhibitor with glucagon-like peptide-1 receptor agonist for the glycemic control of a patient with Prader-Willi syndrome: a case report. Clin Pediatr Endocrinol. 2020;29(2):81–4. https://doi.org/10.1297/cpe.29.81.
4. Morales JS, Valenzuela PL, Pareja-Galeano H, Rincón-Castanedo C, Rubin DA, Lucia A. Physical exercise and Prader-Willi syndrome: a systematic review. Clin Endocrinol (Oxf). 2019;90(5):649–61. https://doi.org/10.1111/cen.13953.
5. Grada AA, Phillips TJ. Lymphedema: pathophysiology and clinical manifestations. J Am Acad Dermatol. 2017;77(6):1009–20. https://doi.org/10.1016/j.jaad.2017.03.022.
6. Laurier V, Lapeyrade A, Copet P, Demeer G, Silvie M, Bieth E, Coupaye M, Poitou C, Lorenzini F, Labrousse F, Molinas C, Tauber M, Thuilleaux D, Jauregi J. Medical, psychological and social features in a large cohort of adults with Prader-Willi syndrome: experience from a dedicated centre in France. J Intellect Disabil Res. 2015;59(5):411–21. https://doi.org/10.1111/jir.12140.
7. Partsch CJ, Lämmer C, Gillessen-Kaesbach G, Pankau R. Adult patients with Prader-Willi syndrome: clinical characteristics, life circumstances and growth hormone secretion. Growth Horm IGF Res. 2000;10(B):S81–5. https://doi.org/10.1016/s1096-6374(00)80015-5.
8. Kim YM, Lee YJ, Kim SY, Cheon CK, Lim HH. Successful rapid weight reduction and the use of liraglutide for morbid obesity in adolescent Prader-Willi syndrome. Ann Pediatr Endocrinol Metab. 2020;25(1):52–6. https://doi.org/10.6065/apem.2020.25.1.52.
9. Horikawa Y, Enya M, Komagata M, Hashimoto KI, Kagami M, Fukami M. Effectiveness of sodium-glucose cotransporter-2 inhibitor as an add-on drug to GLP-1 receptor agonists for glycemic control of a patient with Prader-Willi syndrome: a case report. Diabetes Ther. 2018;9(1):421–6. https://doi.org/10.1007/s13300-018-0369-5.
10. Neeland IJ, Eliasson B, Kasai T, Marx M, Zinman B, Inzucchi SE, et al. The impact of empagliflozin on obstructive sleep apnea, cardiovascular, and renal outcomes: an exploratory analysis of the EMPA-REG OUTCOME trial. Diabetes Care. 2020;43:3007–15. https://doi.org/10.2337/dc20-1096.

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LETTER TO THE EDITOR

Arterial systolic and diastolic blood pressure are not associated with HbA1c levels but arterial systolic blood pressure alone associated with 1-h postchallenge glucose levels

Koji Kashima[1] · Kazuya Okada[2] · Junichi Okada[3] · Kihachi Ohshima[4] · Shuichi Okada[4]

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To the Editor:

In a previous cross-sectional study involving subjects with normal glucose tolerance (NGT), we reported a positive correlation between brachial–ankle pulse wave velocity (baPWV; a measure of arterial stiffness) and 1-h postchallenge glucose (1-h PG) [1]. Moreover, the subpopulation of NGT subjects defined by 1-h PG ≥183 mg/dL (corresponding to impaired glucose tolerance) exhibited arterial stiffness, as evidenced by higher baPWV values, despite fasting plasma glucose (FPG) and 2-h PG levels being within the normal range [2]. NGT subjects who have converted to higher 1-h PG levels from a normal 1-h PG range will develop arterial stiffness [3]. As arterial stiffness is one of the risk factors for elevated arterial blood pressure [4], we hypothesized that not plasma glucose control but 1-h PG level correlates with arterial blood pressure.

To address this issue, we conducted a clinical study on 4051 subjects (3019 men, 1032 women; aged 58.8 ± 9.6 years) who underwent a comprehensive medical checkup at Kiryu Kosei General Hospital during 2008–2018. Study subjects stayed in our hospital on day 1 and were requested to remain in a fasted state overnight (for 12 h). After arterial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed, blood samples were drawn at 8:00 the next morning and 1 h and 2 h after oral administration of 75-g glucose as 75g OGTT on day 2. These individuals had NGT and normal range of arterial blood pressure. All statistical data were analyzed using the SPSS software (version 10.0, SPSS Inc., Chicago, IL, USA). All numerical values are expressed as means ± SD. Dunnett's test was used for the multiple comparisons of variables. Continuous variables were compared by group using the analysis of variance and Wilcoxon rank-sum test for nonnormally distributed data. To estimate the linear correlation between variables, we calculated Pearson's correlation coefficients. All tests for significance and the resulting p values were two-sided, with a level of significance set at 5%.

Mean values were as follows: body mass index (BMI) 23.2 ± 3.1 kg/m^2, SBP 122.9 ± 14.7 mmHg, DBP 76.1 ± 9.7 mmHg, total cholesterol (T-Chol) 201.4 ± 32.1 mg/dL, and triglycerides (TG) 120.2 ± 75.5 mg/dL. The FPG level was 101.6 ± 21.3 mg/dL, 1-h PG level was 152.5 ± 47.4 mg/dL, 2-h PG level was 123.8 ± 36.9 mg/dL, and glycated hemoglobin (HbA1c) level was 5.7% ± 0.65%.

Table 1 shows that SBP did not correlate with FPG and HbA1c levels but weakly correlated with 1- and 2-h PG levels. However, DBP did not correlate with FPG, HbA1c, and 1-h PG levels but weakly correlated with 2-h PG levels. On the other hand, sex, BMI, T-Chol, and TG did not correlate with FPG, 1-h PG, 2-h PG, and HbA1c. Table 2 shows the regression coefficients of the univariate linear regression analysis between SBP and 1-h PG (r = 0.230) and 2-h PG (r = 0.230), respectively, and between DPB and 2-h PG (r = 0.200). Figure 1 illustrates the regression coefficients of the univariate linear regression analysis between SBP and 1-h PG and 2-h PG, respectively. Figure 2 illustrated the regression coefficients of the univariate linear regression analysis between DBP and 2-h PG.

These results indicated that plasma glucose control does not correlate with arterial blood pressure. Although SBP and DBP weakly associated with 2-h PG levels, only SBP weakly correlated with 1-h PG levels. Taken together, among subjects

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Table 1 Analysis of multiple comparisons for factors affecting FPG, 1-h PG, 2-h PG, and HbA1c. SBP and DBP are independently associated with 2-h PG levels, and only SBP is independently correlated with 1-h PG levels. *BMI* body mass index, *T-Chol* total cholesterol, *TG* triglyceride, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *1-h PG* 60-min (1-h) postchallenge plasma glucose, *2-h PG* 120-min (2-h) postchallenge plasma glucose, *HbA1c* glycated hemoglobin, *CI* confidence interval

Multivariable regression analysis between FPG level and the associated variables

	β	95% CI	*p* value
SEX	0.327	-6.320 ~ -5.670	0.915
BMI	4.044	-0.518 ~ -8.606	0.082
T-Chol	4.044	-0.518 ~ 8.606	0.082
TG	-0.540	-2.532 ~ 1.452	0.595
SBP	4.162	-0.340 ~ 2.460	0.519
DBP	0.421	-0.540 ~ 4.270	0.424

Multivariable regression analysis between 2-h PG level and the associated variables

	β	95% CI	*p* value
SEX	0.060	-0.130 ~ 0.250	0.538
BMI	3.517	-0.950 ~ -0.990	0.330
T-Chol	0.017	-0.970 ~ -1.070	0.515
TG	-0.021	-0.850 ~ -1.130	0.776
SBP	-2.750	0.010 ~ 0.350	0.001
DBP	3.517	2.820 ~ 401.89	0.005

Multivariable regression analysis between 1-h PG level and the associated variables

	β	95% CI	*p* value
SEX	-0.025	-0.130 ~ 0.080	0.652
BMI	-1.983	-0.360 ~ -2.700	0.313
T-Chol	1.067	-0.900 ~ -1.140	0.414
TG	2.380	0.980 ~ -1.020	0.322
SBP	-4.536	-0.330 ~ 2.640	0.020
DBP	-5.548	-0.015 – 0.021	0.582

Multivariable regression analysis between HbA1c level and the associated variables

	β	95% CI	*p* value
SEX	-1.429	-2.530 ~ 0.330	0.455
BMI	0.421	-0.540 ~ -4.270	0.424
T-Chol	-0.068	-0.660 ~ -1.330	-0.068
TG	0.009	-0.900 ~ -1.140	0.886
SBP	-0.025	-0.130 ~ 0.080	0.652
DBP	-0.097	-0.340 ~ 2.460	0.849

Table 2 Multiple regression analysis of the relationship between SBP and 1-h PG, between SBP and 2-h PG, and between DPB and 2-h PG. SBP and DBP weakly associated with 2-h PG levels. SBP weakly correlated with 1-h PG levels. *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *CI* confidence interval

Correlation between SBP and 1-h PG level

	r	95% CI
SBP	0.230	-0.061 ~ 0.080

Correlation between SBP and 2-h PG level

	r	95% CI
SBP	0.230	0.082 ~ 0.107
DBP	0.200	0.045 ~ 0.062

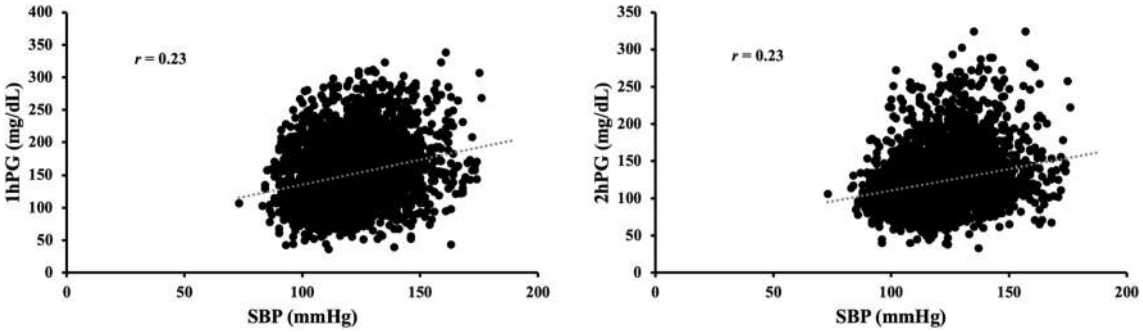

Fig. 1 The analysis of correlation between systolic blood pressure (SBP) and 1-h PG and between SBP and 2-h PG levels. The analysis of correlation between SBP and 1-h PG, and 2-h PG levels is presented. The left figure represents the correlation between SBP and 1-h PG. The right figure represents the correlation between SBP and 2-h PG. 1-h PG: 60-min (1-h) postchallenge plasma glucose. 2-h PG: 120-min (2-h) postchallenge plasma glucose

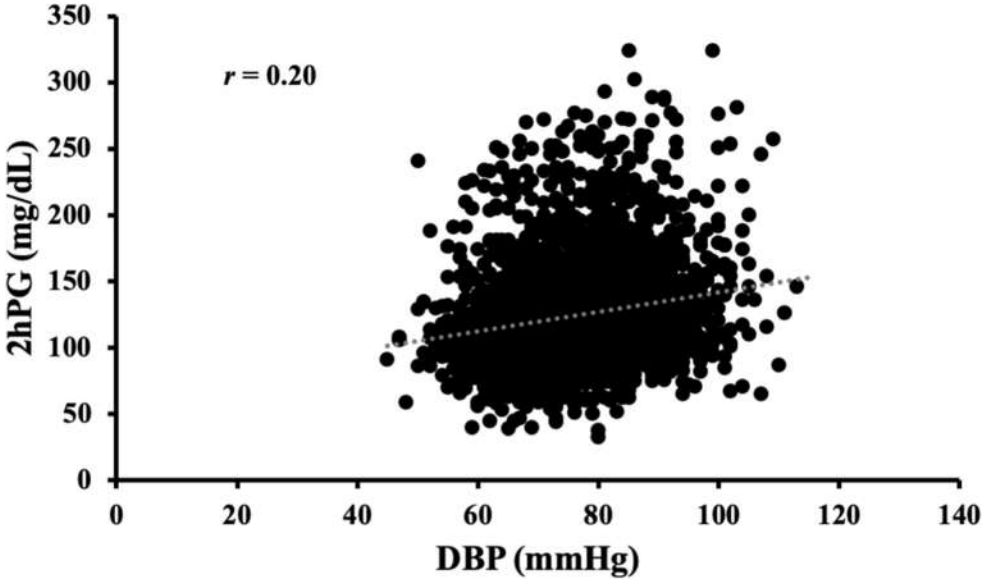

Fig. 2 The analysis of correlation between diastolic blood pressure (DBP) and 2-h PG levels. The analysis of correlation between DBP and 2-h PG levels is presented. 2-h PG: 120-min (2-h) postchallenge plasma glucose

with NGT and normal range of arterial blood pressure, not DBP but SBP weakly associated with 1-h PG levels.

Declarations

Ethics approval and consent to participate The study protocol was reviewed and approved by the review board of Kiryu Kosei General Hospital, and the study was conducted following the guidelines of the Declaration of Helsinki. Subjects provided written informed consent to participate in this clinical study every year.

Conflict of interest The authors declare no competing interests.

References

1. Niijima K, Muranaka Y, Ando T, Okada S, Niijima Y, Hashimoto K, Yamada M, Ohshima K, Mori M, Ono K. Elevated 1-h plasma glucose following 75-g oral glucose load is a predictor of arterial stiffness in subjects with normal glucose tolerance. Diabet Med. 2012;29:e457–60. https://doi.org/10.1111/dme.12026.
2. Ando T, Okada S, Niijima Y, Hashimoto K, Shimizu H, Tsuchiya T, Yamada M, Ohshima K, Mori M, Ono K. Impaired glucose tolerance, but not impaired fasting glucose, is a risk factor for early-stage atherosclerosis. Diabet Med. 2010;27:1430–5. https://doi.org/10.1111/j.1464-5491.2010.03144.x.
3. Saito T, Niijima K, Yamada E, Niijima Y, Okada S, Yamada M. Elevated 1-h post-challenge glucose levels associated with increased arterial stiffness. J Diabetes. 2017;9:102–3. https://doi.org/10.1111/1753-0407.12458.
4. Laurent S, Boutouyrie P. Arterial stiffness and hypertension in the elderly. Front Cardiovasc Med. 2020;7:544302. https://doi.org/10.3389/fcvm.2020.544302 eCollection 2020.

VISION STATEMENT

To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

MISSION STATEMENT

1. Promotion of excellence in diabetes care to make India the Diabetes Care Capital
2. Empowerment of persons living with diabetes
3. Support for diabetes research
4. Dissemination of information and knowledge in diabetes care
5. Advocacy for the cause of diabetology

NEW EXECUTIVE COMMITTEE AND OFFICE BEARERS 2022-2023

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TRAINEE GRANTS (Up to 10 grants)

Research Grants upto INR 200000 to support outstanding thesis/ research work by first year MD/DNB/ PHD students/Research fellows from India.

Eligibility Criteria
All Postgraduates in First year MD, DM /DNB from any of the institutions in the country are eligible to apply

How to apply?
All proposals to be applied online on the RSSDI Website www.rssdi.in

Research proposal should have following proofs-

1. A supporting letter from your guide/ head of department stating that this is a bonafide project for your thesis and also mentioning the dates of you joining the program and expected date of graduation. The guide must also state that he/she will stand guarantee for the work done
2. A detailed budget
3. Thesis proposal approved by the department/appropriate institutional authority
4. Approval by the ethics committee

Selection Process
Proposals will be reviewed by the research committee of the RSSDI.

Disbursement of Grant
A minimum of 25% of the grant amount will be disbursed initially. Further disbursement will be done annually based on submission of progress reports on the work done and utilisation of sanctioned amount. These reports must be filed to the secretary of the RSSDI.

Responsibility:
All grant awardees are expected to present their work at RSSDI Annual Conference during research presentation's session. Failure to file progress reports annually and when requested by the RSSDI and failure to present progress at RSSDI Annual conf may result in the forfeiture of the grant.

All awardees are expected to follow the tenets of responsible and ethical conduct of research. Unethical or fraudulent use of RSSDI research funds will warrant adverse action from the society including

forfeiture of grant, black listing in the society's databases and other legal recourses that are available to the society.

Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

All awardees are encouraged to submit their work to the RSDDI Journal IJDDC

CALL for RESEARCH PROPOSALS for GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI

The proposals may of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization, of funding sought from RSSDI

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
All applications for Research Grants to be made online on RSSDI Website www.rssdi.in:

When to apply
Grant Proposals will be opened for submission online in the first month of every quarter starting from January.
(Jan, Apr, July, Oct)

All research proposals will be reviewed by Research committee over a period of 4-6 weeks & approved proposals will be provided Research Grant after fulfilling all documentation by end of month of every quarter (Mar, Jun, Sept, Dec)

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health-care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmachari Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh

22.	Galaxy Speciality Centre	Sodala, Jaipur
23.	SL Raheja Hospital	Mumbai, Maharashtra

carefully looked into all aspects of this course & has accredited & recognized 22 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

COURSE DETAILS

Name of the Course: Advanced Certificate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given !

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

Session: Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

ANNOUNCEMENTS

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

RSSDI 50th Golden Jubilee Year Celebrations
(look out for more details on our website)

RSSDI JNU certificate course in Diabetes:

Last date of submission of Application Form - 31st December, 2022
Screening Interview - 7th January 2023
Declaration of Exam Result - 10th January 2023
Last date of payment of course fee - 15th January 2023
Commencement of course - 16th January 2023
Prospectus release date - 1st December 2022

Registered with Registrar of News Papers, India vide no. RNI-43019/85

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