ISSN 0973-3930

Volume 42 | Issue 1 | January–March 2022

International Journal of Diabetes in Developing Countries

Official Publication of
Research Society for the
Study of Diabetes in India

Springer

International Journal of Diabetes in Developing Countries
Incorporating Diabetes Bulletin

Aims and Scope

International Journal of Diabetes in Developing Countries targets a readership consisting of clinicians, research workers, paramedical personnel, nutritionists and health care personnel working in the field of diabetes. Original research work and reviews of interest to the above group of readers is considered for publication in the journal.

The journal has a goal of serving as an important resource material in diabetes for its readers, mainly in the developing world.

Copyright Information

Subscription Information

International Journal of Diabetes in Developing Countries is published 4 times a year. Volume 42 (4 issues) of will be published in 2022.

ISSN 0973-3930 print version
ISSN 1998-3832 electronic version

For information on subscription rates please contact Springer Nature Customer Service Center: customerservice@springernature.com

The Americas (North, South, Central America and the Caribbean)
Springer Journal Fulfillment, 233 Spring Street, New York, NY, 10013-1578, USA
Tel. 800-SPRINGER (777-4643); 212-460-1500 (outside North America)

Outside the Americas
Springer Nature Customer Service Center GmbH
Tiergartenstr. 15, 69121 Heidelberg, Germany
Tel.: +49-6221-345-4303

Advertisements

E-mail contact: advertising@springer.com or anzeigen@springer.com (Germany)

Disclaimer

Journal Website

www.springer.com/13410
Electronic edition: link.springer.com/journal/13410

For the actual version of record please always check the online version of the publication.

Office of Publication

Springer Science+Business Media B.V., Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands

International Journal of Diabetes in Developing Countries

Volume 42 · Number 1 · January–March 2022

Further articles can be found at www.springerlink.com

Abstracted/Indexed in *CAB Abstracts, CLOCKSS, CNKI, CNPIEC, Chemical Abstracts Service (CAS), Dimensions, EBSCO Academic Search, EBSCO CINAHL, EBSCO Discovery Service, EBSCO STM Source, EMBASE, EMCare, Google Scholar, IFIS Publishing, Japanese Science and Technology Agency (JST), Journal Citation Reports/Science Edition, Naver, OCLC WorldCat Discovery Service, Portico, ProQuest-ExLibris Primo, ProQuest-ExLibris Summon, SCImago, SCOPUS, Science Citation Index Expanded (SCIE), Semantic Scholar, TD Net Discovery Service, UGC-CARE List (India)*

Instructions for Authors for *Int J Diabetes Dev Ctries* are available at www.springer.com/13410.

Compliance with Ethical Requirements

International journal of Diabetes in Developing Countries requests that all authors comply with Springer's ethical policies. To view our ethics statements please visit the following:

· Conflict of Interest and Ethical Standards: http://www.springer.com/authors?SGWID=0-111-6-791531-0

· Informed Consent: http://www.springer.com/authors?SGWID=0-111-6-608209-0

Statement of Human and Animal Rights: http://www.springer.com/authors?SGWID=0-111-6-608309-0

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):1–2
https://doi.org/10.1007/s13410-022-01065-4

EDITORIAL

MicroRNAs in diabetes mellitus—genetic tools that could transform clinical practice?

S. V. Madhu[1]

Published online: 1 April 2022

MicroRNAs (miRNAs) are a class of small noncoding RNAs which are involved in the regulation of glucose metabolism. They have not only enhanced our understanding of the pathogenesis of type 2 diabetes mellitus (T2DM) but are also emerging as potential novel biomarkers for T2DM and its complications.

miRNAs consist of 18–25 nucleotides only and regulate gene expression of various genes involved in various cellular processes including cell differentiation, apoptosis, and metabolism all of which are critical to the understanding to the pathogenesis of T2DM. miRNAs suppress gene expression in response to different pathophysiological stimuli and thereby constitute a key component of the epigenetic regulation network [1]. miRNAs are relatively stable and are distributed widely in various tissues. They are present mostly intracellularly but are also found in the extracellular space in several body fluids including blood from where they can be extracted and detected by modern techniques. miRNAs are particularly abundant in platelets and hence appear to be intricately involved in diabetes-related vascular complications also. Circulating miRNAs have therefore shown a lot of promise in serving as important biomarkers with diagnostic, prognostic, as well as potential therapeutic significance in T2DM [1].

Several cross-sectional studies have reported an association of miRNAs particularly miR-126 and to some extent miR-375 with glucose dysmetabolism. While a few studies found suppressed miR-126 in T2DM subjects [1, 2] others reported similar suppression in prediabetic subjects also when compared with those with normal glucose tolerance. Prospective studies further confirmed that supressed miR-126 was associated with incident T2DM and also that in prediabetic subjects these levels improve with diet and exercise interventions [3]. miRNAs could therefore be important biomarkers that not only help us understand the pathophysiological processes involved in the development of T2DM but also help in identifying individuals at risk of T2DM and in monitoring the impact of diabetes prevention strategies from time to time. They could become a critical tool in predicting the outcomes of lifestyle or pharmacological strategies in the prevention of T2DM.

Profiling of miRNAs in patients with both type 1 DM and type 2 DM has shown that expression of several miRNAs is altered in both these conditions. About 11 miRNAs have been shown to be dysregulated in T1DM and nearly 40 in T2DM [4]. Measuring miRNAs should therefore serve as important biomarkers to not only identify but also track the progression of diabetes mellitus. Islet-specific miRNAs have been shown to regulate islet cell mass and islet cell function and any alteration in its expression can indicate abnormalities of beta cell mass and insulin secretion [4]. More specifically, these miRNAs regulate beta cell proliferation and apoptosis as well as insulin production and secretion and hence any aberrant islet miRNA expression can result in hyperglycemia and progression of the diabetic state [4]. miRNAs therefore are also potential therapeutic targets which can help reverse beta cell dysfunction and the consequent hyperglycemia. Reversal of type 2 diabetes could also be possible through appropriate and specific targeting of miRNAs.

Another important area where miRNAs appear to be playing a key role and are showing lot of promise is in the area of long-term diabetic complications. MicroRNAs are believed to be interacting closely with epigenetic phenomena and other predisposing risk factors which influence the pathogenesis of diabetic vascular complications. The effects of long-term hyperglycemia on blood vessels appear to be mediated or modified by miRNAs. More specifically, altered expression of miRNAs has been shown to promote inflammation, apoptosis, fibrosis, and angiogenesis all of which are known to be associated with microvascular complications of diabetes. The miRNA

✉ S. V. Madhu
drsvmadhu@gmail.com

[1] Department of Endocrinology, Center for Diabetes, Endocrinology and Metabolism, University College of Medical Sciences, Delhi, India

expression signatures that are associated with the development of diabetic complications can also serve as useful early biomarkers for future complications [5–7].

Based on circulating miRNA profiling in serum samples in humans in several studies, a number of miRNAs have been implicated in the pathogenesis of diabetes as well as diabetes microvascular complications. Some of the important ones are miR-126, miR-21, miR-181, and miR-1179 while others have also been reported to have a potential role [5]. Specific circulating miRNAs have been evaluated as biomarkers for diabetic complications such as retinopathy, diabetic kidney disease, and macrovascular disease. However, most of these studies have used nondiabetic controls, and hence some of the differences in miRNA expression may be due to diabetes itself. Also, miRNA profiling studies have been limited by issues of reproducibility due to potential confounders [6].

MicroRNAs appear to be involved in neovascularization in diabetic microvascular complications. miRNA-126, which plays a key role in angiogenesis and vascular function, has been shown to be downregulated in the retina in experimental diabetes and other hypoxia-induced retinopathy. This is also associated with retinal VEGF levels and treatment with agents to restore retinal miRNA levels resulted in lower VEGF levels and consequent edema, hemorrhages, and apoptosis. Similarly, miRNA-106a leads to reduced VEGF expression with beneficial effects on retinal permeability. A number of other microRNAs, viz. miR-200b, miR-15, miR-150, miR-184, and miR-155, have all been shown to regulate VEGF signalling with potential benefits for DR. These studies point to miRNAs as novel targets for treatment of DR particularly proliferative DR [5, 7].

Many microRNAs have been shown to regulate inflammation, the most prominent of which are miR-146, miR-21, and miR-29 in the context of diabetes complications. Administration of miR-146a has demonstrated anti-inflammatory effects and have been shown to protect against diabetic retinopathy, diabetic peripheral neuropathy, as well as diabetic nephropathy [5].Similarly, several miRs have been found useful in preventing apoptosis. These include miR-155, mir-29, and miR-93, all of which have been linked to epigenetic modifications of podocytes and thereby to diabetic complications particularly nephropathy. Over- or underexpression of miR-216, miR-377, and miR-29 is associated with increased fibrosis and pathological changes seen in diabetic nephropathy in animal models [5]. microRNAs are also believed to be associated with the oxidative stress in diabetes and have been targeted to reduce complications by reducing oxidative stress [8].

In the current issue, Monjezi et al. [9] have evaluated miR-124-3p as a biomarker for diabetic nephropathy besides other markers. This microRNA has not been studied much in humans and these workers studied the expression of miR-124-3p in the peripheral blood mononuclear cells (PBMCs) of diabetic patients with and without nephropathy. They report a 10 fold decrease in expression of miR-124-3p in PBMCs of patients with diabetic nephropathy compared with those without nephropathy. miR-124-3p is a tumor suppressor and is known to be downregulated in several cancers. It is believed to play a role in cell proliferation and apoptosis. If replicated in larger cohorts, miR-124-3p can be a novel biomarker for diabetic nephropathy complementing a number of other miRNAs being evaluated for diabetic nephropathy.

The mechanisms of miRNA regulation and dysregulation of gene expression are highly complex and are yet to be fully understood. Current knowledge based on small studies needs to be supplemented by larger, well-validated studies before we can define their precise role in development of diabetes cardiorenal as well as cardiovascular complications with certainty. Till then we should refrain from overestimating their true potential and should interpret current literature with cautious optimism.

It would therefore be prudent to conclude that miRNA profiling is an emerging area of diabetes research that has immense potential to transform the diagnostic, prognostic, and therapeutic approach in patients with diabetes and diabetic complications. However, large, well-designed prospective studies in the future are needed to translate this potential into clinical use.

References

1. Pordzik J, Jakubik D, Jarosz-Popek J, Wicik Z, Eyileten C, De Rosa S, et al. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review. Cardiovasc Diabetol. 2018;18(1):113. https://doi.org/10.1186/s12933-019-0918-x.
2. Zhang T, Lv C, Li L, Chen S, Liu S, Wang C, et al. Plasma miR-126 is a potential biomarker for early prediction of type 2 diabetes mellitus in susceptible individuals. Biomed Res Int. 2013;2013:761617–6.
3. Liu Y, Gao G, Yang C, Zhou K, Shen B, Liang H, et al. The role of circulating microRNA-126 (miR-126): a novel biomarker for screening prediabetes and newly diagnosed type 2 diabetes mellitus. Int J Mol Sci. 2014;15(6):10567–77.
4. Kim M, Zhang X. The profiling and role of miRNAs in diabetes mellitus. J Diabetes Clin Res. 2019;1(1):5–23. https://doi.org/10.33696/diabetes.1.003.
5. Barutta F, Bellini S, Mastrocola R, Bruno G, Gruden G. MicroRNA and microvascular complications of diabetes. Int J Endocrinol. 2018. https://doi.org/10.1155/2018/6890501.
6. Fan B, On Yan Luk A, Chung Ngor Chan J, Ching Wan Ma R. MicroRNA and diabetic complications: a clinical perspective. Antioxidants Redox Signaling. 2018;29(11):1041–63.
7. Smit-McBride Z, Morse LS. MicroRNA and diabetic retinopathy—biomarkers and novel therapeutics. Transl Med. 2021;9(15):1280.
8. Grieco GE, Brusco N, Licata G, Nigi L, Formichi C, Dotta F, et al. Targeting microRNAs as a therapeutic strategy to reduce oxidative stress in diabetes. Int J Mole Sci. 2019;20(24):6358. https://doi.org/10.3390/ijms20246358.
9. Monjezi A, Khedri A, Zakerkish M, Mohammadzadeh G. Resistin, TNF-α, and microRNA 124-3p expressions in peripheral blood mononuclear cells are associated with diabetic nephropathy. Int J Diabetes Dev Countries. 2022. https://doi.org/10.1007/s13410-021-00966-0. (in current issue).

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):3–28
https://doi.org/10.1007/s13410-022-01063-6

GUIDELINES

RSSDI consensus recommendations for dyslipidemia management in diabetes mellitus

Banshi Saboo[1] · Sanjay Agarwal[2,3,4] · Brij Mohan Makkar[5] · Rajeev Chawla[6] · Sujoy Ghosh[7] · Vijay Viswanathan[8,9,10] · Sunil Gupta[11] · Ch. Vasanth Kumar[12] · Anuj Maheshwari[13] · L. Sreenivasamurthy[14,15] · Rakesh Kumar Sahay[16] · Sanjay Reddy[17] · Shalini Jaggi[18] · Jugal Kishor Sharma[19] · Vijay Panikar[20] · Anand Moses[21] · Bikash Bhattacharjee[22,23] · Pratap Jethwani[24] · Sudhir Bhandari[25] · J. P. S. Sawhney[26] · Shashank Joshi[27] · Saumitra Ray[28] · S. V. Madhu[29] · Bipin Sethi[30]

Published online: 7 April 2022

Abstract

Diabetic dyslipidemia is characterised by low HDL-C and high triglyceride levels. Unlike the Caucasian population, though LDL-C levels are not very high, there is a preponderance of more atherogenic small, dense LDL particles among Indians. Furthermore, apo B levels are elevated. This, unique 'atherogenic dyslipidemia', is frequently encountered in South Asians with diabetes. People with type 2 diabetes are considered to be at high risk for vascular events. Hence, irrespective of other risk factors such as age, male gender, hypertension, family history, smoking, obesity, and polycystic ovary syndrome in women, they must be screened for dyslipidemia.

✉ Sanjay Agarwal
agarwalclinic@gmail.com

Vijay Viswanathan
https://www.mvdiabetes.com; https://www.d-foot.org

J. P. S. Sawhney
jpssawhney@yahoo.com

[1] Diabetes Care & Hormone Clinic, Dia-Care, Ahmedabad, India

[2] Aegle Clinic-Diabetes Care, Pune, India

[3] Department of Medicine and Diabetes, Ruby Hall Clinic, Pune, India

[4] Diabetes & Medicine, Jehangir Hospital, Pune, India

[5] Dr Makkar's Diabetes & Obesity Centre, A-5B/122, Paschim Vihar, New Delhi 110063, India

[6] North Delhi Diabetes Centre, Hony Prof Jaipur National University, 180 Jai Apartment Sec-9 Rohini, New Delhi 110085, India

[7] Department of Endocrinology, Institute of Post Graduate Medical Education and Research Kolkata, Room 9B, 4th floor, Ronald Ross Building, 244 A J C Bose Road, Kolkata 700020, India

[8] M.V. Hospital for Diabetes, Royapuram, Chennai, India

[9] Prof. M. Viswanathan Diabetes Research Centre, (WHO Collaborating Centre for Research, Education and Training in Diabetes), No.4, West Mada Church Street, Royapuram, Chennai, Tamilnadu 600 013, India

[10] D foot International, New Delhi, India

[11] Sunil's Diabetes Care n' Research Centre Pvt. Ltd, 42, Lendra Park, Ramdaspeth, Nagpur, Maharashtra 440010, India

[12] Apollo Hospitals, Hyderabad, India

[13] Department of Medicine, BBD University, Lucknow, India

[14] Life Care Hospital and Research Centre, Bangalore, India

[15] Dr B R Ambedkar Medical College, Bangalore, India

[16] Department of Endocrinology, Osmania Medical College, Hyderabad, India

[17] Center for Diabetes & Endocrine Care, Fortis Hospital, Cunningham Road, Bangalore, India

[18] Lifecare Diabetes Centre, New Delhi, India

[19] FRSSDI, New Delhi, India

[20] Department of Endocrinology and Diabetes, Lilavati Hospital, Mumbai, India

[21] Institute of Diabetology, Madras Medical College, Chennai, 34, Casa Major Road, Egmore, Chennai 600008, India

[22] Sun Valley Diabetes Care & Research Centre, G. S. Road, Guwahati, Assam 781006, India

[23] Diabetes & Obesity Centre, Ulubari, Guwahati 781007, India

[24] Jethwani Hospital, Rajkot, India

[25] S.M.S. Medical College & Hospital, Jaipur, India

[26] Department of Cardiology, Dharma Vira Heart Center, Sir Ganga Ram Hospital, New Delhi 110060, India

[27] Lilavati Hospital and Joshi Clinic, Mumbai, India

[28] Invasive Cardiology, AMRI (S), Kolkata, India

[29] Department of Endocrinology, Centre for Diabetes, Endocrinology & Metabolism, UCMS-GTB Hospital, Delhi, India

[30] Care Hospital, Banjara Hills, Hyderabad, India

Other major ASCVD risk factors include family history of hyperlipidemia, low levels of HDL-C, hypertriglyceridemia, and increased levels of total serum cholesterol level, non-HDL-C, LDL-C, apo B, Lp(a), triglyceride-rich remnants, and small, dense LDL-C. In patients with diabetes, dyslipidemia should be assessed at diagnosis and annually thereafter. In patients with type 1 diabetes, screening for dyslipidemia should be initiated from the age of 12 years. Periodical screening for dyslipidemia is recommended in overweight or obese children with a family history of type 2 diabetes, or those from a predisposed race/ethnicity like Asian, American Indian, etc. Both fasting and non-fasting lipid profiles are important for managing Indian patients with dyslipidemia. For routine screening, a fasting lipid profile is not mandatory; the decision to acquire fasting or non-fasting lipid values must be individually tailored. Apolipoprotein B level is considered an enhanced estimate of an individual's exposure to atherosclerotic lipoproteins, and may be predominantly valuable for assessment of risk in individuals where LDL-C measurement underestimates this burden (those with diabetes mellitus, high triglycerides, obesity, or low LDL-C). The QRISK3 assessment tool algorithm calculates an individual's risk of developing a heart attack or stroke over 10 years, and takes into account ethnicity as a risk factor. Considering the possible genetic influence of Indian ethnicity on CVD, the QRISK3 score exemplifies as the current most accurate CVD screening tool available for the Indian population.

Stratification of ASCVD risk in Indian diabetic patients:

- *High risk:* diabetes with 0–1 other major ASCVD risk factors and no evidence of target organ damage.
- *Very high risk:* diabetes with ≥2 other major ASCVD risk factors or evidence of target organ damage.

High-risk patients necessitate management comparable to that for secondary prevention of CVD. The most important step in defining treatment goals for dyslipidemia in diabetic patients is an extensive assessment of their cardiovascular risk, with LDL-C as the primary target, and non HDL-C, HDL-C, and apo B as secondary targets. A comprehensive strategy is essential in the management of dyslipidemia so as to regulate lipid levels and tackle related metabolic deviations and modifiable risk factors.

Essential considerations to improve lipid profile and glycemic control, and reduce CVD risk:

- Accomplish healthy weight and aerobic activity level,
- Implement an energy-restricted, well-balanced diet,
- No or at most moderate alcohol consumption, and
- Smoking (or any other tobacco use) cessation.

Medical nutrition therapy plays a central part in diabetes management; every individual with diabetes must be actively engaged in self-management, education, and treatment planning with their healthcare team, together with the collective development of an individualised eating plan. Statins are beneficial as a primary or secondary prevention strategy, to reduce the risk of cardiovascular events, in patients with ASCVD or multiple cardiovascular risk factors especially in those with diabetes. Unless contraindicated, first-line cholesterol-lowering therapy includes the use of moderate- to high-intensity statin. Ezetimibe, when combined with statins, provides additive and complementary therapeutic lipid effects, resulting in considerable reductions in LDL-C and significant achievement of target cholesterol levels. It also permits the use of lower dosage of statins without compromising efficacy, reducing the odds of dose-dependent statin adverse effects. Bempedoic acid seems to provide a safe and effective oral therapeutic option for lipid lowering in patients intolerant to statins. PCSK9 inhibitor therapy, in diabetes, induces analogous relative reductions in cardiovascular risk, and is recommended to further reduce LDL-C in patients aged 40–79 years with LDL-C ≥190 mg/dL, with ASCVD risk factors, or other significant additional-high risk markers (including diabetes) and LDL-C ≥100 mg/dL or non-HDL-C ≥130 mg/dL on maximally tolerated statin therapy and/or ezetimibe. Fenofibrate has shown to reduce CVD in diabetic patients with elevated triglycerides and low HDL-C levels. Saroglitazar has well-documented positive effects in the management of diabetic dyslipidemia; not only does it improve lipid parameters (triglycerides, apo B, non-HDL-C), it has a significant impact on glycemic parameters (HbA1c and fasting blood glucose) in dyslipidemic patients. It, hence, appears as a novel therapy for decreasing cardiovascular risk in patients with type 2 diabetes. Omega-3 fatty acids offer additional benefits when administered as an add-on to statins, and could be attributed to the lowering of detrimental chronic inflammatory markers in people with diabetes and high-risk cardiovascular patients. Icosapent ethyl may provide additional risk reduction benefit, beyond a statin, in individuals with ASCVD or diabetes and multiple risk factors and triglyceride ≥150 mg/dL. Considering the evidence in patients with diabetic dyslipidemia combined with the experience and consensus of the experts, we recommend a step-wise approach for the management for diabetic dyslipidemia in the Indian population (Table 7).

Keywords Atherogenic dyslipidemia · Diabetes · Diabetic dyslipidemia · Consensus guideline

Introduction

Dyslipidemia plays a key role in inducing cardiovascular disease (CVD) in persons with type 1 and type 2 diabetes mellitus. The lipid profile in type 1 diabetics with good glycemic control is quite identical to that of the general population. Contrastingly, even with good glycemic control in type 2 diabetes, lipid abnormalities (elevated small dense low-

density lipoprotein cholesterol (LDL), decreased high-density lipoprotein cholesterol (HDL-C), and an increase in triglycerides and non-HDL-C) are frequently noted. Poor glycemic control in type 1 and type 2 diabetes decreases HDL-C levels and increases triglyceride levels with merely meek effects on LDL-C levels [1, 2]. Analogous to global evidence, among Indian patients, the alteration in HDL/LDL ratio in type 2 diabetes is strongly associated with lower HDL and higher LDL levels [3, 4]. In both type 1 as well as type 2 diabetes, an association between atherosclerotic cardiovascular disease (ASCVD) and serum cholesterol and triglyceride levels are commonly encountered. There is an increased risk of coronary heart disease (CHD) at any given level of serum cholesterol in diabetic patients, with an even stronger association with hypertriglyceridemia compared to the general population.

Individuals with type 1 diabetes develop atherosclerosis earlier and with rapid progression, thereby experiencing higher premature mortality as a result of vascular disease, despite higher levels of HDL-C; they seldom exhibit insulin resistance. The exogenous insulin therapy in patients with type 1 diabetes increases the activity of lipoprotein lipase in the skeletal muscle and adipose tissue, which catabolises very low-density lipoprotein cholesterol (VLDL-C), and reduces LDL-C and triglycerides [5]. Dyslipidemia of type 2 diabetes is characterised by low HDL-C and high triglyceride levels. Low HDL-C has been found to be an independent contributor to the development of cardiovascular disease as well as diabetes. There could be a modest surge in LDL-C with poor glycemic control, usually in the small dense LDL sub-fraction, on account of the rise in triglyceride levels [1]. Collectively, these changes could result in accelerated atherosclerosis even before the formal diagnosis of diabetes [6]. In fact, adequate glycemic control in patients with diabetes mellitus has found to aid in a significant decline in triglyceride levels [7]. Hence, optimising glycemic control in patients with diabetes is essential since this will have additional beneficial effects on lipid levels.

Epidemiology

Prevalence of diabetes mellitus

The global prevalence of type 2 diabetes has been growing at an exponential rate [8]. Type 2 diabetes is the most common type of diabetes and accounts for nearly 90% of all diabetes. Almost 463 million adults in 2019 were living with diabetes; by 2045 this number is expected to escalate to 700 million [9]. Latest data from the World Bank demonstrates a global diabetes prevalence of 8.8% in the 20–79 years age group with either type 1 or type 2 diabetes [10]. Recent statistics from the International Diabetes Federation report a prevalence of 8.9% of diabetes in Indian adults [11]. In India, the number of patients with diabetes increased from 26.0 million (95% uncertainty interval [UI], 23.4–28.6) to 65.0 million (95% UI, 58.7–71.1) between 1990 and 2016, with an increase in the prevalence of diabetes in adults aged ≥20 years from 5.5% (95% UI, 4.9–6.1) to 7.7% (95% UI, 6.9–8.4) during the period [12].

Prevalence of dyslipidemia in diabetes mellitus

In 2010, a cross-sectional retrospective analysis of 788 patients with type 2 diabetes in India recorded a prevalence of 85.5% of dyslipidemia among males, and an even higher prevalence of 97.8% among females. Mixed dyslipidemia, defined by high triglycerides, high LDL, and low HDL, was prevalent in 12.1% males and 24.0% females. Combined dyslipidemia was noted in 8.8% males and 9.3% females with high triglycerides and low HDL, 10.2% males and 5.2% females with high triglycerides and high LDL, and 19.4% males and 32.2% females with high LDL and low HDL, whereas isolated single parameter dyslipidemia was detected in 6.4% males and 1.4% females with high triglycerides, 18.2% males and 12.6% females with high LDL, and 10.4% males and 13.1% females with low HDL [13]. The large-scale Indian Council of Medical Research–India Diabetes (ICMR–INDIAB) Study conducted in 16,607 adults revealed increased risks of hypercholesterolemia (OR, 2.47; 95% CI, 1.88–3.24; $p<0.001$), hypertriglyceridemia (OR, 3.41; 95% CI, 2.73–4.26; $p<0.001$), low HDL-C (OR, 1.78; 95% CI, 1.37–2.32; $p<0.001$), and high LDL-C (OR, 2.39; 95% CI, 1.79–3.20; $p<0.001$) in those with dysglycemia [14].

The epidemiological cross-sectional study, SOLID, revealed a prevalence of 48.74% in the control of LDL-C in the Indian diabetic population treated with lipid-lowering drugs [15]. Another recent cross-sectional study by Dayakar et al. reported the incidence of occurrence of hypercholesterolemia to be 58.6% and that of hypertriglyceridemia to be 36.9% in 46 adult patients with type 2 diabetes in a tertiary care centre in Southern India [16].

Bulut et al. found the prevalence of dyslipidemia to be 26.2% in 202 children and adolescents with type 1 diabetes, of which hypercholesterolemia (15.8%) and hyperglyceridemia (12.9%) were the most common. Among those with dyslipidemia, factors such as age, body mass index, glycated hemoglobin (HbA1c), and poor metabolic control were significantly higher. Smoking rate was found to be nearly 14% in those belonging to the pubertal group, with significantly higher dyslipidemia and poor metabolic control ($p<0.05$) [17]. A recent cross-sectional study in underprivileged children and youth in India with poorly controlled type 1 diabetes demonstrated a prevalence of 47.2% of dyslipidemia, with an abnormal lipid profile in 11.9% children below the age of 10 years. High LDL (>2.6 mmol/L; 34.9%) was the most commonly observed lipid abnormality, and was followed by hypercholesterolemia (>5.2 mmol/L; 12.3%), abnormal HDL (<1.1 mmol/L; 12.3%), and hypertriglyceridemia

(>1.5mmol/L in children >10 years and 1.1–1.5 mmol/L in children <10 years; 10.6%) [18].

Recent data from the National Family Health Survey (NFHS-4)/Demographic Health Survey 2015–2016 (a cross-sectional survey of all 29 states and 7 union territories of India) estimated the prevalence of undiagnosed diabetes in India for 750,924 persons aged 15–50 years. They identified 42% of their population with diabetes were 'undiagnosed', with poor detection rates. About 45% of undiagnosed diabetes individuals had access to healthcare. The researchers have recommended combining access to healthcare with routine and rapid low-cost, opportunistic screening of individuals for high glucose levels [19]. The high prevalence of undiagnosed diabetes increases the risk of macro- and microvascular complications of diabetes on account of the poor glycemic control.

There is very little evidence pertaining to the epidemiology of undiagnosed dyslipidemia even in the general population, let alone in patients with diabetes mellitus. A population-based, epidemiological cross-sectional study (part of the Kerman coronary artery disease risk study [KERCADRS]) assessed 5899 (aged 15–75 years) residents of the largest city in southeast of Iran. They reported the prevalence of undiagnosed dyslipidemia as 16.8%, while that of diagnosed dyslipidemia as 13.2%. The overall prevalence of undiagnosed dyslipidemia was found to be higher and significantly influenced by advanced age, obesity, anxiety, and family history of dyslipidemia [20]. On the other hand, lipid profiles (LDL-C) and lipid ratios (LDL-C/HDL-C and TC/HDL-C ratio) have been shown to be potential markers that can perhaps be used to predict glycemic control in patients with type 2 diabetes [21].

Dyslipidemic states

Primary dyslipidemias can take place independent of type 2 diabetes or metabolic syndrome, on account of single or multiple gene mutations resulting in abnormal serum lipid levels [22]. *Hypertriglyceridemia* is generally defined as fasting serum triglyceride levels of ≥150 mg/dL (1.7 mmol/L), despite the 'optimal' <100 mg/dL fasting triglyceride concentration that confers minimal risk of incident as well as recurrent ASCVD [23]. Even though the prevalence of *hypercholesterolemia* does not rise in diabetes mellitus, mortality from coronary heart disease (CHD) intensifies exponentially as a function of serum cholesterol; lowering of cholesterol levels with statins in diabetic patients' moderates the relative cardiovascular risk [24].

Mixed dyslipidemia is a state with increased levels of LDL cholesterol and triglycerides and reduced HDL cholesterol levels; this state is often encountered in individuals with diabetes and metabolic syndrome [25]. *Dysbetalipoproteinemia*, an unusual familial dyslipidemia, is characterised by nearly similarly raised triglyceride and serum cholesterol levels as a result of accrued remnant lipoproteins in apolipoprotein E2/E2 homozygotes. This condition has been associated with a higher risk for premature CVD. Diagnosis of this dyslipidemic state should be considered either in those with mixed dyslipidemia with a relatively low concentration of apolipoprotein B (apo B) compared to the total cholesterol concentration or in cases of substantial disparity between calculated LDL and direct LDL cholesterol concentrations [26].

Familial hypercholesterolemia (FH), a genetic disorder of lipoprotein metabolism, is a dyslipidemic state with an eminent surge in plasma total-cholesterol levels with detrimental cardiovascular consequences that embark in childhood. It epitomises the phenotypic manifestation of abnormal lipoprotein metabolism triggered by an assortment of genetic abnormalities [27]. *Familial combined hyperlipidemia* (FCH), a common metabolic disorder, is characterised by an upsurge in cholesterolemia and/or triglyceridemia in at least two members of the same family, intra-individual and intra-familial variability of the lipid phenotype, combined with an elevated risk of premature CHD [28]. Untreated FCH has been linked with early-onset CVD; LDL-C levels directly correlate with CVD across a number of populations [22]. *Familial dysbetalipoproteinemia* (also known as type III hyperlipoproteinemia or remnant removal disease), another genetic lipid disorder, is characterised by hyperlipidemia, mutations in the apolipoprotein E gene, and an increased CVD risk [29].

Familial chylomicronemia syndrome (FCS), lysosomal acid lipase deficiency, familial hypoalphalipoproteinemia, β-sitosterolemia, and lipodystrophy are few other uncommon genetic dyslipidemic syndromes [30]. Clinicians could refer such patients to specialists for further investigations (e.g. genetic testing) and appropriate management.

Secondary causes of lipid disorders

It is imperative to identify secondary causes of dyslipidemia before initiating or intensifying treatment. Treating the underlying condition might improve the dyslipidemia, plummeting the need for therapy. Recognising the co-morbidity could amend consequent treatment decisions. In fact, certain dyslipidemias may appear to be refractory to treatment in the presence of an unrecognised secondary cause.

The most common causes of dyslipidemia include diabetes mellitus, excessive alcohol intake, hypothyroidism, liver disease, renal disorders, obesity, ageing, postprandial lipemia, metabolic syndrome, pregnancy, smoking, dysproteinemia, acute stress, and drugs (oestrogen medications, human immunodeficiency virus (HIV) therapy, antipsychotic medications, steroids, immunosuppressive agents, etc.) [31, 32]. Addressal of poor glycemic control, obesity, diets high in refined carbohydrates, alcohol excess, lack of exercise, and smoking as secondary causes of dyslipidemia are advocated [33].

Once diagnosed, secondary causes of dyslipidemia should be excluded in order to rule out individuals that could possibly be treated or cured with approaches other than triglyceride- or cholesterol-lowering therapies. Initially, a complete medical, family, and nutrition history must be recorded, followed by a physical examination to ascertain additional risk factors. Common laboratory tests useful in excluding a secondary cause of dyslipidemia comprise of urinalysis, glucose, TSH, plasma creatinine, protein electrophoresis, alkaline phosphatase, and transaminases. Moreover, all prescriptions, dietary supplements, and over-the-counter medications should be noted. Monitoring of lipid levels must be continued after diagnosing the secondary cause of dyslipidemia, since certain conditions increase the risk of ASCVD, thereby warranting more aggressive lipid-lowering therapy [30].

Atherogenic diabetic dyslipidemia

Hyperglycemia, adipocytokines, and insulin deficiency or resistance could contribute to the modifications in lipid metabolism in patients with diabetes [6]. The pattern of dyslipidemia is not the same in Indians. Contrary to that seen in Caucasians, though LDL-C levels are not very high, the preponderance of more atherogenic small, dense LDL particles is greater among Indians. Furthermore, HDL-C levels are low while levels of triglycerides and apo B are elevated. This pattern, known as 'atherogenic dyslipidemia', is frequently encountered in South Asians with diabetes. The pattern and prevalence of concomitant cardiovascular risk factors moderating the impact of dyslipidemia on cardiovascular risk (e.g. truncal obesity, diabetes and metabolic syndrome) also vary in Indians [5, 24, 34]. In a recent cross-sectional study in naïve Indian diabetic patients, the prevalence of atherogenic diabetic dyslipidemia was 34%, 73% of whom had high HbA1c levels (>8%). The authors also observed a staggering 89.2% patients newly diagnosed with diabetes demonstrating a high prevalence of dyslipidemia [35]. Molar concentrations of lipoprotein a [Lp(a)] have been found to be dose-dependently associated with CAD risk, peripheral artery disease, aortic valve stenosis, heart failure, and lifespan [36].

Role of HDL rise in diabetes mellitus

The cardiovascular protective role of HDLs is generally attributed to their role in reverse cholesterol transport, endothelium-dependent vasorelaxant effects, and anti-inflammatory, antithrombotic, and anti-oxidative abilities. These are, however, compromised in diabetic states, on account of glycation of the HDL protein, oxidative alteration, and the conversion of the HDL proteome into a pro-inflammatory protein. The capability of HDL to subdue inflammatory signals is known to considerably decrease in such patients [37, 38]. Since HDL function is disconcerted in patients with diabetes, HDL-C levels, in isolation, may perhaps not reveal the risk of CVD in diabetes accurately [1].

High-density lipoprotein concentration, composition, and metabolism as well as functionality vary substantially in patients with diabetes compared to the general population. People with type 1 diabetes with nephropathy and type 2 diabetes have low HDL-C. In these states, the activity of cholesteryl ester transfer protein (CETP) increases while that of serum paraoxonase-1 (PON-1) is decreased. This impairment in the functionality of HDL is due to glycation of HDL protein constituents [39].

Though evidence demonstrates normal or even higher plasma concentrations of HDL-C in type 1 diabetes than type 2, there is an increased incidence of CVD in type 1. This enigma could be elucidated by alterations in the abnormal cholesteryl ester/triglycerides ratio, lower phospholipid content, decline in the capacity to stimulate cholesterol efflux from macrophages, compromised anti-inflammatory and anti-oxidant activities, and other probable atherogenic properties disturbing HDL functional properties in patients with type 1 diabetes [40]. In type 2 diabetes, HDL is enhanced in triglycerides, and hence responsible for the higher catabolism of HDL particles. Besides, HDL particles are glycated in this state and an upfront correlation between glycation of apo A-I and plasma glucose level has been noted. Glycation of apo A-I has been shown to bring about a reduction in the solidity of the lipid–apoprotein interaction in addition to that of the apoprotein self-association, expediting dissociation of the former complex and distressing the organisational cohesion of HDL particles. Such structural amendments reduce the binding of HDL to its receptor [37].

Subsequent to the comprehension that higher HDL levels might not always render improved HDL function, newer treatment approaches emphasise not only on enriching HDL levels, but also on augmenting its function, and consist of inhibition of HDL modification (like vitamin E), HDL substitution (like apo-AI mimetics), and rise of HDL (like CETP inhibitors). HDL mimetic agents, for e.g. reconstituted HDL, apo-AI, apo-AI Milano, and apo-AI mimetic peptides signify an innovative therapeutic objective enhancing HDL functionality by not just improving reverse cholesterol transport, but also their antithrombotic, anti-oxidative, and anti-inflammatory properties [39, 41]. The Strong Heart study (n=3,216) noted a 1.32-fold higher hazard ratio (95% CI, 1.06–1.64) for CHD among diabetic adults with high triglycerides and low HDL-C, than those with normal triglycerides and normal HDL levels [42].

Lipoprotein (a) and apolipoprotein B

Lipoprotein (a), bearing a structure similar to plasminogen, attaches to the plasminogen receptor, resulting in amplified thrombosis. Data illustrates Lp(a) measurement to offer clinically significant enhanced risk reclassification in specific situations, and must be in cases with an estimated 10-year risk of ASCVD, or those with high-to-moderate risk [43, 44]. The recent ESC guidelines endorse a one-off measurement of Lp(a) to stratify patients with considerably high inherited Lp(a) levels posing a substantial lifetime risk of ASCVD, those with a family history of premature CVD. Moreover, it can help in defining treatment strategies in individuals whose projected risk is on the verge of risk categories [45].

A recent case–control study of 1,43,087 Icelanders (n=17,715 with CAD; 8,734 with T2D) validated an increased risk of type 2 diabetes with low concentrations of Lp(a). The researchers also revealed a projected reduction in CAD risk without an increase in the risk of type 2 diabetes with a pharmacologic decline in Lp(a) concentration [36]. Another study assessing the relationship between Lp(a) and type 2 diabetes in 2,040 patients with and without CAD, found an independent association between elevated Lp(a) levels with the presence and severity of CAD [46].

Singla et al. designed a case–control study to examine (i) Lp(a) levels in 60 age- and sex-matched patients with type 2 diabetes and (ii) their association with LDL:HDL ratio and glycemic control. When compared to the control group, the diabetic group had significantly higher levels of Lp(a) and LDL:HDL ratio. However, there was no association with LDL:HDL ratio or the degree of glycemic control. The authors concluded that elevated levels of Lp(a) do not reflect the glycemic status and are non-dependent of the rise in LDL:HDL ratio [47]. Other researchers have suggested low Lp(a) levels in type 2 diabetes to be beneficial and at the same time unhealthy, and linked to undesirable cardiometabolic phenotype, inferior glycemic control, lesser β-cell function, and amplified microvascular damage in spite of being related to evident reduction in CAD [48].

Furthermore, plasma levels of certain apolipoproteins are altered in type 2 diabetes with CVD or other complications. A number of apolipoprotein polymorphisms have been linked with lipid metabolism and/or diabetes susceptibility [49]. Apo B recognises high-risk dyslipidemic phenotypes in patients with type 2 diabetes, which are not identified by standard lipid profile. Adding apo B to standard lipid profile may perhaps assist in appropriate introduction of lipid-lowering therapy in undetected high-risk patients, thereby plummeting mortality and morbidity due to future cardiovascular complications [50]. Apo B measurement could also aid in assessing cardiovascular risk in those diabetic people with hypertriglyceridemia or CHD, who have already accomplished LDL-C and non-HDL-C targets [51].

The current ESC recommendations advocate Apo B as an enhanced estimate of a person's exposure to atherosclerotic lipoproteins, and may be predominantly valuable for assessment of risk in cases where LDL-C measurement underestimates this burden, for instance people with high triglycerides, diabetes mellitus, obesity, or very low LDL-C [45].

Need for screening for dyslipidemia in diabetes

Who should be tested?

People with type 2 diabetes are considered to be at high risk for vascular events. As a result, regardless of other risk factors on history (age, gender, hypertension, family history, smoking) or physical examination (hypertension, obesity, polycystic ovary syndrome in women), they ought to be screened for dyslipidemia. In patients with type 1 diabetes, screening for dyslipidemia should be initiated from the age of 12 years. In cases of known family history of hypercholesterolemia, early CVD or if the family history is unknown, screening should begin at the age of 2 years. If results are within normal limits, screening should be repeated every 5 years, until adulthood, and annually thereafter [5].

The American Association of Clinical Endocrinologists and American College of Endocrinology (AACE/ACE) guidelines for management of dyslipidemia and prevention of cardiovascular disease recommend annual screening for all adult individuals with T1DM or T2DM for dyslipidemia. In the absence of ASCVD risk factors, middle-aged individuals should be screened for dyslipidemia at least once every 1 to 2 years. Annual screening for dyslipidemia is recommended for older adults with 0 to 1 ASCVD risk factor [52]. The recent RSSDI advocates simultaneous screening and treatment for modifiable risk factors for CVD like dyslipidemia, hypertension, alcohol consumption, and smoking, in patients with prediabetes. In patients with diabetes, dyslipidemia should be assessed at diagnosis and annually thereafter. Periodical screening for dyslipidemia is recommended in overweight or obese children with a family history of type 2 diabetes, or those from a predisposed race/ethnicity like Asian, American Indian, etc. [53].

What should be tested?

Basic physical examination must include patient's height and weight, waist circumference, blood pressure, and peripheral and carotid pulses. Laboratory evaluations should include fasting lipid profile (total cholesterol, HDL-C, triglycerides, LDL-C, and calculated non-HDL-C), comprehensive medical

panel (including uric acid), HbA1c, and thyroid-stimulating hormone. Non-fasting lipid levels are effective in initial screening; non-HDL-C is a reasonable screening test. Non-HDL-C should routinely be calculated in diabetic patients owing to the higher prevalence of elevated triglycerides and small-dense LDL. Assessment of apo B or LDL particles, Lp(a), and high-sensitivity C-reactive protein should also be considered, when deemed necessary. Diagnostic procedures could include resting electrocardiogram, treadmill, chemical, and/or nuclear stress tests, if required [5, 7, 30, 54].

Dyslipidemia testing

Fasting vs non-fasting

In postprandial hyperlipidemia, also known as postprandial hypertriglyceridemia, there is an increase in triglyceride-rich chylomicron remnants and hypertriglyceridemia is protracted. This condition, which induces atherogenesis in the postprandial period, is a vital residual risk factor particularly in patients with diabetes mellitus and metabolic syndrome [55–57]. Guidelines advocate the gold standard, viz. fasting levels of lipids for atherogenic risk assessment, on account of the sensitivity of triglyceride levels to the postprandial state, predominantly in patients with insulin resistance [58]. Fasting triglycerides are said to be more apt for calculating LDL-C, as a number of dietary factors influence triglycerides and hence escalate after a meal, unlike apo B and non-HDL-C levels [59].

The requisite for fasting pressurises patients' as well as laboratory facilities that necessitate accommodation of such patients in the wee hours [58]. Fasting for 8 h or more customarily occurs a few hours prior to breakfast, disparate to the non-fasting state that prevails over 24 h, and in doing so, captures improvised levels of atherogenic lipoproteins. In the latter state, plasma comprises of atherogenic lipoproteins of hepatic descent in the fasting state as well as those of originating from the intestines [60]. Non-fasting lipid assessment is rational in numerous clinical settings as LDL-C can be precisely assessed using modern techniques and that prediction of ASCVD risk is analogous with fasting or non-fasting lipid values. Permitting the alternative for non-fasting lipid assessment may reduce a barrier to lipid testing and enable appropriate ASCVD risk assessment with the eventual prospective effect of plummeting the ubiquitous burden of ASCVD [61].

With the elimination of the requirement to return another day for a fasting lipid profile, non-fasting lipid assessment has the potential to reduce overall costs, decreasing missed work time thereby enhancing patient satisfaction and compliance with lipid testing [59, 60, 62]. Patients with diabetes on antihyperglycemic agents (particularly long-acting basal insulin or sulphonylureas) are at a higher risk of developing hypoglycemia in the fasting state [63]. When using non-fasting lipid profiles to decide commencement of a statin or titration of its dose in people with borderline LDL cholesterol, there is a need to consider the lower LDL cholesterol observed mainly 0–4 h after a meal, owing to liberal fluid intake and haemodilution, predominantly in patients with diabetes [56].

Non-fasting LDL-C is by and large valid but for elevated triglyceride levels; the prandial state does not affect non-HDL-C or apo B measurements even when triglyceride levels are not in the normal range [64, 65]. Nonetheless, certain high-risk patients or those with severe hypertriglyceridemias being treated to low LDL-C levels might need fasting lipid panels for an exact diagnosis and to regulate therapeutic monitoring. Since patients with well-controlled LDL-C but discordantly high apo B continue to face a greater risk of ASCVD, a non-fasting lipid profile could reveal a more precise average lipid exposure [61]. The advantages of fasting and non-fasting lipid testing are summarised in Table 1.

A fasting lipid profile should be acquired when non-fasting triglyceride levels are >440 mg/dL (>5 mmol/L) [62]. In individuals with non-fasting non-HDL-C level of ≥220 mg/dL, a familial cause of hyperlipidemia ought to be suspected and assessed further. Among those with features suggestive of familial hyperlipidemia or a family history of premature ASCVD, screening and follow-up must be performed with fasting lipid panels. Though follow-up fasting triglyceride in cases of non-fasting

Table 1 Advantages of fasting and non-fasting lipid testing

Fasting	Non-fasting
• Gold standard for atherogenic risk assessment	• Detects plasma lipid levels of both atherogenic lipoproteins of hepatic descent and those originating from the intestines
• Highly sensitive to detect triglyceride levels especially in patients with insulin resistance	• Preferred in patients with diabetes due to the increased risk of hypoglycaemia with fasting
• Preferable in certain high-risk patients or those with severe hypertriglyceridemias for exact diagnosis and therapeutic monitoring	• Low-density lipoprotein cholesterol can be precisely assessed using modern techniques
• Preferable when non-fasting triglyceride is >440 mg/dL	• Reduces patient barrier to testing
• To be considered after 2–4 weeks when non-fasting triglyceride is ≥200 mg/dL	• Increases patient convenience and compliance
	• Decreases strain on laboratory facilities

Table 2 Suggested cut-off points of serum lipid levels in fasting and non-fasting states

Lipid parameter	Fasting (mg/dL)	Non-fasting (mg/dL)
Total cholesterol	≥190	≥190
Triglycerides	≥150	≥175
HDL-C	≤40	≤40
Non-HDL-C	≥145	≥150
LDL-C	≥115	≥115
Remnant cholesterol	≥30	≥35
Lipoprotein (a)	≥50	≥50
Apolipoprotein B	≥100	≥100

Abbreviations: *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol

triglyceride level 175 mg/dL is not obligatory, patients must be advised for healthy lifestyle changes, while in those with non-fasting triglyceride level ≥200 mg/dL, it is helpful to follow up with a fasting lipid panel after 2–4 weeks [66, 67]. Table 2 enlists the suggested cut-off points of serum lipid levels in fasting and non-fasting states adapted from [62, 68].

Recent studies over the last couple of decades have reported similar prognostic values of non-fasting LDL-C to fasting LDL-C [69–72]. Lipids must be checked every 3 months or more frequently, as deemed necessary, in patients at moderate-to-extreme risk [30]. An increase in the use of non-fasting lipid testing could result in better patient awareness, detection, surveillance, and dyslipidemia control at a population level leading to a substantial decline in the ASCVD burden [61]. The decision to acquiring fasting or non-fasting lipid values must be tailored on an individual basis. On the whole, there is enough evidence in favour of the use of non-fasting testing for dyslipidemia in clinical practice to evaluate and manage the risk of ASCVD.

Non-HDL should be calculated in every subject (LAI)

- Non-HDL-C, which is equal to total cholesterol minus HDL-C, includes all circulating atherogenic lipoproteins and is therefore a more accurate predictor of ASCVD risk, particularly in patients who have elevated triglycerides (e.g. diabetics, obese persons, those with metabolic syndrome) and those already on statin therapy.
- The LAI recommends non-HDL-C as a co-primary target, as important as LDL-C, for lipid-lowering therapy.
- Monitoring of non-HDL-C will provide a simple, practical tool for treatment decisions relating to lipid-lowering therapy since it does not require a fasting blood sample and takes care of both LDL-C and triglyceride targets.
- In all individuals, the non-HDL-C level should be kept within 30 mg/dL of LDL-C levels.

Assessment of cardiovascular risk

Cardiovascular risk scores are considered valuable tools in diabetes management, predominantly when the score is established in an identical population. Scores that categorise risk well are suitable for detecting people at highest risk, where therapy can be directed. On the contrary, the method of predicting risk precisely to offer prognostic information is aided better by risk scores that compute absolute risk accurately [73].

Even though a prudent objective in the clinical management of dyslipidemia is achieving normal lipid levels, there is a need to set more aggressive goals for individuals at higher risk. There are a multitude of risk scores that assess cardiovascular risk considering diabetes as a risk factor (Table 3). Despite some variability in calibration in various subgroups, together with gender, race, and diabetes, most studies have found no statistically significant difference in the overall

Table 3 Total CVD risk assessment systems that considered diabetes as a risk factor

System	Risk assessed
Framingham models	10-year risk of CHD events [74]
ASSIGN (CV risk estimation model from the Scottish Intercollegiate Guidelines Network)	10-year risk of first CVD event [75]
QRISK3	10-year risk of CVD event [76]
Prospective Cardiovascular Munster Study (PROCAM)	Two separate scores calculate 10-year risk of major coronary events and cerebral ischemic events [77]
Reynolds Risk Score	10-year risk of incident myocardial infarction, stroke, coronary revascularisation, or CV death [78]
CUORE	10-year risk of first CVD event [79]
Pooled Cohort equations	10-year risk of CVD event [80]
Globorisk	10-year risk of CVD mortality [81]

Abbreviations: *CHD*, coronary heart disease; *CVD*, cardiovascular disease

Table 4 Coronary calcium scoring recommendations by the National Lipid Association [87]

Age groups	Additional considerations	Recommendations
30–39 years	Long-standing diabetes mellitus (type 1 diabetes, >20 years; type 2 diabetes, >10 years) and risk factors or microangiopathy	CAC scoring may be reasonable to aid in ASCVD risk stratification and statin treatment shared decision making.
40–75 years	LDL-C level: 70–189 mg/dL	Moderate or high intensity statin is indicated, regardless of CAC score
	When decision to initiate statin therapy has been made	Choose a high intensity statin when CAC score >100
>75 years	When decision to employ a statin for primary prevention is uncertain	CAC scoring is reasonable to aid in statin treatment shared decision making

Abbreviations: *ASCVD*, atherosclerotic cardiovascular disease; *CAC*, coronary calcium scoring; *LDL-C*, low-density lipoprotein cholesterol

prediction of CVD risk in those with or without diabetes, authenticating the use of risk calculators in people with diabetes. On the other hand, diabetes itself confers an amplified risk for ASCVD. It should hence be acknowledged that these risk calculators do not account for either the duration of diabetes or the presence of diabetes complications.

Different categorisations of the Framingham risk score are reported to have a potent relationship with the various components of metabolic syndrome [82]. However, since the Framingham Heart Study did not include patients with diabetes, the question regarding the applicability of the score to gauge CVD risk in patients with diabetes arises. Stephens and colleagues retrospectively analysed data of 1176 patients with diabetes attending the diabetes clinic at University College London Hospitals NHS Trust from 1990 to 2001 to observe the efficacy of the Joint British Societies Risk Chart (JBSRC), the CardioRisk Manager (CRM) calculator, the PROCAM calculation and the UKPDS risk engine (specific to diabetes) for risk prediction in patients with diabetes. The researchers concluded that, though these methods have reasonable discrimination, they tend to underestimate future CHD and CVD events [83].

Patients with ASCVD, type 1 or type 2 diabetes, steep levels of individual risk factors, or those with chronic kidney disease are generally considered at very-high or high total cardiovascular risk. Risk estimation models are not essential for such people; all risk factors warrant active management. In fact, risk scores developed for the general population are not endorsed for cardiovascular risk assessment in this subgroup [45]. The United Kingdom Prospective Diabetes Study (UKPDS) risk engine offers estimates of CHD risk for the primary prevention of CHD in patients with type 2 diabetes. This diabetes-specific model integrates glycemia, systolic blood pressure, and lipid levels as risk factors, over and above age, sex, ethnic group, smoking status, and time since diagnosis of diabetes [84].

The QRISK2 assessment tool has now been updated to the QRISK3 assessment tool. The new algorithm calculates an individual's risk of developing a heart attack or stroke over 10 years, and takes into account ethnicity as a risk factor, in addition to age, sex, body mass index, smoking status, type of diabetes, lipid levels, hypertension, family history of CVD, CKD, among others. Considering the possible genetic influence of Indian ethnicity on CVD, the QRISK3 score exemplifies as the current most accurate CVD screening tool available for the Indian population [85, 86].

Coronary artery calcium (CAC) scoring is an extensively available, cost-effective, rapid, safe test that better identifies those at risk for ASCVD and assists superior reclassification of the risk, particularly when used in combination with global risk scoring systems [87]. A plethora of studies have recognised measurement of CAC to enhance the prediction

Table 5 ASCVD risk categories and treatment goals for dyslipidemia in patients with diabetes

	High risk	Very-high-risk	Extreme risk
Definition	Diabetes with no other risk factors	Diabetes with ≥1 major risk factor(s) for ASCVD	Diabetes with established clinical ASCVD
LDL-C (mg/dL)	<100	<70	<55
Non-HDL-C (mg/dL)	<130	<100	<80
Apolipoprotein B (mg/dL)	<90	<80	<70
Triglycerides (mg/dL)	<150	<150	<150

Abbreviations: *ASCVD*, atherosclerotic cardiovascular disease; *DM*, diabetes mellitus; *LDL-C*, low-density lipoprotein cholesterol; *non-HDL-C*, high-density lipoprotein cholesterol

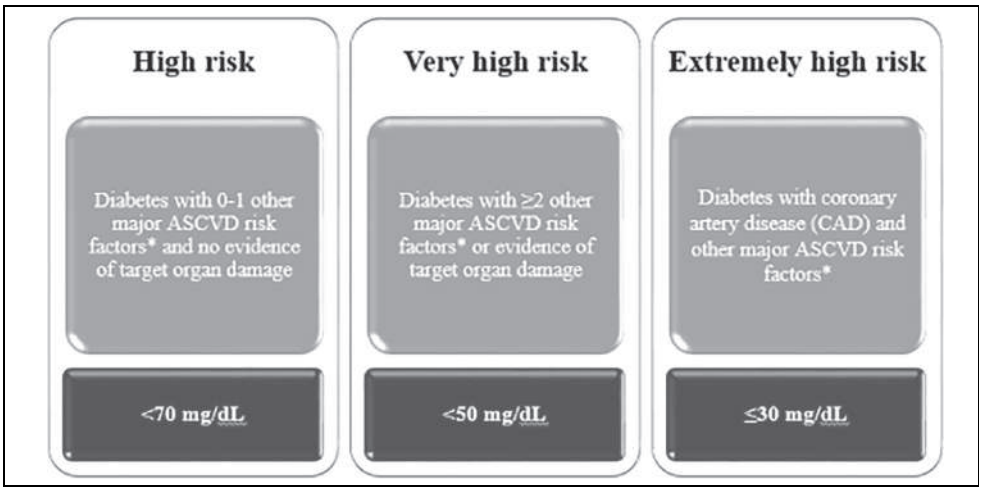


Fig. 1 Treatment goals for LDL-C for patients with diabetes across categories of total cardiovascular disease risk [7]. *Major ASCVD risk factors include age ≥45 years in males and ≥55 years in females, family history of premature ASCVD, current cigarette smoking or tobacco use, high blood pressure, or low HDL-C. *Abbreviations*: ASCVD, atherosclerotic cardiovascular disease; LDL-C, low-density lipoprotein cholesterol

of cardiovascular events in asymptomatic patients with type 2 diabetes. In fact, it has been established as an independent predictor of future ASCVD events in patients with diabetes and is consistently superior to the Framingham Risk Score as well as the UKPDS risk engine [88–90]. Conversely, the DIAD study revealed no clinical advantage to routine screening of asymptomatic patients with type 2 diabetes [91].

The recent recommendations from the National Lipid Association on coronary calcium scoring for patients with diabetes are summarised in Table 4.

ASCVD risk category and treatment goals

The AACE has defined five risk categories considering the number and severity of major ASCVD risk factors, viz. diabetes mellitus, family history of hyperlipidemia, fasting/postprandial hypertriglyceridemia, increased levels of total serum cholesterol level, non-HDL-C, LDL-C, apo B, Lp(a), triglyceride-rich remnants, and small, dense LDL-C, and low levels of HDL-C, including others (Table 5) [30].

The European Society of Cardiology (ESC) recommends more intensive reduction of LDL-C across the cardiovascular risk categories. In addition, they advocate LDL-C goal of <1.0 mmol/L (<40 mg/dL) for patients with ASCVD experiencing a consequent vascular event within 2 years while on maximally tolerated statin therapy. For secondary prevention, in patients at very-high risk, a ≥50% reduction LDL-C and LDL-C goal of <1.4 mmol/L (<55 mg/dL) may be considered [45].

In patients with diabetes, cardiovascular risk categories applicable to Indians include (a) high risk, i.e. patients with long-standing diabetes mellitus, especially with other cardiovascular risk factors or with target organ damage, and (b) moderately high risk, i.e. patients with recent onset diabetes mellitus with no other major cardiovascular risk factor and no evidence of target organ damage [5]. The recent Expert Consensus Statement on dyslipidemia by the Lipid Association of India stratifies Indian patients with diabetes as either 'high risk', 'very-high risk', or 'extremely high risk' (Fig. 1) [7].

High-risk patients necessitate management comparable to that for secondary prevention of CVD [92]. The most important step in defining treatment goals for diabetes patients is an extensive assessment of their cardiovascular risk, with LDL-C as the primary target, and non-HDL-C, HDL-C, and apo B as secondary targets [5, 93]. The AACE endorses non-HDL-C goal as an even better indicator of ASCVD risk compared to LDL-C [30]. The ESC recommends apo B analysis for risk assessment, predominantly in those with diabetes mellitus, high triglycerides, obesity or metabolic syndrome, or very low levels of LDL-C. If available, apo B can be used as the primary measurement for screening, diagnosis, and management, and preferred over non-HDL-C in this population [45]. Since all atherogenic particles contain an apo B_{100} molecule, it might deliver precise estimation of atherogenicity. Besides, apo B measurement helps in evaluating the success of lipid-lowering therapy, as it may continue to remain above the target even after achieving the LDL-C goal [30].

In children with type 1 diabetes, a dispute concerning the goals for lipid levels exists. Where the AACE and American Academy of Pediatrics suggest softer LDL-C targets (normal <110 mg/dL, high >130 mg/dL, borderline 110–130 mg/dL), the International Society for Pediatric and Adolescent Diabetes recommends LDL-C target of <100 mg/dL [5].

Management of diabetic dyslipidemia

Goals of therapy

A comprehensive strategy is essential in the management of dyslipidemia so as to regulate lipid levels and tackle related metabolic deviations and modifiable risk factors. Lifestyle

modifications such as smoking cessation, physical activity, medical nutrition therapy, sleep evaluation, and mental health conditions play a crucial part in dyslipidemia management [30]. The basis of such management should encompass an individual's principal phenotype and comprise of treatment regimens demonstrated to reduce cardiovascular events. Despite the need to individualise the choice to initiate drug therapy, regardless of basal plasma cholesterol levels, statin therapy must be considered in high-risk patients with diabetes. Owing to the intricacy of diabetic dyslipidemia, there usually is a need for multiple agents to accomplish therapeutic goals [24].

Non-pharmacological therapy

Lifestyle management continues to form the basis of all lipid-reduction therapies [30]. Healthy behavior interventions are a vital element of diabetes management and CVD prevention strategies. Essential considerations to improve overall lipid profile and glycemic control, and reduce CVD risk include accomplishing a healthy weight and aerobic activity level, implementing an energy-restricted, well-balanced diet, moderating alcohol consumption and smoking cessation [5, 94–98].

Changes in lifestyle behavior, such as weight loss, regular physical activity, and medical nutrition therapy, may assist in decreasing ASCVD risk factors. In order to enhance lipid profile and decrease the risk of developing ASCVD in patients with diabetes, the ADA recommends lifestyle modifications aiming towards weight loss (as and when indicated), increased physical activity, and adapting to a Mediterranean style or Dietary Approaches to Stop Hypertension (DASH) eating pattern, with reduced intake of saturated- and trans-fat, and increased intake of dietary n-3 fatty acids, viscous fibre, and plant stanols/sterols [99]. Modifications in lifestyle can reduce triglyceride levels by up to 50% [7].

Physical activity

Physical inactivity has been linked with glucose intolerance, hypertension, waist circumference, and obesity, as well as dyslipidemia. Explicit improvements in lipid levels with regular exercise embrace higher HDL-C, decreased VLDL-C and triglycerides, and decline in high-sensitivity C-reactive protein (hsCRP), and even increase the LDL-C particle size thereby making it less permeable. Constant reinforcement for regular physical activity is recommended in non-adherent individuals. In order to improve adherence, healthcare personnel could adapt diverse approaches like personalised advice, instructor-led exercise classes, and ascertaining barriers to adherence, in addition to routine consultation and follow-up [30]. A meta-analysis of randomised controlled trials (RCT) demonstrated progressive resistance training to reduce total cholesterol, total cholesterol to HDL-C ratio, non-HDL-C, LDL-C, and triglycerides in adults [96].

Yoga

Yoga, a lifestyle intervention that uses an integrated approach, aims at reducing raised lipid levels in diabetic patients [100]. Gordon et al. suggested the beneficial role of yoga in the management of dyslipidemia in 35 patients with end-stage renal disease. They noted a significant decrease in total cholesterol after 4 months (−4.58%; p=.0001), triglycerides (−6.26%; p=0.0001), LDL-C (−11.32%; p=0.0001), and total cholesterol/HDL-C ratio (−12.26%; p=0.047) [101]. A recent stratified translational research (NMB-2017 India) trial assessed the efficacy of a validated yoga protocol on dyslipidemia in patients (n=17,012) with diabetes. After 3 months of intervention, 60% patients attained normal total cholesterol (<200 mg/dL), 73.7% patients achieved normal LDL, normal triglyceride levels were accomplished by 63% patients, and 43.7% returned to normal HDL (>45 mg/dL). The authors concluded the implementation of yoga to significantly mitigate the hyperlipidemic states in patients with diabetes [102].

Sleep and optimism

Cappuccio et al. conducted a systematic review and meta-analysis of 15 prospective studies (n=4,74,684; follow-up=6.9–25 years) to examine the relationship between duration of sleep and morbidity and mortality from CHD, stroke, and total CVD. They found both short (relative risk [RR], 1.48; 95% CI, 1.22–1.80; p<0.0001) and long (RR, 1.38; 1.15–1.66; p=0.0005) durations of sleep to be predictors of cardiovascular outcomes [103]. Allowing for optimism in terms of lipids could propose novel approaches for prevention and intervention to improve cardiovascular health. The Midlife in the United States (MIDUS) study investigated the relationship of optimism with total cholesterol, HDL-C, LDL-C, and triglycerides. The authors' reported an association between greater optimism with greater HDL-C and lower triglycerides, and no significant association with LDL-C or total cholesterol [104].

Medical nutrition therapy

Medical nutrition therapy (MNT) plays a central part in diabetes management; every individual with diabetes must be actively engaged in self-management, education, and treatment planning with their healthcare team, together with the collective development of an individualised eating plan [105]. Nutrition intervention must be tailored to each patient's age, type of diabetes, pharmacological regime, lipid levels, and medical issues [99]. Vital elements of MNT include assessment, nutrition diagnosis, interventions like education and counselling, and monitoring with ongoing follow-up so as

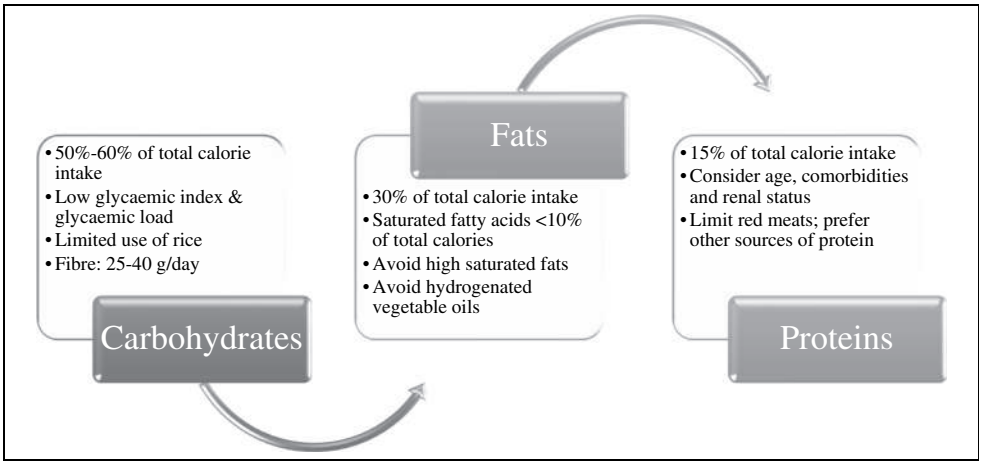


Fig. 2 Recommendation for medical nutrition therapy in patients with type 2 diabetes

support durable lifestyle alterations, estimate outcomes, and amend interventions, whenever necessary [105].

Global clinical practice guidelines for type 2 diabetes from the ADA, IDF, and AACE highlight the significance of incorporating MNT in type 2 diabetes management as a first-line therapy [30, 99]. The goals of MNT are to endorse and support healthful eating patterns, accentuating an assortment of nutrient-dense foods in appropriate portion sizes, in order to improve overall health and:

- Accomplish and sustain body weight goals,
- Achieve personalised lipid, glycemic, and blood pressure targets, and
- Defer or avert diabetic complications.

A tailored MNT programme, delivered by a registered dietician nutritionist, is obligatory to achieve therapeutic targets in type 1 or type 2 diabetes, prediabetes, and gestational diabetes mellitus. In overweight or obese patients with prediabetes and diabetes, lifestyle adaptation to attain and retain a minimum weight loss of 5% is recommended. As there is no distinct ideal nutritional distribution of calories for people with diabetes, meal plans must be personalised bearing the total calorie and metabolic targets in mind [99]. There is a need for healthcare professionals in India to consider cultural, regional, economic, and agricultural aspects while customising meal plans, since these aspects have a remarkable influence on the reception of MNT by Indian patients [53].

An eating plan emphasising elements of a Mediterranean-style eating pattern rich in monounsaturated and polyunsaturated fats may be considered to improve glucose metabolism and lower cardiovascular disease risk [99]. Keeping in mind clinically relevant observations in the Indian population, the Research Society for the Study of Diabetes in India/Endocrine Society of India (RSSDI-ESI) endorse the implementation of dietician-guided MNT as a fundamental constituent of diabetes management (Fig. 2). They advocate a diet rich in fruits, leafy vegetables, nuts, fibres, whole grains, unsaturated fats, pulses, legumes, unprocessed vegetables, and low fat dairy. Salt consumption must be restricted to <5 g/day and artificial sweeteners <2–3 g/day [53]. Dietary fructose, if consumed >10% of total energy intake, regardless of its low glycemic index, results in hypertriglyceridemia. For that reason, there is a need for a vigilant nutritional evaluation concentrating on fructose intake for individuals with hypertriglyceridemia [5].

The Diabetes in India Nutrition Guidelines Study was a 12-month prospective cluster RCT that compared the outcomes of patients with type 2 diabetes who received dieticians' usual care (n=154) to those who received Evidence-Based Nutrition Practice Guidelines care (n=85). When treated with Evidence-Based Nutrition Practice Guidelines, patients were significantly more likely to achieve LDL-C (mean change from baseline, −11 ± 20 mg/dL), HDL-C (+1.6 ± 4.9), and triglyceride (−74 ± 224) targets over a period of 1 year [106]. A multisite study verifying the efficacy of registered dietician nutritionist interventions in the management of glycemic control and diabetic dyslipidemia reviewed 392 patients with type 2 diabetes completing diabetes self-management education (DSME) and MNT at four regional centres in Alabama from 2013 to 2014. Following DSME and MNT, 62% of patients reached a glycemic target of HbA1c≤7%, compared to 32% patients at baseline (p<0.001). Moreover, there were substantial reductions from baseline to 1-year follow-up in triglyceride levels (162±74 mg/dL [4.19±1.91 mmol/L] vs 109±36 mg/dL [2.82 ±0.92 mmol/L]; p<0.001) and triglyceride-to-HDL ratio (4.07 ±2.41 vs 2.48±1.26; p<0.001), with significant improvement in HDL (45±13 mg/dL [1.16±0.34 mmol/L] vs 48±11 mg/dL [1.24±0.28 mmol/L]; p=0.05) [107].

Pharmacological management

Pharmacological management is recommended when interventions to improve dietary changes and metabolic control are not successful in achieving the recommended lipid targets

[5]. Existing evidence for curtailing the atherogenic impact of lipid aberrations in diabetes is to emphasise on attaining very low plasma LDL-C concentrations, characteristically with statin-based therapy [64]. Second-line LDL-C-lowering therapies should be considered in individuals who do not achieve the recommended lipid targets regardless of maximally tolerated statin therapy or in those with statin intolerance [108]. The latest ADA guidelines recommend intensification of lifestyle therapy and optimisation of glycemic control for diabetic patients with increased triglyceride (≥150 mg/dL [1.7 mmol/L]) and/or low HDL cholesterol (<40 mg/dL [1.0 mmol/L] for men, <50 mg/dL [1.3 mmol/L] for women) levels [99]. Meta-analyses of various lipid-lowering therapies revealed ~20% per 1 mmol/L reduction in LDL-C in the incidence of major vascular events, irrespective of the baseline LDL-C level. Though people with type 2 diabetes were estimated to have a relative risk reduction comparable to non-diabetic patients, being at higher absolute risk, the absolute benefit was predicted to be greater [109, 110].

LDL-C-lowering therapy

Statins Statin therapy is indicated not only in patients with genetic causes of dyslipidemia (e.g. familial hypercholesterolemia) but also in primary and secondary prevention of ASCVD. Moreover, usage of statin therapy in high-risk individuals like those with diabetes duration of more than 40 years and high-risk primary prevention is generally accepted to offer benefit. Nonetheless, there is evidence in patient cohorts where treatment with statin has not been proven to be effective (in cases of haemodialysis or heart failure) or not studied (e.g. malignancy, end of life) [111]. The latest AACE guidelines recommend the use of a moderate- to high-intensity statin as first-line cholesterol-lowering therapy, unless contraindicated. Nonetheless, even with aggressive statin monotherapy, substantial residual risk continues to exist in primary prevention patients with multiple cardiovascular risk factors [30]. Major statin trials that particularly included people with diabetes have shown substantial benefits of statin therapy on CVD events [109].

The ESC suggests delaying statin therapy in asymptomatic patients with diabetes, until the age of 30 years. However, in the presence of ambient levels of LDL-C, microalbuminuria, and end-organ damage, statin therapy could be considered using a personalised approach [45]. On the other hand, the AHA recommends a judicious introduction of statin therapy in adults in the 20–39 years age group with diabetes mellitus, with albuminuria (≥30 mcg of albumin/mg creatinine), long duration of diabetes (≥20 years of type 1 diabetes; ≥10 years of type 2 diabetes), estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, neuropathy, retinopathy, or ankle-brachial index (<0.9). In adults between 40 and 75 years of age with diabetes, moderate-intensity statin therapy is indicated, irrespective of the estimated 10-year ASCVD risk. In diabetic patients aged 40 to 75 years with LDL-C ≥70 mg/dL (≥1.8 mmol/L), moderate-intensity statin therapy should be initiated without calculating 10-year ASCVD risk. In this group, but with LDL-C levels between 70 and 189 mg/dL (1.7–4.8 mmol/L), it is rational to evaluate the 10-year risk of a first ASCVD event. A high-intensity statin is considered reasonable in people with diabetes at higher risk, specifically those with manifold risk factors or those in the 50–75 years age group, in order to decrease LDL-C levels by 50% or more [54]. The ESC 2019 Guidelines have recognised the fact that though the relative risk reduction of CV events with statin therapy is same in diabetics as in non-diabetics, but as the risk of events is higher in diabetics, the absolute benefit is more in diabetic population and the number needed to treat is lower [45].

The recent ADA recommendations are in concurrence with those of AHA. For primary prevention, moderate-intensity statin therapy with lifestyle therapy is advocated in patients with diabetes without ASCVD aged 40–75 years. In those with diabetes with additional ASCVD risk factors in the 20–39 years age group, it may be judicious to start statins with lifestyle therapy. In diabetics at higher risk, particularly those aged 50–70 years with multiple ASCVD risk factors, using high-intensity statin therapy is considered reasonable. In those with diabetes and 10-year ASCVD risk of ≥20%, adding ezetimibe to maximally tolerated statin therapy to reduce LDL-C by >50% may be reasonable. For secondary prevention, the ADA recommends high-intensity statin therapy in addition to lifestyle therapy for patients of all ages with diabetes and ASCVD. For those with diabetes and ASCVD considered very high risk using specific criteria, adding additional LDL-lowering therapy could be considered if LDL-C level is ≥70 mg/dL on maximally tolerated statin dose; ezetimibe may be preferred due to lower cost. In diabetes adults above >75 years of age already on statins, continuing statin treatment may be reasonable. The statin dosing intensities recommended for use in clinical practice for patients with diabetes are high-intensity statin regimens (that achieve nearly ≥50%

Table 6 Recommended high-intensity and moderate-intensity once-daily statin therapy

Intensity of statin therapy	Drug	Dose (mg)
High-intensity	Atorvastatin	40–80
	Rosuvastatin	20–40
Moderate-intensity	Atorvastatin	10–20
	Rosuvastatin	5–10
	Simvastatin	20–40
	Pravastatin	40–80
	Lovastatin	40
	Fluvastatin extended-release	80
	Pitavastatin	1–4

reduction in LDL-C) and moderate-intensity statin therapy (that achieves 30–49% reductions in LDL-C) (Table 6) [99].

Intensification of statin therapy is recommended before initiating a combination therapy. Statin therapy may be considered in both type 1 and type 2 diabetic patients aged >30 years of age with LDL-C level >2.5 mmol/L and/or established end-organ damage, with the exclusion of pregnancy [45]. By and large, low-dose statin therapy is not prescribed for patients with diabetes except in cases where it is the only dose of tolerable statin [112]. Naeem and colleagues reviewed cardiovascular outcome trials with statins in people with diabetes and suggested a substantial benefit in plummeting cardiovascular events as part of primary prevention, whereas for secondary prevention, intensive lipid-lowering therapies with high-dose statins were found to be superior than standard lipid-lowering regimens in further decreasing cardiovascular events; however, higher doses might not be tolerated owing to a surge in adverse events [113].

The prospective, randomised, placebo-controlled Collaborative Atorvastatin Diabetes Study (CARDS) noted reductions in major cardiovascular events (37%), acute coronary heart disease–related events (36%), coronary revascularisations (31%), and stroke (48%) in patients with diabetes [114]. In the individual patient data-meta-analysis of statin therapy in At risk Groups: Effects of Rosuvastatin, atorvastatin, and simvastatin (VOYAGER) database with 27.5% patients with diabetes, rosuvastatin was more efficacious than atorvastatin and simvastatin, in lowering LDL-C and reaching a target level of <70 mg/dL for LDL-C. Furthermore, it was more effective in raising HDL-C than atorvastatin [115]. Pitavastatin is a potent moderate- to high-intensity β-Hydroxy β-methylglutaryl-CoA (HMG-CoA; statin) with LDL-C-lowering effects analogous to atorvastatin or rosuvastatin. Pitavastatin also offers a sustained increase of HDL-C levels, a vital element of diabetic dyslipidemia. Moreover, the pleiotropic effects of pitavastatin, which moderate the metabolic changes linked to adiposity and enhance glucose metabolism, separate it from other statins. This may perhaps not escalate the risk of new-onset diabetes. Therefore, pitavastatin could be preferred in the management of dyslipidemia in patients with diabetes or those at risk of developing diabetes [116–118].

Besides rising HDL-C levels, pitavastatin appears to enhance HDL function and reduce the development of atherosclerotic plaques by transforming HDL-related inflammation and oxidation, frequently encountered in patients with metabolic syndrome and type 2 diabetes. Furthermore, pitavastatin has been identified with distinctive pharmacological features that render wide-ranging activities on apo-A- and apo-B-containing lipoproteins, when compared to other statins. Numerous studies confirmed pitavastatin (1–4 mg) to be well-tolerated, with considerable improvements in LDL-C and triglyceride levels to a degree similar or greater than those of atorvastatin, simvastatin, or pravastatin, regardless of patients' diabetic status. While most statins display varying effects on HDL-C levels, patients treated with pitavastatin show clinically significant rises in HDL-C, which are usually sustained and even increased in the long run [119–122].

A prospective, comparative, randomised, controlled, double-blind, clinical trial by Patil et al. investigated the efficacy and safety of pitavastatin against atorvastatin in 100 dyslipidemic patients with hypertension, diabetes and/or CAD. By the end of 8 weeks, there were significant improvements in HDL-C (+11.00% vs +5.35%; $p<0.001$) and LDL-C/HDL-C ratio (−48.68% vs. −44.71%) with pitavastatin than atorvastatin [123]. The recent Scope for Atherosclerotic Cardiovascular Disease Risk Reduction study conducted in South India found a significant proportion of patients with high ASCVD risk who could benefit from statin therapy did not receive it [124].

Statin intolerance Though statins are generally safe and well-tolerated, for people at an intensified risk of ASCVD, the advantages prevail over the odds of adverse effects. Yet, a considerable proportion of statin-treated patients may encounter statin intolerance; this intolerance to statins or the inability to reach LDL-C targets could restrict the use of intensive statin therapy in such individuals [45].

Statin intolerance is the occurrence of (1) adverse symptoms perceived by the patient to be unacceptable, and/or (2) laboratory abnormalities suggesting undue risk, which are attributed to statin therapy and lead to its discontinuation [125]. The most common symptoms of the statin-induced myopathy include muscular pain, weakness, cramps, or stiffness, and may be caused by advanced age >75 years, female gender, abdominal obesity and metabolic syndrome, frailty, smaller body size, Asian ethnicity, alcohol consumption, vitamin D deficiency, excessive physical activity, uncontrolled hypothyroidism, chronic kidney disease, liver disease, family history of statin intolerance and personal history of intolerance to other statins and lipid-lowering therapies, metabolic muscle disorders, and treatments that elevate circulating levels of statins and/or their active metabolites (e.g., erythromycin, fluconazole) [125–127]. In such scenarios, smaller statin doses and/or less potent statins with lower incidence of myopathy (e.g., pitavastatin, extended-release fluvastatin), with vigilant dose up-titration should be considered [45].

In high-risk patients who are intolerant to statins, an amalgamation of lifestyle measures and non-statin drugs must be prescribed to attain LDL-C levels as close as possible to the established goal [126]. The diverse statin-based approaches suggested to manage muscle symptoms include moving to another statin, down-titrating the dose (de-challenge) or reducing the frequency (intermittent dosages), or re-challenging with the same statin. In order to achieve LDL-C goals with minimal or no muscle complaints, if well-tolerated the doses can be slowly up-titrated. Twice-weekly or alternate-day

dosing can be preferred in those who are unable to tolerate daily-dose statins. In cases where statins are not at all tolerated, other lipid-lowering agents as monotherapy or added to the maximum tolerated statin dose are suggested [127].

Ezetimibe Ezetimibe is an inhibitor of intestinal cholesterol absorption. On combination with statins, this drug provides additive and complementary therapeutic lipid effects, resulting in considerable reductions in LDL-C and significant achievement of target cholesterol levels [128]. Adding ezetimibe to statin therapy permits the use of lower dosage of statins without compromising efficacy, reducing the odds of dose-dependent statin adverse effects. Furthermore, ezetimibe seems to have a neutral effect on glucose metabolism; rather a beneficial effect on glycemic control when used for >3 months [93, 128, 129]. Most guidelines advocate the addition of ezetimibe when targets are not achieved with the maximum tolerated dose of statin [45]. The AHA suggests adding ezetimibe to maximally tolerated statin therapy to reduce LDL-C levels by ≥50% in people with diabetes and 10-year ASCVD risk of ≥20% [54].

A pre-specified subgroup analysis demonstrated a greater decrease in LDL-C levels with a combination of simvastatin–ezetimibe in patients with type 2 diabetes than those without diabetes (−16.6 vs. −14.3 mg/dL; p=0.003). Therapy with the combination showed significant relative risk reductions in myocardial infarction (−24%) and ischemic stroke (−39%) in the diabetic sub-population [130]. In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), the subset of patients with diabetes (27%; n=4933) had a higher rate of major vascular events than those without diabetes (46% vs. 31% 7-year Kaplan–Meier rate vs. placebo). Ezetimibe seemed specifically efficacious in diabetes, with a 15% (95% CI, 6–22%) relative risk reduction and 5.5% absolute risk reduction [131].

The Recognized Effect of Statin and Ezetimibe therapy for Achieving the LDL-C goal (RESEARCH) study was a randomised, multicentre, open-label, prospective study that assessed the 52-week long-term effect of ezetimibe as an add-on therapy in 109 type 2 diabetic patients with hypercholesterolemia, not attaining LDL-C target value despite first-line dose statin (10 mg atorvastatin or 1 mg pitavastatin) therapy. Ezetimibe exhibited a robust advantage in lowering LDL-C and achieving goal LDL-C values than with doubling the dosage of statin. What is more is that sd-LDL displayed noticeable steady decrease when ezetimibe was added to the statin [132]. In a recent multicentre, open-label, parallel-group study by Lee et al., 134 patients with type 2 diabetes were randomised to receive either a combination of rosuvastatin 5 mg/ezetimibe 10 mg once daily or rosuvastatin 10 mg once daily monotherapy for a period of 8 weeks. Compared to rosuvastatin monotherapy, ezetimibe as an add-on led to significant reductions in the apo B/A1 ratio (−46.14 ± 1.58% vs. −41.30 ± 1.58%; p=0.03). Besides, the proportion of patients achieving >50% reduction in LDL-C in the comprehensive lipid target significantly varied (76.5% and 73.5%, rosuvastatin/ezetimibe group; 47.1% and 45.6%, rosuvastatin group; p<0.001) among the groups [133].

Evidence suggests combination therapy of ezetimibe–statin may be a convenient strategy in people with diabetes at a residual risk of major adverse cardiovascular outcomes. In a meta-analysis and meta-regression of seven trials with 28,191 patients (7,298 with diabetes; 25.9%), ezetimibe was linked to a superior decline of MACE risk (pooled relative risk, 0.84 vs. 0.93; $P_{\text{heterogeneity}}$=0.012) in those with diabetes than in those without diabetes, particularly when added to statins (β=0.87, p=0.038) [134].

Bempedoic acid Bempedoic acid is a small molecule ATP-citrate lyase inhibitor being developed as a once-daily, first-in-class, oral drug in the management of hypercholesterolemia [135, 136]. This prodrug is activated by a liver enzyme (not present in skeletal muscle) and inhibits ATP-citrate lyase, which is an enzyme upstream of β-hydroxy β-methylglutaryl-coenzyme A reductase, in the cholesterol biosynthesis pathway. This molecule seems to provide a safe and effective oral therapeutic option for lipid lowering in patients intolerant to statins.

The phase 3, double-blind, placebo-controlled Cholesterol Lowering via bempedoic acid, an ACL-Inhibiting Regimen (CLEAR) Serenity study randomised 345 patients with hypercholesterolemia and a history of intolerance to ≥2 statins to bempedoic acid (180 mg; n=234) or placebo (n=111) once daily over 24 weeks. By week 12, treatment with bempedoic acid significantly lowered LDL-C (placebo-corrected difference, −21.4%; 95% CI, −25.1% to −17.7%; p<0.001), non-HDL-C (−17.9%), total cholesterol (−14.8%), apo B (−15.0%), and hsCRP (−24.3%; p<0.001 for all comparisons) [137].

A pooled analysis of four phase 3 clinical trials assessed the effect of bempedoic acid on glycemic control and new-onset diabetes in patients with hypercholesterolemia receiving stable lipid-lowering therapy. Compared to placebo, the use of bempedoic acid led to significantly lower HbA1c levels at week 12 in those with diabetes at baseline (mean placebo-corrected change: −0.19%; nominal p<0.0001) [138]. A recent systematic review and meta-analysis of 11 trials compared the use of bempedoic acid with either placebo or no treatment for primary prevention of cardiovascular events in 4,391 statin-intolerant patients with

hypercholesterolemia. Treatment with bempedoic acid was associated with a decrease in composite cardiovascular outcome (RR, 0.75; 95% CI, 0.56–0.99; I^2=0%) as well as LDL-C (mean difference, −22.91; 95% CI, −27.35 to −18.47; I^2=99%). The novel drug was also associated with decline in rates of new-onset or worsening diabetes (RR, 0.65; 95% CI, 0.44–0.96; I^2=23%) [110]. In addition to similar reductions in lipid profiles, another recent systematic review and meta-analysis recorded a strong association of bempedoic acid treatment with a decreased risk of new onset or worsening diabetes (OR, 0.59; 95% CI, 0.39–0.90; p=0.01) [136].

Proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors Human monoclonal antibodies, which target PCSK9, have demonstrated a reduction in LDL-C levels by 55–72% in diverse high-risk patient groups [139]. Treatment with PCSK9 inhibitors in diabetes induces analogous relative reductions in cardiovascular risk. This class of drugs does not raise blood glucose levels, unlike that seen with statins. A systematic review suggested aggressive use of treatment with PCSK9 inhibitors in patients with diabetes with a target of accomplishing and sustaining goal LDL-C levels even lower than that proposed for non-diabetic patients [140]. Aggressive lowering of LDL-C with human PCSK9 monoclonal antibodies has been established with a very favourable safety profile. Based on evidence from diverse clinical trials, LDL lowering with PCSK9 inhibitors is endorsed for high-risk patients with LDL-C levels ≥70 mg/dL on maximally tolerated oral therapies comprising of statins and/or ezetimibe [139].

As per the recent 2021 Canadian Cardiovascular Society Guidelines, patients with clinically evident ASCVD and diabetes mellitus derive the largest secondary prevention benefit from intensification of statin therapy along with a PCSK9 inhibitor [141]. The National Lipid Association Expert Panel recommend considering PCSK9 inhibitor therapy to further reduce LDL-C in patients aged 40–79 years with LDL-C ≥190 mg/dL, no uncontrolled ASCVD risk factors, or other significant additional-high risk markers (including diabetes) and on-treatment LDL-C ≥100 mg/dL or non–HDL-C ≥130 mg/dL on maximally tolerated statin therapy and/or ezetimibe [142]. These drugs are generally well-tolerated and offer substantial LDL-C lowering in patients with diabetes mellitus and hyperlipidemia when added to the maximally tolerated statin therapy, without affecting glycemic control or increasing the risk of developing diabetes mellitus in pre-existing diabetes mellitus; in fact, they can prevent or reduce further cardiovascular events [143, 144].

Alirocumab as well as evolocumab have demonstrated effective lowering of LDL-C in high cardiovascular risk patients, including those with diabetes mellitus. In the Further cardiovascular OUtcomes Research with PCSK9 inhibition in 27,564 subjects with Elevated Risk (FOURIER) trial, inhibition of PCSK9 with evolocumab in the presence of statin therapy reduced LDL-C levels to a median of 30 mg/dL (0.78 mmol/L) in addition to a fall in the risk of cardiovascular events [145]. A prespecified analysis of the FOURIER trial investigated the efficacy and safety of evolocumab by diabetes status and the effect of evolocumab on glycemia and risk of developing diabetes. The data affirmed the efficacy and safety of evolocumab in patients with atherosclerotic disease with and without diabetes. Neither did evolocumab intensify the risk of new-onset diabetes, nor did it aggravate glycemia [146].

The ODYSSEY DM-DYSLIPIDEMIA trial demonstrated the superiority of alirocumab to usual care in lowering non-HDL-C (−32.5% difference; 97.5% CI, −38.1 to −27.0; p<0.0001) in patients with type 2 diabetes and mixed dyslipidemia on maximally tolerated statin. Besides, alirocumab also significantly reduced LDL-C (−43.0%), apo B (−32.3%), total cholesterol (−24.6%), and LDL particle number (−37.8%) [147]. A systematic review and meta-analysis reported no association of PCSK9 inhibitors with risk of incident diabetes (RR, 1.00; 95% CI, 0.93–1.07; p=0.96; I^2=0%; RD, 0.001; 95% CI, −0.004 to 0.006; p=0.75; I^2=11%; $P_{interaction}$=0.02) [148].

Triglyceride-lowering therapy

In patients with ASCVD or diabetes and multiple risk factors and triglyceride levels of ≥150 mg/dL, icosapent ethyl (IPE) may provide additional risk reduction benefit beyond a statin. If triglyceride level is ≥500 mg/dL, prior to considering a fibrate or other non-statin drug, a statin with or without ezetimibe must be preferred. The primary objective in such cases is to decrease the risk of acute pancreatitis by lowering triglyceride level. Treatment should be initiated with a non-statin drug (e.g., fenofibrate) and later add statin to achieve LDL-C and non-HDL-C targets. Among non-statin drugs, omega-3 fatty acids, especially IPE (4 g/day) is preferred, since it has found to reduce adverse cardiovascular events in those with ASCVD or diabetes and multiple risk factors. In people with very high levels of triglycerides, fibrates must be initiated with simultaneous identification and control of secondary causes [7].

Fibrates The hallmark of diabetic dyslipidemia is increased triglycerides and low HDL-C levels. The exact benefits of fibrates on these parameters are still controversial. Although a meta-analysis in >11,000 diabetic patients showed that fibrates significantly decreased the risk of non-fatal myocardial infarction by 21%, there was no effect on the risk of overall or cardiovascular mortality [149]. The effects on fibrates in people with type 2 diabetes without elevated levels

of triglycerides were demonstrated to be much lesser on increasing HDL-C (5%) and lowering triglycerides (20%) in longer duration studies [150, 151]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study showed a significant change in lipoprotein levels with fenofibrate in patients with diabetes. Furthermore, there were improvements in glycemic parameters especially in women. It was also effective in reducing total CVD event risk in women with type 2 diabetes, especially those with dyslipidemia [152]. Fenofibrate therapy, in a statin-free cohort with type 2 diabetes (n=171) of the FIELD study, demonstrated long-term benefits on VLDL-C and HDL-C. There was a noticeable decrease in large VLDL particles associated with smaller HDL particles [150].

In the follow-up of Action to Control Cardiovascular Disease in Diabetes (ACCORD) lipid study conducted in >4500 patients, fenofibrate therapy was shown to reduce CVD in diabetic patients with elevated triglycerides and low HDL-C levels (HR, 0.73; 95% CI, 0.56–0.95) [151]. The Diabetes Atherosclerosis Intervention Study (DAIS), conducted in 204 patients with type 2 diabetes, found significant reduction in triglyceride levels with fenofibrate, remnant-like particle cholesterol (RLP-C) and activity of lipoprotein-associated phospholipase A2, along with an increase in the HDL-C levels [153].

Saroglitazar Saroglitazar has well-documented positive effects in the management of diabetic dyslipidemia due to its dual mode of action, viz. agonistic activity on PPAR-α and PPAR- . It not only improves lipid parameters (triglycerides, apo B, non-HDL-C), but also has a significant impact on glycemic parameters (HbA1c and fasting blood glucose) in dyslipidemic patients. It is devoid of conventional side effects of fibrates and pioglitazone. On account of its unique insulin sensitising action, the potential of hypoglycemic effect is low; however, it could occur when combined with other agents like sulphonylureas or insulin [154]. The improvements in lipid parameters with saroglitazar are especially valuable in dyslipidemia patterns commonly seen in Indians. Improvement in insulin sensitivity delivers glycemic control. The innovative chemical entity is a 'first in class' drug to be approved anywhere in the world demonstrating higher efficacy in decreasing triglycerides and non-HDL-C, with a twofold action on both dyslipidemia and hyperglycemia [155].

Long-term real-world evidence of up to 58 weeks, in more than 5000 Indian patients with diabetic dyslipidemia, suggested that saroglitazar improved both lipid and glycemic parameters without major adverse effects [156]. Another randomised, double-blind, placebo-controlled trial in 30 treatment-naive type 2 diabetes patients with serum triglyceride >150 mg/dL found saroglitazar to effectively reduce hypertriglyceridemia and improve insulin sensitivity along with β-cell function by reducing gluco-lipotoxicity, probably directly through PPAR-γ agonism. The latest PRESS XII (Phase III) study, with a primary endpoint of HbA1c reduction, involving >1000 patients with type 2 diabetes found a significant decrease in triglycerides, LDL-C, VLDL-C, total cholesterol, and non-HDL-C, associated with an important increase in HDL-C (<0.016). When added to metformin therapy, saroglitazar resulted in improved glucose control and lipid levels over 56 weeks. It thus appears as a novel therapy for decreasing the cardiovascular risk in patients with type 2 diabetes [157].

Omega-3 fatty acids Due to a considerably diverse mode of action, compared to other lipid-lowering drugs, omega-3 fatty acids offer additional benefits when administered as an add-on to statins. Furthermore, it lacks clinically significant drug interactions with statins and, unlike fibrates and niacin, does not deleteriously affect liver function [158]. Icosapent ethyl, which is available as a prescription form of eicosapentaenoic acid (EPA) ethyl ester, is indicated as an adjunct to diet to reduce triglyceride levels in patients with severe (≥500 mg/dL) hypertriglyceridemia [159]. The recent 2021 Canadian Cardiovascular Society Guidelines strongly recommend the use of IPE to reduce cardiovascular event risk in patients with ASCVD, or those with diabetes and ≥1 CVD risk factors, with an elevated fasting triglyceride of 1.5–5.6 mmol/L in spite of treatment with maximally tolerated statin therapy [141].

The Reduction of Cardiovascular Events with EPA-Intervention Trial (REDUCE-IT) was among the most significant clinical trials in recent history as it evaluated the potential benefits of EPA on cardiovascular outcomes in patients with hypertriglyceridemia. Nearly 8000 patients, already receiving a statin and LDL-C levels of 1.0–2.6 mmol/L (41–100 mg/dL) with cardiovascular risk factors like persistent hypertriglyceridemia (1.7–5.6 mmol/L; 150–499 mg/dL), and either established ASCVD or diabetes mellitus were analysed. Administration of EPA, although at a higher dose (2 g b.i.d.), was associated with ~25% relative risk reduction (p<0.001) in MACE compared to placebo (mineral oil) [160, 161]. ANCHOR, a 12-week phase 3 RCT, examined the effects of IPE 2 g/day or 4 g/day in >700 patients (73%, diabetes mellitus) with hypertriglyceridemia (200–500 mg/dL; although with normalised LDL-C, 40-100 mg/dL) on statin therapy. Significantly favourable effects on lipid parameters were noted with 4 g IPE, with no deterioration of glucose parameters in patients with diabetic dyslipidemia; the positive effects were enhanced in patients with poorly controlled diabetes [159].

A meta-analysis established that omega-3 fatty acids were associated with a significant decrease in apo AII (−8.0 mg/dL; 95% CI, −12.71 to −3.29, p=0.0009), triglycerides (−44.88 mg/dL; 95% CI, −82.6, −7.16, p<0.0001), and HDL (−2.27 mg/dL; 95% CI, −3.72 to −0.83; p=0.002) in the diabetic population compared to their control counterparts. These beneficial effects of omega-3 fatty acids could be attributed to the lowering of detrimental chronic inflammatory markers in people with diabetes and high-risk cardiovascular patients [162].

Special populations

Type 1 diabetes

Statins are recommended as first-line drug therapy in patients with type 1 diabetes with dyslipidemia as they are at high or very-high total CVD risk. If goals are not achieved with the maximum tolerated statin doses, adding ezetimibe is recommended. For patients at very-high risk not achieving their goal on a maximum tolerated dose of statin and ezetimibe, combining a PCSK9 inhibitor is recommended for secondary prevention. For very high-risk patients (with ASCVD or another major risk factor) not achieving their goals on a maximum tolerated dose of statin and ezetimibe, a combination with a PCSK9 inhibitor is recommended [45].

Pediatrics

In a metabolically unhealthy child with type 2 diabetes and obesity, a number of cardiovascular risk factors aggregate and intensify the risk of morbidity and mortality later in life [163]. The International Society for Pediatric and Adolescent Diabetes (ISPAD) and ADA advocate lipid screening either once glycemic control is achieved or after 3 months of starting therapy and yearly thereafter. Initial therapy should include optimising glucose control and MNT to avoid trans fats, and restricting calorie intake from fat to 25–30%, ~10% from monounsaturated fats, saturated fat to <7%, and cholesterol to <200 mg/day.

Recommended lipid goals include LDL-C <100 mg/dL, HDL-C >35 mg/dL, and triglycerides <150 mg/dL. A statin may be considered after the age of 10 years, in cases where despite lifestyle changes and MNT, LDL-C is >160 mg/dL (4.1 mmol/L), or LDL-C is >130 mg/dL (3.4 mmol/L) along with ≥1 CVD risk factors. Fibrates are commended when fasting triglycerides are >400 mg/dL or non-fasting triglycerides are >1000 mg/dL. Considering their demonstrated safety and efficacy in adolescents, fibrates are the preferred drug of choice for hypertriglyceridemia in this population. Despite the presence of aberrant levels of atherogenic triglyceride-rich lipoproteins, apo B, and non-HDL-C in pediatric type 2 diabetes, these are not measured for risk assessment or management [164, 165].

Pregnancy

Irregular maternal lipids during pregnancy are linked to adverse pregnancy outcomes for both the mother and the infant; maternal lipids in pregnancies are complicated by diabetes [166]. Regardless of the well-known clinical outcomes and advantages of a number of lipid-lowering therapies on atherogenic lipid profiles, there is a dearth of evidence in pregnancy. As a matter of fact, pregnant women are usually not included in clinical trials. Consequent to this, there are inadequate recommendations on the treatment of significant dyslipidemia in pregnant women, let alone in those with diabetes.

Lipid-lowering drugs should not be given when pregnancy is planned, during pregnancy, or during the breastfeeding period. Statin therapy is not recommended in pre-menopausal patients with diabetes who are considering pregnancy or not using adequate contraception. However, for severe FH patients, bile acid sequestrants (which are not absorbed) and/or LDL apheresis may be considered [45]. Monitoring is commended at every trimester or within 6 weeks of treatment initiation, and close follow-up of the mother is strongly advised [167].

Elderly

The latest ESC guidelines recommend statin therapy in the elderly considering the predictable risk level and baseline LDL-C, keeping in mind the patients' health condition and risk of drug interactions. In people aged >75 years, if at high-risk or above, statin treatment may be considered for primary prevention. In cases of substantial renal impairment and/or potential for drug interactions, statin therapy should be initiated at a low dose and later up-titrated to attain LDL-C goals [45]. The AHA guidelines advocate continuation of statin therapy in those aged >75 years with diabetes mellitus and already on statin therapy [54].

The effects of statin therapy are independent of age, and are governed by the baseline ASCVD risk and absolute reduction in LDL-C. A meta-analysis of randomised trials of statin therapy noted a proportional reduction of 25% (RR, 0.75; 95% CI, 0.73–0.78) in the risk of coronary revascularisation procedures with statin therapy or a more intensive statin regimen/1.0 mmol/L lower LDL-C, which did not significantly vary across age groups (p_{trend}=0.6) [168]. Another meta-analysis by the CTT trial investigators revealed an explicit reduction in the risk of major vascular events with statin as well as non-statin LDL-C-lowering therapy among patients aged

75 years and older as that in younger patients. In addition to decreasing morbidity and mortality, there were no offsetting safety concerns with lipid-lowering therapies in this population [169].

Where there is substantial evidence supporting the use of statins for secondary prevention in the elderly population, that for statins as primary prevention is less convincing. Even with scarce data on older people with diabetes, there appears to be no substantial difference depending on their diabetes status [170].

Hepatic conditions

The concurrence of type 2 diabetes and non-alcoholic fatty liver disease (NAFLD), also characterised by atherogenic dyslipidemia, exacerbates metabolic profile, thereby intensifying cardiovascular risk. The underlying disturbances of which include activation of hepatic de novo lipogenesis, hepatic overproduction of large triglyceride-rich very LDL and deferred clearance of triglyceride-rich lipoproteins; amplified secretion of these lipids into circulation results in diabetic dyslipidemia. Subsequently, all these factors, pooled with hyperglycemia, intensify CVD risk [171].

Hepatocellular damage is frequently used to assess the activity of plasma alanine aminotransferase (ALT). Mild ALT elevation is noted in 0.5–2% patients on statin therapy and is more common with high doses or potent statins. Such mild increase in ALT is not linked to changes in liver function or true hepatotoxicity. Since progression to liver failure is exceptional, guidelines no longer recommend routine ALT monitoring during statin treatment [45, 172]. Such trivial rise in ALT levels in asymptomatic statin users is clinically irrelevant. Statin therapy does not aggravate liver disease in those with mild ALT elevation on account of steatosis or NAFLD [173, 174]. Treatment with statins resulting in clinically apparent liver injury is very unusual and probably a class effect of statins [175]. Regardless of original observations of raised liver enzymes in clinical studies, the US FDA concluded that statins, as a class, do not negatively impact the liver; liver monitoring is hence not required. Nonetheless, statins are contraindicated in patients with active liver disease [176].

All guidelines acknowledge that lifestyle modifications play a vital role in the management of NAFLD but any medicines prescribed explicitly for NAFLD should be considered an off-label treatment [177]. In EVIDENCE IV—a phase II, randomised, USA-based study—saroglitazar 4 mg compared to placebo showed significant mean ALT reduction (−44.3 vs 4.1 %), HOMA IR (−5.1 vs −2.5), triglycerides (−70.3 vs −3.4), total cholesterol (−24.2 vs −4.4), and mean liver fat content (−4.2% vs −0.3%) ($p<0.05$ for all) [178]. A recently published, real-world study from India found saroglitazar to significantly improve transaminases and glycemic control as well as lipid parameters in NAFLD patients with diabetic dyslipidemia [179]. Saroglitazar could hence be considered a potential therapeutic option achieving the unmet need in the management of NAFLD.

Renal impairment

Statin therapy has not been associated with clinically substantial decline in renal function. Dose adjustment, keeping in mind the eGFR, may be judicious in those with severe kidney dysfunction on intensive statin regimes [175]. Additional analyses of the Japanese long-term prospective post-marketing surveillance LIVALO Effectiveness and Safety (LIVES) Study demonstrated an improvement in HbA1c levels in individuals with type 2 diabetes post long-term pitavastatin therapy and a significant upturn in eGRF in patients with chronic kidney disease [180]. A recent meta-analysis found both atorvastatin and rosuvastatin to improve GFR, whereas when compared to rosuvastatin, atorvastatin was more effective in reducing proteinuria [181].

With diabetes being a major cause of chronic renal failure worldwide, renal transplant has emerged as a dominant therapeutic option in the high-risk diabetic population with end-organ damage. The post-transplant period is complicated by pre-existing risk factors in these patients, which include severe insulin resistance, higher triglyceride levels, lower HDL-c, abnormalities in fibrinolysis and coagulation and endothelial dysfunction, thereby increasing cardiovascular mortality [182]. Moreover, the use of inhibitors of the mammalian target of rapamycin (m-TOR) immunosuppressants like sirolimus and everolimus further deteriorates hyperlipidemia [183].

The new drug inclisiran is an inhibitor of the mRNA transduction of the PCSK9 gene. It could be considered in clinical practice for the overall management of patients with dyslipidemia on account of its sustained action and evident ability to lower LDL-C [184, 185]. Results from the ORION-1 RCT pointed towards the possibility of PCSK9-targeted small interfering RNA (siRNA)–driven strategies as a novel therapeutic option for managing dyslipidemia both in the presence and absence of diabetes [186]. Inclisiran has been found to be safe in patients with mild, moderate, or severe renal impairment without the need for adjustments in dose or dosing regimen in patients with established ASCVD and in those at high risk for subsequent major adverse

cardiovascular events [185]. As the common cause of renal transplant is diabetic kidney disease, inclisiran could be included in the future for the management of dyslipidemia in diabetic patients with solid organ transplant (liver, kidney, etc.)

Taking into account the evidence available in patients with diabetic dyslipidemia and the experience and consensus of the experts, we recommend a step-wise approach for the management for diabetic dyslipidemia in the Indian population (Table 7).

Table 7 Step-wise management strategy for Indian patients with diabetic dyslipidemia

Step-wise management strategy for Indian patients with diabetic dyslipidemia		
Stratify risk for ASCVD	**Age** <40 years ≥40 years	**ASCVD risk** Moderate risk High risk
	ASCVD risk score	Use the QRISK3 tool
Set lipid goals as per ASCVD risk	**LDL-C** goals for patients at: Moderate risk High risk Very high risk	**Target goal** Baseline to 30% Baseline to <50% Baseline to >50% OR Equivalent to HDL-C
	Triglycerides	<150 mg/dL
Initiate and escalate lipid lowering drugs based on established goals	**LDL-C** *Statins* Moderate-intensity High-intensity *Ezetemibe* *PCSK9 inhibitors*	**Management** Atorvastatin 10-20 mg Rosuvastatin 5-10 mg Simvastatin 20-40 mg Pitavastatin 2-4 mg Atorvastatin 40-80 mg Rosuvastatin 20-40 mg When goal not met with high-intensity statins In patients with established ASCVD
	Triglycerides Statins Fibrates* Saroglitazar* Omega 3-fatty acids*	At moderate- or high-intensity per CVD risk *Can be initiated when triglyceride is >200mg/dL

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *PCSK9,* Proprotein convertase subtilisin-kexin type 9.

Acknowledgment We acknowledge the assistance provided by consultant medical writers, Dr. Aafreen Saiyed and Dr. Suraj Ghag, in the preparation of these Guidelines.

Declarations

Competing interests The authors declare no competing interests.

References

1. Feingold KR, Grunfeld C. Diabetes and dyslipidemia. Endotext [Internet]. 2019 Jan 3.
2. Spratt KA. Managing diabetic dyslipidemia: aggressive approach. J Am Osteopathic Assoc. 2009;109(5_suppl_1):S2–7.
3. Gowtham K, Gandhe MB, Salwe KJ, Srinivasan AR. HDL/LDL ratio as a risk factor in type 2 diabetes mellitus. Adv Lab Med Int. 2012;2:9–18.
4. Chhatriwala MN, Patel MP, Patel DS, Shah HN. Relationship between dyslipidemia and glycemic status in type-2 diabetes mellitus. Natl J Lab Med. 2019;8(4):BO01–4.
5. Chandra KS, Bansal M, Nair T, Iyengar SS, Gupta R, Manchanda SC, et al. Consensus statement on management of dyslipidemia in Indian subjects. Indian Heart J. 2014;66(Suppl 3):S1.
6. Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes dyslipidemia. Diabetes Therapy. 2016;7(2):203–19.
7. Puri R, Mehta V, Iyengar SS, Narasingan SN, Duell PB, Sattur GB, et al. Lipid Association of India expert consensus statement on management of dyslipidemia in Indians 2020: Part III. J Assoc Phys India. 2020;68(11 [Special]):8–9.
8. Khan MA, Hashim MJ, King J, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes—global burden of disease and forecasted trends. J Epidemiol Glob Health. 2020 Mar;10(1):107–11.
9. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2019;157:107843.
10. The World Bank [Website]. Diabetes prevalence. Accessed on 04th December 2019. Available from: https://data.worldbank.org/indicator/SH.STA.DIAB.ZS?end=2019&name_desc=false&start=2019&type=shaded&view=map&year=2019.
11. International Diabetes Federation. Updated on: 3rd March 2020. Cited on: 24th November 2020. Available from: https://idf.org/our-network/regions-members/south-east-asia/members/94-india.html.
12. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, et al. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. Lancet Glob Health. 2018;6(12):e1352–62.
13. Parikh RM, Joshi SR, Menon PS, Shah NS. Prevalence and pattern of diabetic dyslipidemia in Indian type 2 diabetic patients. Diabetes Metab Syndr. 2010;4(1):10–2.
14. Joshi SR, Anjana RM, Deepa M, Pradeepa R, Bhansali A, et al. Prevalence of dyslipidemia in urban and rural India: the ICMR–INDIAB Study. PLoS ONE. 2014;9(5):e96808. https://doi.org/10.1371/journal.pone.0096808.
15. Mithal A, Majhi D, Shunmugavelu M, Talwarkar PG, Vasnawala H, Raza AS. Prevalence of dyslipidemia in adult Indian diabetic patients: a cross sectional study (SOLID). Indian J Endocrinol Metabol. 2014;18(5):642.
16. Dayakar E, Sree CS, Sanjay E. Study on the prevalence of dyslipidemia in type 2 diabetes mellitus. Int J Adv Med. 2019;6:786–9.
17. Bulut T, Demirel F, Metin A. The prevalence of dyslipidemia and associated factors in children and adolescents with type 1 diabetes. J Pediatr Endocrinol Metab. 2017;30(2):181–7.
18. Shah N, Khadilkar A, Gondhalekar K, Khadilkar V. Prevalence of dyslipidemia in Indian children with poorly controlled type 1 diabetes mellitus. Pediatr Diabetes. 2020;21(6):987–94.
19. Claypool KT, Chung MK, Deonarine A, Gregg EW, Patel CJ. Characteristics of undiagnosed diabetes in men and women under the age of 50 years in the Indian subcontinent: the National Family Health Survey (NFHS-4)/Demographic Health Survey 2015–2016. BMJ Open Diab Res Care. 2020;8(1):e000965.
20. Najafipour H, Shokoohi M, Yousefzadeh G, Azimzadeh BS, Kashanian GM, Bagheri MM, et al. Prevalence of dyslipidemia and its association with other coronary artery disease risk factors among urban population in Southeast of Iran: results of the Kerman coronary artery disease risk factors study (KERCADRS). J Diabetes Metab Disord. 2016;15(1):49.
21. Artha IM, Bhargah A, Dharmawan NK, Pande UW, Triyana KA, Mahariski PA, et al. High level of individual lipid profile and lipid ratio as a predictive marker of poor glycemic control in type-2 diabetes mellitus. Vasc Health Risk Manag. 2019;15:149.
22. Halcox J, Misra A. Type 2 diabetes mellitus, metabolic syndrome, and mixed dyslipidemia: how similar, how different, and how to treat? Metab Syndr Relat Disord. 2015;13(1):1–21.
23. Simha V. Management of hypertriglyceridemia. BMJ. 2020 Oct12;371:m3109.
24. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(3):150–9.
25. Panikar V. Chapter 101: mixed dyslipidemia. Medicine Update. 2008;18:764.
26. Kei A, Miltiadous G, Bairaktari E, Hadjivassiliou M, Cariolou M, Elisaf M. Dysbetalipoproteinemia: two cases report and a diagnostic algorithm. World J Clin Cases. 2015;3(4):371.
27. Varghese MJ. Familial hypercholesterolemia: a review. Ann Pediatr Cardiol. 2014;7(2):107.
28. Gaddi A, Cicero AF, Odoo FO. Practical guidelines for familial combined hyperlipidemia diagnosis: an up-date. Vasc Health Risk Manag. 2007;3(6):877.
29. Koopal C, Marais AD, Visseren FL. Familial dysbetalipoproteinemia: an underdiagnosed lipid disorder. Curr Opin Endocrinol Diabetes Obes. 2017;24(2):133–9.
30. Handelsman Y, Jellinger PS, Guerin CK, Bloomgarden ZT, Brinton EA, Budoff MJ, et al. Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the management of dyslipidemia and prevention of cardiovascular disease algorithm—2020 executive summary. Endocr Pract. 2020;26(10):1196–224.
31. Vodnala D, Rubenfire M, Brook RD. Secondary causes of dyslipidemia. Am J Cardiol. 2012;110(6):823–5.
32. Kolovou GD, Anagnostopoulou KK, Kostakou PM, Bilianou H, Mikhailidis DP. Primary and secondary hypertriglyceridaemia. Curr Drug Targets. 2009;10(4):336–43.
33. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J. 2011;32(11):1345–61.
34. Musunuru K. Atherogenic dyslipidemia: cardiovascular risk and dietary intervention. Lipids. 2010;45(10):907–14.
35. Krishnamurthy V, Kerekoppa AR, Prabhakar B. Cross-sectional study of pattern of dyslipidemia and prevalence of atherogenic diabetic dyslipidemia in newly detected diabetic patients. Asian J Med Sci. 2019;10(6):45–9.
36. Gudbjartsson DF, Thorgeirsson G, Sulem P, Helgadottir A, Gylfason A, Saemundsdottir J, et al. Lipoprotein (a) concentration

and risks of cardiovascular disease and diabetes. J Am Coll Cardiol. 2019;74(24):2982–94.
37. Vergès B. Lipid modification in type 2 diabetes: the role of LDL and HDL. Fundam Clin Pharmacol. 2009;23(6):681–5.
38. 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):1–9.
39. Younis NN, Durrington PN. HDL functionality in diabetes mellitus: potential importance of glycation. Clin Lipidol. 2012;7(5):561–78.
40. Ganjali S, Dallinga-Thie GM, Simental-Mendía LE, Banach M, Pirro M, Sahebkar A. HDL functionality in type 1 diabetes. Atherosclerosis. 2017;267:99–109.
41. Farbstein D, Levy AP. HDL dysfunction in diabetes: causes and possible treatments. Expert Rev Cardiovasc Ther. 2012;10(3): 353–61.
42. Lee JS, Chang PY, Zhang Y, Kizer JR, Best LG, Howard BV. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the Strong Heart Study. Diabetes care. 2017;40(4):529–37.
43. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Extreme lipoprotein (a) levels and improved cardiovascular risk prediction. J Am Coll Cardiol. 2013;61(11):1146–56.
44. Willeit P, Kiechl S, Kronenberg F, Witztum JL, Santer P, Mayr M, et al. Discrimination and net reclassification of cardiovascular risk with lipoprotein (a): prospective 15-year outcomes in the Bruneck Study. J Am Coll Cardiol. 2014;64(9):851–60.
45. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111–88.
46. Zhang HW, Zhao X, Guo YL, Gao Y, Zhu CG, Wu NQ, et al. Elevated lipoprotein (a) levels are associated with the presence and severity of coronary artery disease in patients with type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis. 2018;28(10):980–6.
47. Singla S, Kaur K, Kaur G, Kaur H, Kaur J, Jaswal S. Lipoprotein (a) in type 2 diabetes mellitus: relation to LDL: HDL ratio and glycemic control. Int J Diabetes Dev Ctries. 2009;29(2):80.
48. Hermans MP, Ahn SA, Rousseau MF. The mixed benefit of low lipoprotein (a) in type 2 diabetes. Lipids Health Dis. 2017;16(1): 171.
49. Zhang P, Gao J, Pu C, Zhang Y. Apolipoprotein status in type 2 diabetes mellitus and its complications. Mol Med Rep. 2017;16(6):9279–86.
50. Kanani FH, Alam JM. Apolipoprotein B in type 2 diabetics—a cross sectional study in a tertiary care set-up. J Pak Med Assoc. 2010;60(8):653.
51. Lee B, Pratumvinit B, Thongtang N. The role of apoB measurement in type 2 diabetic patients. Clin Lipidol. 2015;10(2):137–44.
52. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. Endocr Pract. 2017;23(s2):1–87.
53. Chawla R, Madhu SV, Makkar BM, Ghosh S, Saboo B, Kalra S. RSSDI-ESI clinical practice recommendations for the management of type 2 diabetes mellitus 2020. Indian J Endocr Metab. 2020;24:1–122.
54. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139:e1082–143.
55. O'Keefe JH, Bell DS. Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. Am J Cardiol. 2007;100(5):899–904.
56. Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. Clin Chem. 2011;57(3):482–9.
57. Nakamura K, Miyoshi T, Yunoki K, Ito H. Postprandial hyperlipidemia as a potential residual risk factor. J Cardiol. 2016;67(4): 335–9.
58. Anderson TJ, Mancini GJ, Genest J Jr, Grégoire J, Lonn EM, Hegele RA. The new dyslipidemia guidelines: what is the debate? Can J Cardiol. 2015;31(5):605–12.
59. de Vries MA. Novel pro-and anti-atherogenic effects of apolipoprotein B-containing lipoproteins: To feast or to fast? 2017.
60. Nordestgaard BG. A test in context: lipid profile, fasting versus nonfasting. J Am Coll Cardiol. 2017 Sep 18;70(13):1637–46.
61. Rahman F, Blumenthal RS, Jones SR, Martin SS, Gluckman TJ, Whelton SP. Fasting or non-fasting lipids for atherosclerotic cardiovascular disease risk assessment and treatment? Curr Atheroscler Rep. 2018;20(3):14.
62. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944–58.
63. Aldasouqi S, Sheikh A, Klosterman P, Kniestedt S, Schubert L, Danker R, et al. Hypoglycemia in patients with diabetes on antidiabetic medications who fast for laboratory tests. Diabetes Care. 2011;34(5):e52.
64. Mancini GJ, Hegele RA, Leiter LA. Dyslipidemia. Can J Diabetes. 2018;42:S178–85.
65. Sathiyakumar V, Park J, Golozar A, Lazo M, Quispe R, Guallar E, et al. Fasting versus nonfasting and low-density lipoprotein cholesterol accuracy. Circulation. 2018;137(1):10–9.
66. Stone NJ, Robinson JG, Lichtenstein AH, Merz CN, Blum CB, Eckel RH, et al. ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;63(25 Part B):2889–934.
67. Driver SL, Martin SS, Gluckman TJ, Clary JM, Blumenthal RS, Stone NJ. Fasting or nonfasting lipid measurements: it depends on the question. J Am Coll Cardiol. 2016;67(10):1227–34.
68. Yoshida H. Determination of fasting and non-fasting cholesterol levels of low-and high-density lipoproteins with homogenous assays: a promising reliable way to assessment of dyslipidemia. J Atheroscler Thromb. 2017;24(6):569–71.
69. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118(10):993.
70. Doran B, Guo Y, Xu J, Weintraub H, Mora S, Maron DJ, et al. Prognostic value of fasting versus nonfasting low-density lipoprotein cholesterol levels on long-term mortality: insight from the National Health and Nutrition Examination Survey III (NHANES-III). Circulation. 2014;130(7):546–53.
71. Fatima S, Ijaz A, Sharif TB, Khan DA, Siddique A. Accuracy of non-fasting lipid profile for the assessment of lipoprotein coronary risk. J Coll Physicians Surg Pak. 2016;26:954–7.
72. Andrade C. Nonfasting lipid profile may suffice to manage dyslipidemia. Indian J Psychol Med. 2020;42(3):316–7.
73. Chamnan P, Simmons RK, Sharp SJ, Griffin SJ, Wareham NJ. Cardiovascular risk assessment scores for people with diabetes: a systematic review. Diabetologia. 2009;52(10):2001.

74. D'agostino RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care. Circulation. 2008;117(6):743–53.
75. Woodward M, Brindle P, Tunstall-Pedoe H. Adding social deprivation and family history to cardiovascular risk assessment: the ASSIGN score from the Scottish Heart Health Extended Cohort (SHHEC). Heart. 2007;93(2):172–6.
76. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. BMJ. 2017;23:357.
77. Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. Circulation. 2002;105(3):310–5.
78. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007;297(6):611–9.
79. Ferrario M, Chiodini P, Chambless LE, Cesana G, Vanuzzo D, Panico S, et al. Prediction of coronary events in a low incidence population. Assessing accuracy of the CUORE Cohort Study prediction equation. Int J Epidemiol. 2005;34(2):413–21.
80. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'agostino RB, Gibbons R, et al. ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;63(25 Part B):2935–59.
81. Hajifathalian K, Ueda P, Lu Y, Woodward M, Ahmadvand A, Aguilar-Salinas CA, et al. A novel risk score to predict cardiovascular disease risk in national populations (Globorisk): a pooled analysis of prospective cohorts and health examination surveys. Lancet Diabetes Endocrinol. 2015;3(5):339–55.
82. Jahangiry L, Farhangi MA, Rezaei F. Framingham risk score for estimation of 10-years of cardiovascular diseases risk in patients with metabolic syndrome. J Health Popul Nutr. 2017;36(1):1–6.
83. Stephens JW, Ambler G, Vallance P, Betteridge DJ, Humphries SE, Hurel SJ. Cardiovascular risk and diabetes. Are the methods of risk prediction satisfactory? Eur J Cardiovasc Prev Rehabil. 2004;11(6):521–8.
84. Stevens RJ, Kothari V, Adler AI, Stratton IM, Holman RR, United Kingdom Prospective Diabetes Study (UKPDS) Group. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). Clin Sci. 2001;101(6):671–9.
85. QRISK®3 score [Website]. Version 2018.0. Last Updated on: 13th August 2018. Cited on: 19th April 2021. Available from: https://qrisk.org/three/index.php.
86. Ghosal S, Sinha B, Ved J, Biswas M. Quantitative measure of asymptomatic cardiovascular disease risk in type 2 diabetes: evidence from Indian outpatient setting. Indian Heart J. 2020;72(2):119–22.
87. Orringer CE, Blaha MJ, Blankstein R, Budoff MJ, Goldberg RB, Gill EA, et al. The National Lipid Association scientific statement on coronary artery calcium scoring to guide preventive strategies for ASCVD risk reduction. J Clin Lipidol. 2021 Jan-Feb;15(1):33–60
88. Elkeles RS, Godsland IF, Feher MD, Rubens MB, Roughton M, Nugara F, et al. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. Eur Heart J. 2008;29(18):2244–51.
89. Raggi P, Shaw LJ, Berman DS, Callister TQ. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. J Am Coll Cardiol. 2004;43:1663–9.
90. Anand DV, Lim E, Hopkins D, Corder R, Shaw LJ, Sharp P, et al. Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. Eur Heart J. 2006;27:713–21.
91. Young LH, Frans JT, Chyun DA, Davey JA, Barrett EJ, Taillefer R, et al. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. JAMA. 2009;301(15):1547–55.
92. Reiner Ž, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Eur Heart J. 2011;32(14):1769–818.
93. Wu H, Shang H, Wu J. Effect of ezetimibe on glycemic control: a systematic review and meta-analysis of randomized controlled trials. Endocrine. 2018;60(2):229–39.
94. Kendall CW, Jenkins DJ. A dietary portfolio: maximal reduction of low-density lipoprotein cholesterol with diet. Curr Atheroscler Reports. 2004;6(6):492–8.
95. Jenkins DJ, Kendall CW, Faulkner DA, Nguyen T, Kemp T, Marchie A, et al. Assessment of the longer-term effects of a dietary portfolio of cholesterol-lowering foods in hypercholesterolemia. Am J Clin Nutr. 2006;83:582–91.
96. Kelley GA, Kelley KS. Impact of progressive resistance training on lipids and lipoproteins in adults: a meta-analysis of randomized controlled trials. Prev Med. 2009;48(1):9–19.
97. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the study of obesity. Circulation. 2009;120(16):1640–5.
98. Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, Look AHEAD Research Group, et al. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. Diabetes Care. 2011;34(7):1481–6.
99. American Diabetes Association. 5. Facilitating behavior change and well-being to improve health outcomes: standards of Medical Care in Diabetes 2021. Diabetes Care. 2021;44(Suppl. 1):S53–72.
100. Shantakumari N, Sequeira S. Effects of a yoga intervention on lipid profiles of diabetes patients with dyslipidemia. Indian Heart J. 2013;65(2):127–31.
101. Gordon L, McGrowder DA, Pena YT, Cabrera E, Lawrence-Wright M. Effect of exercise therapy on lipid parameters in patients with end-stage renal disease on hemodialysis. J Lab Physicians. 2012;4(1):17.
102. Nagarathna R, Tyagi R, Kaur G, Vendan V, Acharya IN, Anand A, et al. Efficacy of a validated yoga protocol on dyslipidemia in diabetes patients: NMB-2017 India trial. Medicines. 2019;6(4):100.
103. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J. 2011;32(12):1484–92.
104. Boehm JK, Williams DR, Rimm EB, Ryff C, Kubzansky LD. Relation between optimism and lipids in midlife. Am J Cardiol. 2013;111(10):1425–31.
105. Evert AB, Dennison M, Gardner CD, Garvey WT, Lau KH, MacLeod J, et al. Nutrition therapy for adults with diabetes or prediabetes: a consensus report. Diabetes Care. 2019;42(5):731–54.
106. Myers EF, Trostler N, Varsha V, Voet H. Insights from the Diabetes in India Nutrition Guidelines Study: adopting

innovations using a knowledge transfer model. Top Clin Nutr. 2017;32(1):69.
107. Marincic PZ, Salazar MV, Hardin A, Scott S, Fan SX, Gaillard PR, et al. Diabetes self-management education and medical nutrition therapy: a multisite study documenting the efficacy of registered dietitian nutritionist interventions in the management of glycemic control and diabetic dyslipidemia through retrospective chart review. J Acad Nutr Diet. 2019;119(3):449–63.
108. Hegele RA, Gidding SS, Ginsberg HN, McPherson R, Raal FJ, Rader DJ, et al. Nonstatin low-density lipoprotein–lowering therapy and cardiovascular risk reduction—statement from ATVB council. Arterioscler, Thromb, Vasc Biol. 2015;35(11):2269–80.
109. Trialists CT. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet. 2008;371(9607):117–25.
110. Wang N, Fulcher J, Abeysuriya N, Park L, Kumar S, Di Tanna GL, et al. Intensive LDL cholesterol-lowering treatment beyond current recommendations for the prevention of major vascular events: a systematic review and meta-analysis of randomised trials including 327,037 participants. Lancet Diabetes Endocrinol. 2020;8(1):36–49.
111. Hadjiphilippou S, Ray KK. Cholesterol-lowering agents: statins—for everyone? Circ Res. 2019;124(3):354–63.
112. Khalil S, Khayyat S, Al-Khadra Y, Alraies MC. Should all diabetic patients take statin therapy regardless of serum cholesterol level? Expert Rev Cardiovasc Ther. 2019;17(4):237–9.
113. Naeem F, McKay G, Fisher M. Cardiovascular outcomes trials with statins in diabetes. Br J Diabetes. 2018;18(1):7–13.
114. Armani A, Toth PP. The CARDS trial: diabetic patients dealt a winning hand. Curr Atheroscler Rep. 2006;8(5):429–32.
115. Karlson BW, Barter PJ, Palmer MK, Lundman P, Nicholls SJ. Comparison of the effects of different statins and doses on lipid levels in patients with diabetes: results from VOYAGER. Nutr Metab Cardiovasc Dis. 2012;22(9):697–703.
116. Kawai Y, Sato-Ishida R, Motoyama A, Kajinami K. Place of pitavastatin in the statin armamentarium: promising evidence for a role in diabetes mellitus. Drug Des Devel Ther. 2011;5:283.
117. Barrios V, Escobar C. Clinical benefits of pitavastatin: focus on patients with diabetes or at risk of developing diabetes. Future Cardiol. 2016;12(4):449–66.
118. Martín-Timón I, Sevillano-Collantes C, García-Domínguez M, Marín-Peñalver JJ, Ugalde-Abiega B, del Cañizo-Gómez FJ. Update on the management of diabetic dyslipidaemia. EMJ Diabet. 2018;6(1):53–61.
119. Masana L. Pitavastatin in cardiometabolic disease: therapeutic profile. Cardiovasc Diabetol. 2013;12(1):1–8.
120. Mita T, Nakayama S, Abe H, Gosho M, Iida H, Hirose T, et al. Comparison of effects of pitavastatin and atorvastatin on glucose metabolism in type 2 diabetic patients with hypercholesterolemia. J Diabetes Investig. 2013;4(3):297–303.
121. Gumprecht J, Gosho M, Budinski D, Hounslow N. Comparative long-term efficacy and tolerability of pitavastatin 4 mg and atorvastatin 20–40 mg in patients with type 2 diabetes mellitus and combined (mixed) dyslipidaemia. Diabetes Obes Metab. 2011;13(11):1047–55.
122. Hoy SM. Pitavastatin: a review in hypercholesterolemia. Am J Cardiovasc Drugs. 2017;17(2):157–68.
123. Patil CY, Baig MS, Doifode SM. Assessing the efficacy and safety of pitavastatin compared to atorvastatin in dyslipidemic patients: a double blind randomized controlled trial. Int J Basic Clin Pharmacol. 2016;5:834–40.
124. Jayakumari C, Jabbar PK, Soumya S, Jayakumar RV, Das DV, Girivishnu G, et al. Lipid profile in Indian patients with type 2 diabetes: the scope for atherosclerotic cardiovascular disease risk reduction. Diabetes Spectr. 2020;33(4):299–306.
125. Stulc T, Ceška R, Gotto AM. Statin intolerance: the clinician's perspective. Curr Atheroscler Rep. 2015;17(12):1–7.
126. Bitzur R, Cohen H, Kamari Y, Harats D. Intolerance to statins: mechanisms and management. Diabetes Care. 2013;36(Supplement 2):S325–30.
127. Alonso R, Cuevas A, Cafferata A. Diagnosis and management of statin intolerance. J Atheroscler Thromb. 2019 Mar 1;26(3):207–15.
128. Ferreira AM, da Silva PM. Defining the place of ezetimibe/atorvastatin in the management of hyperlipidemia. Am J Cardiovasc Drugs. 2017 Jun;17(3):169–81.
129. Barkas F, Elisaf M, Liberopoulos E, Klouras E, Liamis G, Rizos EC. Statin therapy with or without ezetimibe and the progression to diabetes. J Clin Lipidol. 2016 Mar 1;10(2):306–13.
130. Bohula EA, Giugliano RP, Cannon CP, Zhou J, Murphy SA, White JA, et al. Achievement of dual low-density lipoprotein cholesterol and high-sensitivity C-reactive protein targets more frequent with the addition of ezetimibe to simvastatin and associated with better outcomes in IMPROVE-IT. Circulation. 2015;132(13):1224–33.
131. Giugliano RP, Cannon CP, Blazing MA, Nicolau JC, Corbalán R, Špinar J, et al. Benefit of adding ezetimibe to statin therapy on cardiovascular outcomes and safety in patients with versus without diabetes mellitus: results from IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial). Circulation. 2018 Apr 10;137(15):1571–82.
132. Sakamoto K, Kawamura M, Watanabe T, Ashidate K, Kohro T, Tanaka A, et al. Effect of ezetimibe add-on therapy over 52 weeks extension analysis of prospective randomized trial (RESEARCH study) in type 2 diabetes subjects. Lipids Health Dis. 2017 Dec;16(1):1–9.
133. Lee J, Hwang YC, Lee WJ, Won JC, Song KH, Park CY, et al. Comparison of the efficacy and safety of rosuvastatin/ezetimibe combination therapy and rosuvastatin monotherapy on lipoprotein in patients with type 2 diabetes: multicenter randomized controlled study. Diabetes Ther. 2020 Feb;17:1–3.
134. Hong N, Lee YH, Tsujita K, Gonzalez JA, Kramer CM, Kovarnik T, et al. Comparison of the effects of ezetimibe-statin combination therapy on major adverse cardiovascular events in patients with and without diabetes: a meta-analysis. Endocrinol Metab. 2018 Jun;33(2):219.
135. Wang X, Zhang Y, Tan H, Wang P, Zha X, Chong W, et al. Efficacy and safety of bempedoic acid for prevention of cardiovascular events and diabetes: a systematic review and meta-analysis. Cardiovasc Diabetol. 2020 Dec;19(1):1–9.
136. Cicero AF, Fogacci F, Hernandez AV, Banach M. Lipid and Blood Pressure Meta-Analysis Collaboration (LBPMC) Group and the International Lipid Expert Panel (ILEP). Efficacy and safety of bempedoic acid for the treatment of hypercholesterolemia: A systematic review and meta-analysis. PLoS Med. 2020;17(7):e1003121.
137. Laufs U, Banach M, Mancini GJ, Gaudet D, Bloedon LT, Sterling LR, et al. Efficacy and safety of bempedoic acid in patients with hypercholesterolemia and statin intolerance. J Am Heart Assoc. 2019;8(7):e011662.
138. Leiter L, Banach M, Catapano A, Duell P, Gotto A, Laufs U, et al. Bempedoic acid and glycemic control: a pooled analysis of 4 phase 3 clinical trials. J Clin Lipidol. 2020;14(4):577–9.
139. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The evolving future of PCSK9 inhibitors. J Am Coll Cardiol. 2018;72(3):314–29.
140. Monami M, Sesti G, Mannucci E. PCSK9 inhibitor therapy: a systematic review and meta-analysis of metabolic and cardiovascular outcomes in patients with diabetes. Diabetes Obes Metab. 2019;21(4):903–8.

141. Pearson GJ, Thanassoulis G, Anderson TJ, Barry AR, Couture P, Dayan N, et al. 2021 Canadian Cardiovascular Society guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2021;37:1129–50.
142. Orringer CE, Jacobson TA, Saseen JJ, Brown AS, Gotto AM, Ross JL, et al. Update on the use of PCSK9 inhibitors in adults: recommendations from an Expert Panel of the National Lipid Association. J Clin Lipidol. 2017;11(4):880–90.
143. Handelsman Y, Lepor NE. PCSK9 inhibitors in lipid management of patients with diabetes mellitus and high cardiovascular risk: a review. J Am Heart Assoc. 2018;7(13):e008953.
144. Kosmas CE, Skavdis A, Sourlas A, Papakonstantinou EJ, Genao EP, Uceta RE, et al. Safety and tolerability of PCSK9 inhibitors: current insights. Clin Pharmacol. 2020;12:191.
145. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Eng J Med. 2017;376(18):1713–22.
146. Sabatine MS, Leiter LA, Wiviott SD, Giugliano RP, Deedwania P, De Ferrari GM, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. Lancet Diabetes Endocrinol. 2017;5(12):941–50.
147. Ray KK, Leiter LA, Müller-Wieland D, Cariou B, Colhoun HM, Henry RR, et al. Alirocumab vs usual lipid-lowering care as add-on to statin therapy in individuals with type 2 diabetes and mixed dyslipidaemia: the ODYSSEY DM-DYSLIPIDEMIA randomized trial. Diabetes Obes Metab. 2018;20(6):1479–89.
148. Khan SU, Rahman H, Okunrintemi V, Riaz H, Khan MS, Sattur S, et al. Association of lowering low-density lipoprotein cholesterol with contemporary lipid-lowering therapies and risk of diabetes mellitus: a systematic review and meta-analysis. J Am Heart Assoc. 2019;8(7):e011581.
149. Saha SA, Arora RR. Fibrates in the prevention of cardiovascular disease in patients with type 2 diabetes mellitus–a pooled meta-analysis of randomized placebo-controlled clinical trials. Int J Cardiol. 2010;141(2):157–66.
150. Hiukka A, Leinonen E, Jauhiainen M, Sundvall J, Ehnholm C, Keech AC, et al. Long-term effects of fenofibrate on VLDL and HDL subspecies in participants with type 2 diabetes mellitus. Diabetologia. 2007;50(10):2067–75.
151. Elam MB, Ginsberg HN, Lovato LC, Corson M, Largay J, Leiter LA, et al. Association of fenofibrate therapy with long-term cardiovascular risk in statin-treated patients with type 2 diabetes. JAMA Cardiol. 2017;2(4):370–80.
152. d'Emden MC, Jenkins AJ, Li L, Zannino D, Mann KP, Best JD, et al. Favourable effects of fenofibrate on lipids and cardiovascular disease in women with type 2 diabetes: results from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetologia. 2014;57(11):2296–303.
153. Tsunoda F, Asztalos IB, Horvath KV, Steiner G, Schaefer EJ, Asztalos BF. Fenofibrate, HDL, and cardiovascular disease in type-2 diabetes: the DAIS trial. Atherosclerosis. 2016;247:35–9.
154. Joshi SR. Saroglitazar for the treatment of dyslipidemia in diabetic patients. Expert Opin Pharmacother. 2015;16(4):597–606.
155. Sai VN, Pasula S, Sumathi S, Sreekanth M, Rao AS, Prasad BD. The clinical aspects of saroglitazar and its side effects. J Drug Deliv Ther. 2020;10(2):208–12.
156. Kaul U, Parmar D, Manjunath K, Shah M, Parmar K, Patil KP, et al. New dual peroxisome proliferator activated receptor agonist—saroglitazar in diabetic dyslipidemia and non-alcoholic fatty liver disease: integrated analysis of the real world evidence. Cardiovasc Diabetol. 2019;18(1):1–1.
157. Krishnappa M, Patil K, Parmar K, Trivedi P, Mody N, Shah C, et al. Effect of saroglitazar 2 mg and 4 mg on glycemic control, lipid profile and cardiovascular disease risk in patients with type 2 diabetes mellitus: a 56-week, randomized, double blind, phase 3 study (PRESS XII study). Cardiovasc Diabetol. 2020;19(1):1–3.
158. Backes J, Anzalone D, Hilleman D, Catini J. The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids Health Dis. 2016;15(1):1–2.
159. Brinton EA, Ballantyne CM, Bays HE, Kastelein JJ, Braeckman RA, Soni PN. Effects of icosapent ethyl on lipid and inflammatory parameters in patients with diabetes mellitus-2, residual elevated triglycerides (200–500 mg/dL), and on statin therapy at LDL-C goal: the ANCHOR study. Cardiovasc Diabetol. 2013;12(1):1-0.
160. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Effects of icosapent ethyl on total ischemic events: from REDUCE-IT. J Am Coll Cardiol. 2019;73(22):2791–802.
161. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Eng J Med. 2019;380(1):11–22.
162. Natto ZS, Yaghmoor W, Alshaeri HK, Van Dyke TE. Omega-3 fatty acids effects on inflammatory biomarkers and lipid profiles among diabetic and cardiovascular disease patients: a systematic review and meta-analysis. Sci Rep. 2019;9(1):1-0.
163. Sunil B, Ashraf AP. Dyslipidemia in pediatric type 2 diabetes mellitus. Curr Diab Rep. 2020;20(10):1–9.
164. Zeitler P, Arslanian S, Fu J, Pinhas-Hamiel O, Reinehr T, Tandon N, et al. ISPAD clinical practice consensus guidelines 2018: type 2 diabetes mellitus in youth. Paedr diabetes. 2018;19:28–46.
165. American Diabetes Association. 13, Children and adolescents: standards of medical care in diabetes− 2020. Diabetes Care. 2020;43(Supplement 1):S163–82.
166. Barrett HL, Nitert MD, McIntyre HD, Callaway LK. Normalizing metabolism in diabetic pregnancy: is it time to target lipids? Diabetes Care. 2014;37(5):1484–93.
167. Wild R, Weedin EA, Wilson D. Dyslipidemia in pregnancy. Cardiology clinics. 2015;33(2):209–15.
168. Trialists CT. Articles Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials. Lancet. 2019;393:407–15.
169. Gencer B, Marston NA, Im K, Cannon CP, Sever P, Keech A, et al. Efficacy and safety of lowering LDL cholesterol in older patients: a systematic review and meta-analysis of randomised controlled trials. Lancet. 2020;396(10263):1637–43.
170. Ponce OJ, Larrea-Mantilla L, Hemmingsen B, Serrano V, Rodriguez-Gutierrez R, Spencer-Bonilla G, et al. Lipid-lowering agents in older individuals: a systematic review and meta-analysis of randomized clinical trials. J Clin Endocrinol Metab. 2019;104(5):1585–94.
171. Matsuzaka T, Shimano H. New perspective on type 2 diabetes, dyslipidemia and non-alcoholic fatty liver disease. J Diabetes Investig. 2020;11(3):532–4.
172. Marcum ZA, Griend JP, Linnebur SA. FDA drug safety communications: a narrative review and clinical considerations for older adults. Am J Geriatr Pharmacother. 2012;10(4):264–71.
173. Chalasani N, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. Gastroenterology. 2004;126:1287–92.
174. Dongiovanni P, Petta S, Mannisto V, Mancina RM, Pipitone R, Karja V, et al. Statin use and non-alcoholic steatohepatitis in at risk individuals. J Hepatol. 2015;63:705–12.
175. Mach F, Ray KK, Wiklund O, Corsini A, Catapano AL, Bruckert E, et al. Adverse effects of statin therapy: perception vs. the evidence–focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. Eur Heart J. 2018;39(27):2526–39.

176. Bays HE, Cohen DE, Chalasani N, Harrison SA. An assessment by the Statin Liver Safety Task Force: 2014 update. J Clin Lipidol. 2014;8:S47–57.
177. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: a systematic review with comparative analysis. World J Gastroenterol. 2018;24(30):3361–73.
178. Gawrieh S, Noureddin M, Loo NM, Mohseni R, Awasty VR, Cusi K, et al. A phase 2, prospective, multicenter, double-blind, randomized study of saroglitazar magnesium 1 mg, 2 mg or 4 mg versus placebo in patients with nonalcoholic fatty liver disease and/or nonalcoholic steatohepatitis (EVIDENCES IV). Hepatology. 2019;70(6):1484A–5A.
179. Goyal O, Nohria S, Goyal P, Kaur J, Sharma S, Sood A, et al. Saroglitazar in patients with non-alcoholic fatty liver disease and diabetic dyslipidemia: a prospective, observational, real world study. Scientific Reports. 2020;10(1):1–9.
180. Teramoto T. Pitavastatin: clinical effects from the LIVES Study. Atheroscler Suppl. 2011;12(3):285–8.
181. Wu Y, Wang Y, An C, Dong Z, Liu H, Zhang Y, et al. Effects of rosuvastatin and atorvastatin on renal function–meta-analysis. Circulation J. 2012;76(5):1259–66.
182. Rangel ÉB, de Sá JR, Melaragno CS, Gonzalez AM, Linhares MM, Salzedas A, et al. Kidney transplant in diabetic patients: modalities, indications and results. Diabetol Metab Syndr. 2009;1(1):1–7.
183. Breda A, Budde K, Figueiredo A, García EL, Olsburgh J, Regele H, et al. EAU guidelines on renal transplant – update 2021. Edn. presented at the EAU Annual Congress Milan Italy 2021. ISBN 978-94-92671-13-4.
184. Scicchitano P, Milo M, Mallamaci R, De Palo M, Caldarola P, Massari F, et al. Inclisiran in lipid management: a literature overview and future perspectives. Biomed Pharmacother. 2021;143:112227.
185. Wright RS, Collins MG, Stoekenbroek RM, Robson R, Wijngaard PL, Landmesser U, et al. Effects of renal impairment on the pharmacokinetics, efficacy, and safety of inclisiran: an analysis of the ORION-7 and ORION-1 studies. InMayo Clinic Proceedings. 2020;95(1):77–89.
186. Leiter LA, Teoh H, Kallend D, Wright RS, Landmesser U, Wijngaard PL, et al. Inclisiran lowers LDL-C and PCSK9 irrespective of diabetes status: the ORION-1 randomized clinical trial. Diabetes Care. 2019;42(1):173–6.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):29–39
https://doi.org/10.1007/s13410-021-00973-1

REVIEW ARTICLE

Type 2 diabetes mellitus and the risk of hip and vertebral fractures: a systematic review and meta-analysis of cohort studies

Ning Wang[1] · Min Zhang[2] · Jindou Ji[1] · Dong Li[1] · Liyou Hu[1] · Jing Meng[1] · Bo Yu[3]

Received: 21 December 2020 / Accepted: 26 June 2021 / Published online: 13 July 2021

Abstract

Background Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease. The association between diabetes mellitus and fracture risk is unclear. Currently, T2DM is not an independent risk factor for low-energy fractures in elderly patients. This study aimed to explore the association between T2DM and the risk of hip and vertebral fractures.

Method PubMed, MEDLINE, and Cochrane library databases were searched for articles on T2DM and fracture risk. The final study sample from the literature was determined using predefined inclusion criteria, and a meta-analysis, including heterogeneity testing, publication bias analysis, and subgroup analysis, of relevant data was undertaken using STATA software.

Results Seventeen studies, involving 365,185 participants, 6539 hip fracture events, and 1381 vertebral fracture events were included in this research. The adjusted relative risk of T2DM and hip or vertebral fracture were 1.46 (95% confidence interval [CI] 1.31–1.61) and 1.00 (95% CI 0.81–1.18), respectively, which showed that T2DM is positively related to hip fracture although not significantly associated with vertebral fractures.

Conclusion T2DM was positively associated with an increased risk of hip fracture but not with vertebral fracture. Bone health in patients with T2DM requires more attention, and further research is warranted.

Keywords Type 2 diabetes · Hip fracture · Vertebral fracture · Fracture risk · Meta-analysis

Introduction

Type 2 diabetes mellitus (T2DM) comprises a group of metabolic diseases characterized by hyperglycemia that is caused by defects in insulin secretion or action [1, 2] and is one of the most prevalent chronic diseases worldwide. Approximately 366 million people were diagnosed with diabetes until 2011, and this number is expected to increase to 552 million worldwide by 2030 [3]. Hip and vertebral fractures are common types of osteoporotic fractures in the elderly, and long-term bed rest due to fractures can be fatal for the elderly. Type 2 diabetes patients have a significantly increased risk of fractures, Vilaca et al. reported an increased risk of non-vertebral fracture in patients with T2DM (RR 1.19; 95% CI, 1.11–1.28), while Moayeri et al. reported an increased risk of any fracture in patients with T2DM (RR 1.05; 95% CI 1.04–1.06) [4, 5], but, paradoxically, their bone mineral density (BMD) is often higher than or close to the normal range [6–9]. There is no unified explanation for this, although some studies indicate that patients with T2DM have higher glycemic levels and bone accumulation of advanced glycation end products (AGEs) due to insulin resistance. These factors will lead to changes in bone microstructure and increase the porosity of cortical bone [10–12]. The bone turnover rate in patients with T2DM tends to decrease [6, 7], with a resultant increase in bone fragility. Complications of T2DM such as disturbed vision, cardiac arrhythmia, poor balance, and poorer walking performances increase the risk for falls in T2DM patients [6, 13–15]. Therefore, the combined effects of intra- and extra-skeletal factors make T2DM patients more likely to

Ning Wang and Min Zhang contributed equally to this work.

✉ Bo Yu
YBshengzhongyi@126.com

[1] Shandong University of Traditional Chinese Medicine, Jinan 250014, China

[2] Department of Nursing, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan 250014, China

[3] Department of Orthopaedics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jingshi Road16369, Jinan 250014, China

have non-traumatic fractures. Type 1 diabetes has a greater impact on hip fractures than does T2DM [16–21]. In recent years, the estimated size of the association between T2DM and risk of hip fracture has varied, and gender specificity has become particularly prominent [22–24]. The reported risk that T2DM confers with regard to vertebral fractures is controversial and even contradictory [25, 26]. Therefore, it is necessary to conduct a detailed meta-analysis to study the association between T2DM and fractures of the hip or vertebrae.

We undertook this research and conducted a meta-analysis of cohort studies among participants with T2DM. Moreover, we conducted a subgroup analysis based on sex and geographical region to explore the impact of relevant factors on the study outcomes. In addition, we used publication bias testing and sensitivity analysis to determine the source of heterogeneity.

Materials and methods

Search strategy and data sources

A comprehensive online literature search was conducted to identify studies related to hip and vertebral fractures in patients with T2DM. We undertook a systematic literature search of PubMed, MEDLINE, and Cochrane library using the following keywords: (1) "type 2 diabetes mellitus," "diabetes, OR diabetes mellitus, OR T2DM;" (2) "hip fractures" OR "vertebral fractures" OR "fracture;" and (3) "cohort study OR prospective study." The start and end dates of the search were January 1995 and February 2020, respectively, without any restrictions on the language and time of publication. To avoid omission of relevant literature, the reviewers conducted multiple reviews of all relevant citations. Moreover, manual searches of reference lists of relevant studies were conducted to identify additional eligible studies.

Study selection

Two reviewers (WN and MZ) independently evaluated the selected studies. Disagreements between data inclusion and data interpretation were resolved through arbitration, and a consensus was reached through discussion between the two reviewers. The inclusion criteria for studies in our research were as follows: (1) cohort study; (2) subjects with hip or vertebral fractures or both; (3) research on fractures, with non-pathological fractures as the main observation results, is warranted; and (4) related research that reported the adjusted

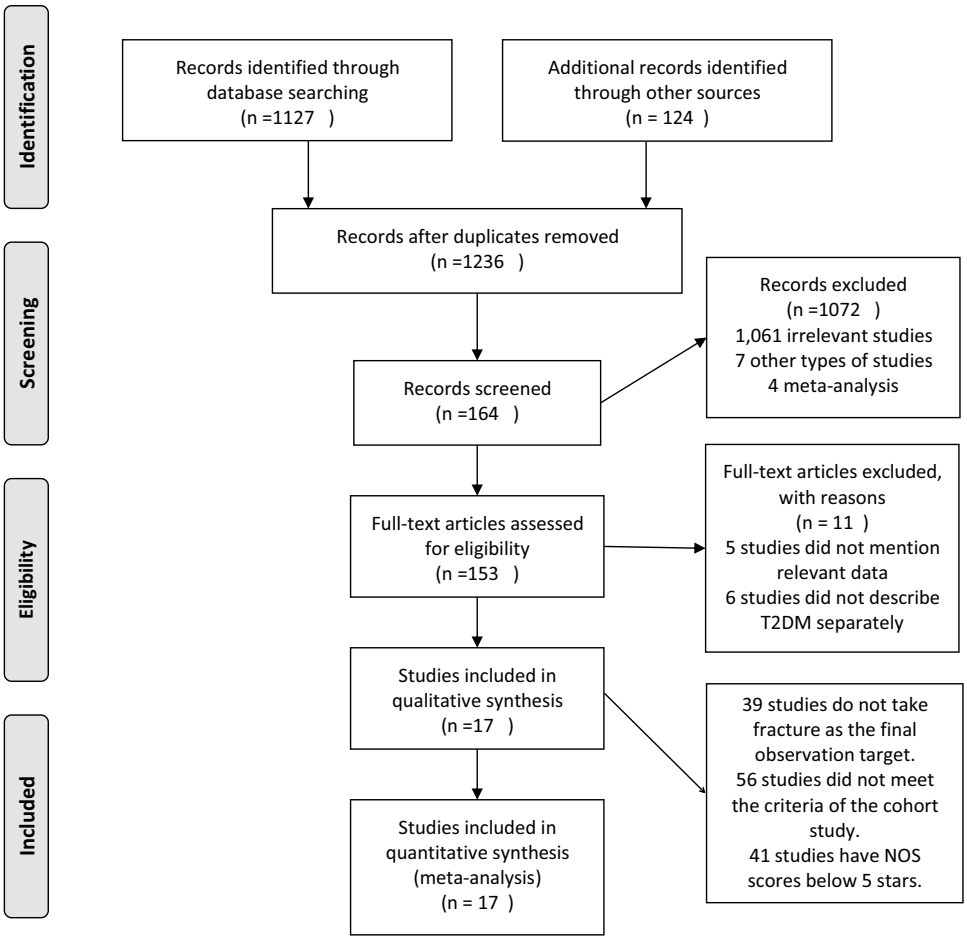
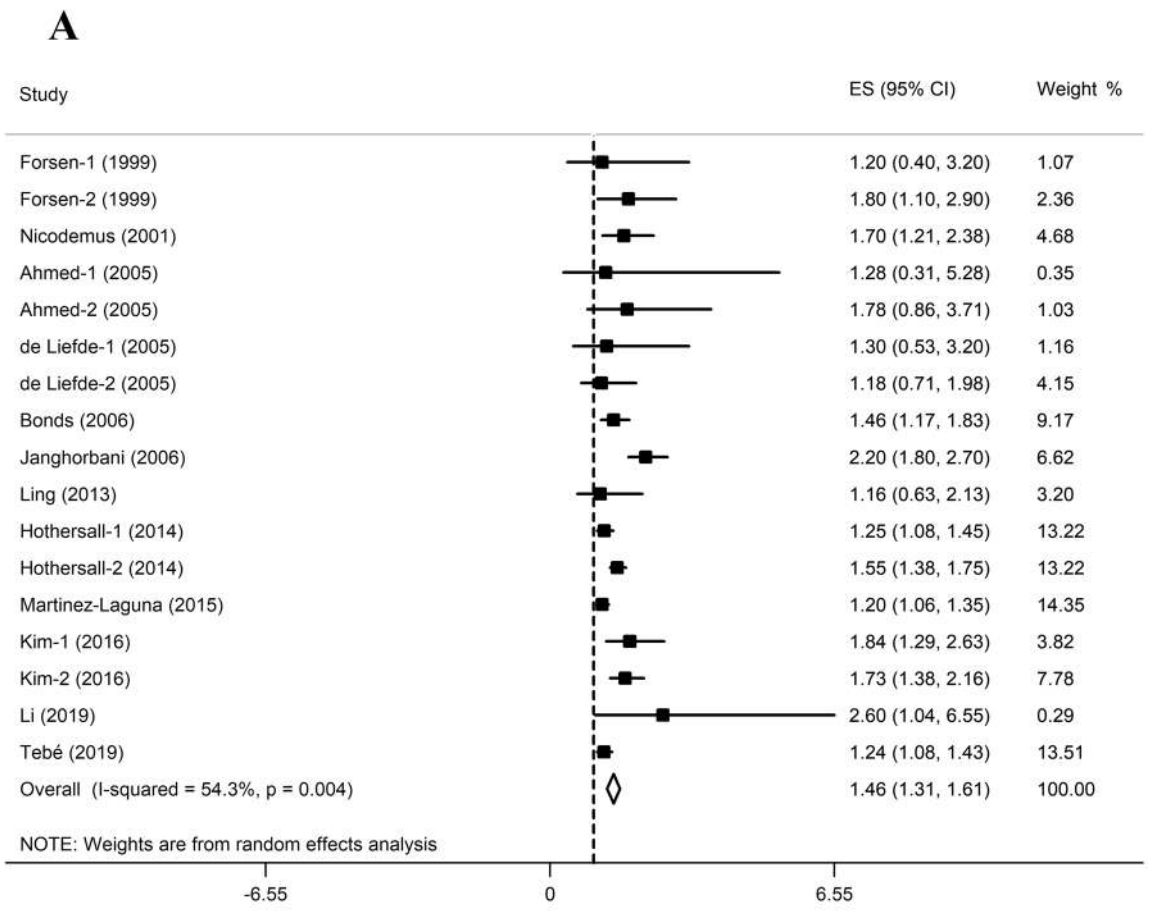


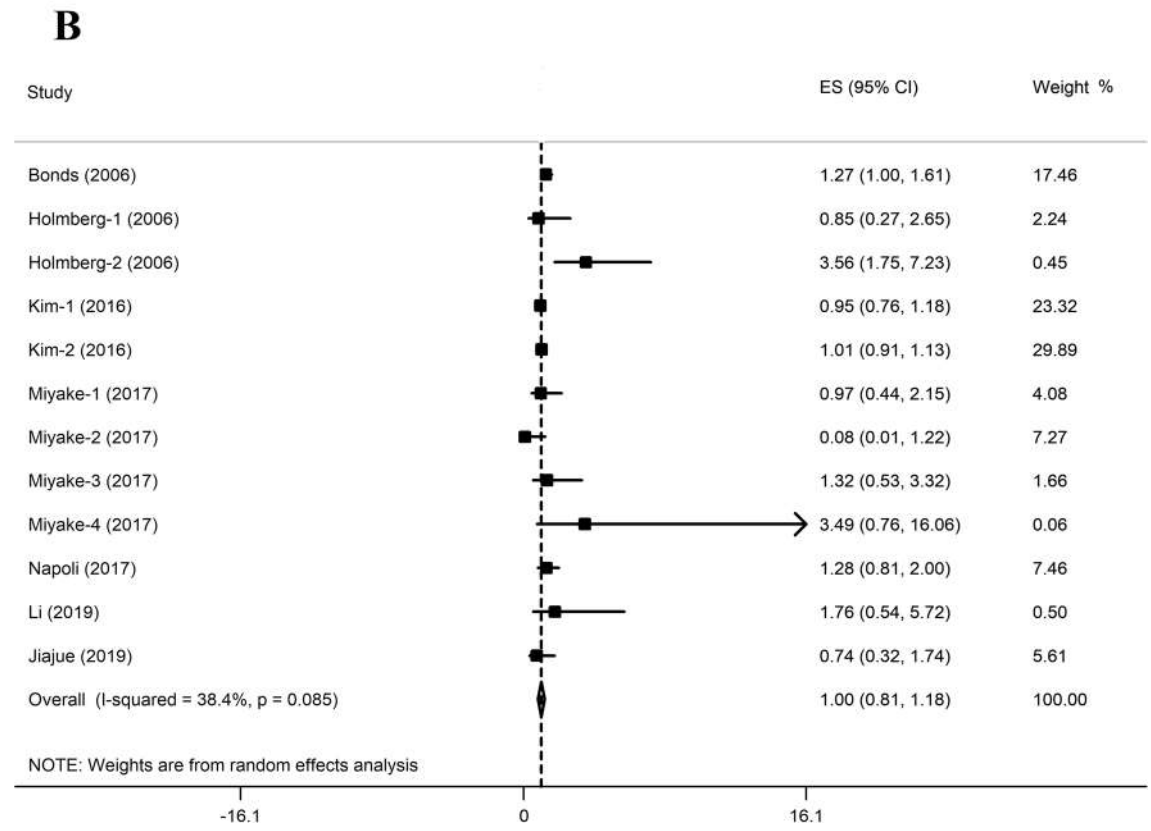
Fig. 1 PRISMA 2009 flow diagram

Table 1 Characteristics of the included studies

	Year	HF(M\F\T)	VF(M\F\T)	Sex	Age	Country	Period	Follow-up (year)	Adjustment
Martinez-Laguna	2015	**\444	*	33,147\25,336	62.71 ± 11.9	Spain	2006–2011	2.63	Body mass index, previous fracture, and use of oral corticosteroids
Li	2019	**\6	**\3	55\83	69.4 ± 8.2	Canada	1995–1997	9.2 ± 4.5	Age, sex, and BMD femoral neck *T*-scores
Wallander	2016	106\225\331	*	5038\6006	80.8 ± 8.2	Swenden	2008–2014	1.3	Age,sex,weight,height,previous fracture,rheumatoid arthritis,glucocorticoid and alendronate use and Charlson comorbidity index,self-reported known fall injury
Ling	2013	**\11	*	102\115	68.5 ± 7.8	Holand	1990–1993	12.2 ± 4.2	BMD-adjusted model, adjusted for age, sex, height, weight, and femoral neck BMD
Kim	2016	66\179\245	138\544\682	7868\9242	≥ 50	Korean	2002–2010	6	Age, household income, osteoporosis, and comorbidities
Ahmed	2005	2\9\11	*	89\120	68.2	Norway	1994–1995	7	Age, BMI, smoking, and metabolic features
Bonds	2006	*\128\128	*\99\99	*\5285	64.9 ± 7.0	US	1993–1998	7	Age; ethnicity; weight; height; time-dependent history of falls; previous fracture; history of osteoporosis; trouble seeing at baseline; alcohol or tobacco use; calcium and vitamin D intake; exercise; bisphosphonate, estrogen, steroid, insulin, SERM, or thyroid hormone use
de Liefde	2005	9\28\37	*	309\483	73.8 ± 9.2	Holland	1990–1993	6.8 ± 2.3	Age, gender, BMI, smoking, serum creatinine, visual acuity, falling frequency, lower limb disability
Forsen	1999	21\69\90	*	581\835	≥ 50	Norway	1984–1986	9	Age, BMI and daily smoking
Hothersall	2014	454\967\1421	*	180,841	40–84	UK	2005–2008	3	Age, calendar year, SIMD, and for the overall estimate, an SIMD-age interaction
Janghorbani	2006	*\125\125	*	*\8348	61.7 ± 8.2	US	1980–Now	22	Age, BMI, physical activity, menopausal status and estrogen use, smoking and daily intake of calcium, vitamin D, and protein
Jiajue	2019	*	*\8\8	*\186	65	China	2008–2009 2013–2014	5.2 ± 1.0	Age, YSM, BMI, LS BMD, and any previous fractures
Miyake	2017	*	89\54\143	222\189	66.6 ± 9.5	Japan	1997–2009	6.79	Age (plus gender for total subjects),HbA1c, BMI, duration of diabetes, and serum creatinine, systolic blood pressure and LDL-C, treatment for osteoporosis
Nicodemus	2001	*\38\38	*	*\1682	62.3	US	1986–1997	9.53	Age, smoking (former, current, never), estrogen use (former, current, never), BMI, and waist-to-hip ratio
Holmberg	2006	192\143\335	219\166\385	22,444\10,902	M:44 F:48	Germany	M:1974–1984 F:1977–1992	M:16 F:11	Age
Napoli	2017	*	61*\61	875*	73.7 ± 5.6	US	2000–2002	N\A	Age, race, clinic site, BMI, spine vs. BMD
Tebé	2019	**\3317	*	20,907\23,895	72.19 ± 4.38	Spain	2006–2013	8	Adjusted for age, previous major fracture, previous ischemic heart disease, previous cerebrovascular disease, previous nephropathy, corticoid, prescriptions, anti-osteoporosis drugs, and calcium + vitamin D

HF, hip fracture; *VF*, vertebral fracture; *M*, male; *F*, female; *T*, total fracture; *not available


A
Study
ES (95% CI)
Weight %
Forsen-1 (1999) 1.20 (0.40, 3.20) 1.07
Forsen-2 (1999) 1.80 (1.10, 2.90) 2.36
Nicodemus (2001) 1.70 (1.21, 2.38) 4.68
Ahmed-1 (2005) 1.28 (0.31, 5.28) 0.35
Ahmed-2 (2005) 1.78 (0.86, 3.71) 1.03
de Liefde-1 (2005) 1.30 (0.53, 3.20) 1.16
de Liefde-2 (2005) 1.18 (0.71, 1.98) 4.15
Bonds (2006) 1.46 (1.17, 1.83) 9.17
Janghorbani (2006) 2.20 (1.80, 2.70) 6.62
Ling (2013) 1.16 (0.63, 2.13) 3.20
Hothersall-1 (2014) 1.25 (1.08, 1.45) 13.22
Hothersall-2 (2014) 1.55 (1.38, 1.75) 13.22
Martinez-Laguna (2015) 1.20 (1.06, 1.35) 14.35
Kim-1 (2016) 1.84 (1.29, 2.63) 3.82
Kim-2 (2016) 1.73 (1.38, 2.16) 7.78
Li (2019) 2.60 (1.04, 6.55) 0.29
Tebé (2019) 1.24 (1.08, 1.43) 13.51
Overall (I-squared = 54.3%, p = 0.004) 1.46 (1.31, 1.61) 100.00
NOTE: Weights are from random effects analysis
-6.55
0
6.55


B
Study
ES (95% CI)
Weight %
Bonds (2006) 1.27 (1.00, 1.61) 17.46
Holmberg-1 (2006) 0.85 (0.27, 2.65) 2.24
Holmberg-2 (2006) 3.56 (1.75, 7.23) 0.45
Kim-1 (2016) 0.95 (0.76, 1.18) 23.32
Kim-2 (2016) 1.01 (0.91, 1.13) 29.89
Miyake-1 (2017) 0.97 (0.44, 2.15) 4.08
Miyake-2 (2017) 0.08 (0.01, 1.22) 7.27
Miyake-3 (2017) 1.32 (0.53, 3.32) 1.66
Miyake-4 (2017) 3.49 (0.76, 16.06) 0.06
Napoli (2017) 1.28 (0.81, 2.00) 7.46
Li (2019) 1.76 (0.54, 5.72) 0.50
Jiajue (2019) 0.74 (0.32, 1.74) 5.61
Overall (I-squared = 38.4%, p = 0.085) 1.00 (0.81, 1.18) 100.00
NOTE: Weights are from random effects analysis
-16.1
0
16.1

◂**Fig. 2** Fracture risk in the elderly patients with T2DM

values for relative risks (RRs), odds ratios (ORs), or hazard ratios (HRs) with 95% confidence intervals (CIs). The exclusion criteria were as follows: (1) research other than cohort studies; (2) no data on hip or vertebral fractures; (3) no data on RR, OR, or HR with 95% CI; (4) no separate description of two types of diabetes; and (5) reports from the same cohort. We excluded other types of studies to limit the possibility of bias, as various study-related confounding factors may have conferred bias upon the study results.

Data extraction

Two authors (WN and ZM) independently extracted relevant data. Another author made the final decision for the inclusion of the study by reading the full text of the research. Discrepancies were resolved through discussion with the other authors. The abstracted data included the first author's surname, year of publication, study design, the geographical location of the participants, sex and age range of participants, duration of follow-up, number of participants, number of hip and vertebral fractures, adjusted variables, RR, OR, or HR with 95% CI values.

Statistical analyses

RRs were used as a common correlation measure between the studies, and the HRs and ORs were converted to RRs [27–30]. Cochran Q ($p < 0.10$ indicated statistically significant heterogeneity) and I^2 (values > 50% indicated statistically significant heterogeneity) statistics were used to evaluate heterogeneity between studies [31]. The geographical location and sex of participants were considered to be possible sources of heterogeneity for subgroup analysis. Sensitivity analyses were conducted by excluding certain low-quality studies, undertaking reanalysis, and then comparing the results from before and after the exclusion to explore the impact of the eliminated experiments and the characteristics or types of studies that contributed to the total effect. Publication bias was evaluated by visual inspection of the funnel plot as well as Egger and Begg tests. Statistical analysis of all data was conducted using STATA 12 (Stata Corporation, College station, TX).

Results

Search results

The results of study selection are shown in Fig. 1. Overall, 1251 articles were retrieved on the initial search, of which 1076 studies were excluded (1061 irrelevant studies, 15 duplicate articles). After reading the article titles and abstracts, 38 potentially eligible studies were selected. Thereafter, the selected literature was evaluated and studies that did not meet the inclusion criteria were excluded, i.e., 3 cross-sectional studies, 4 case–control studies; 5 studies that did not mention relevant RRs; 6 literature reviews that did not separately describe fracture types or types of diabetes; and 4 meta-analysis. Finally, 17 relevant studies were deemed eligible for inclusion in this meta-analysis [16–18, 24, 26, 32–43].

Study characteristics and quality assessment

The 17 studies included in this meta-analysis reported data from 365,185 patients with T2DM, of whom 7920 suffered fracture events. The study and participant characteristics are summarized in Table 1. The cohort studies either reported only hip fractures (10 studies) or multiple fracture events, including the hip and vertebrae (7 studies). All included studies were published between 1999 and 2019, with 7 and 10 studies published before and after 2010, respectively. Moreover, 5 studies were conducted in North America, 9 in Europe, and the rest in Asia. One study included only men, 4 included only women, and the other studies included both sexes. The follow-up period ranged from 1.3 to 22 years. All fractures in the included studies were examined by imaging and confirmed by clinicians. Study quality was evaluated by the Newcastle–Ottawa Scale (NOS). All included studies had assessment scores indicative of high study quality (5–9 stars, nine-star NOS).

Hip fracture risk in the elderly patients with T2DM

There were 6541 hip fractures reported in 14 studies with a total study population of 363,713. As shown in Fig. 2A, the multivariable-adjusted RR of hip fracture was 1.46 (95% CI 1.31–1.61) for participants with T2DM. A sensitivity analysis of hip fracture events in patients with T2DM revealed a combined RR value of 1.36 (95% CI 1.29–1.44), indicating that no significant change in effect size was found with the exclusion of any study (Fig. 3A).

Vertebral fracture risk in the elderly patients with T2DM

Seven studies with a total of 57,351 participants reported a total of 1381 vertebral fractures. The multivariable-adjusted RR of vertebral fractures was 1.00 (95% CI 0.81–1.18; Fig. 2B) for participants with T2DM. There was low heterogeneity between these studies ($p = 0.085$; $I^2 = 38.4\%$). A sensitivity analysis of vertebral fracture events in patients

with T2DM revealed a combined RR value of 1.01 (95% CI 0.92–1.11), indicating that there was no significant change in result value with the exclusion of any study (Fig. 3B).

Subgroup analysis

Table 2 presents the results of subgroup analysis. To explore the source of heterogeneity between studies, we conducted subgroup analyses of studies of hip and vertebral fractures after categorizing them based on sex and geographical region. For hip fractures, the sex-based subgroup analysis showed that the RR (95% CI) values were 1.24 (1.14–1.33) for males and 1.50 (1.31–1.68) for females, whereas for vertebral fractures, these were 1.00 (0.81–1.18) for males and 0.92 (0.54–1.30) for females. Similarly, region-based subgroup analysis showed that RR did not differ significantly for hip fractures between geographical regions. The RR (95% CI) values were 1.53 (1.24–1.82), 1.92 (1.62–2.23), and 1.30

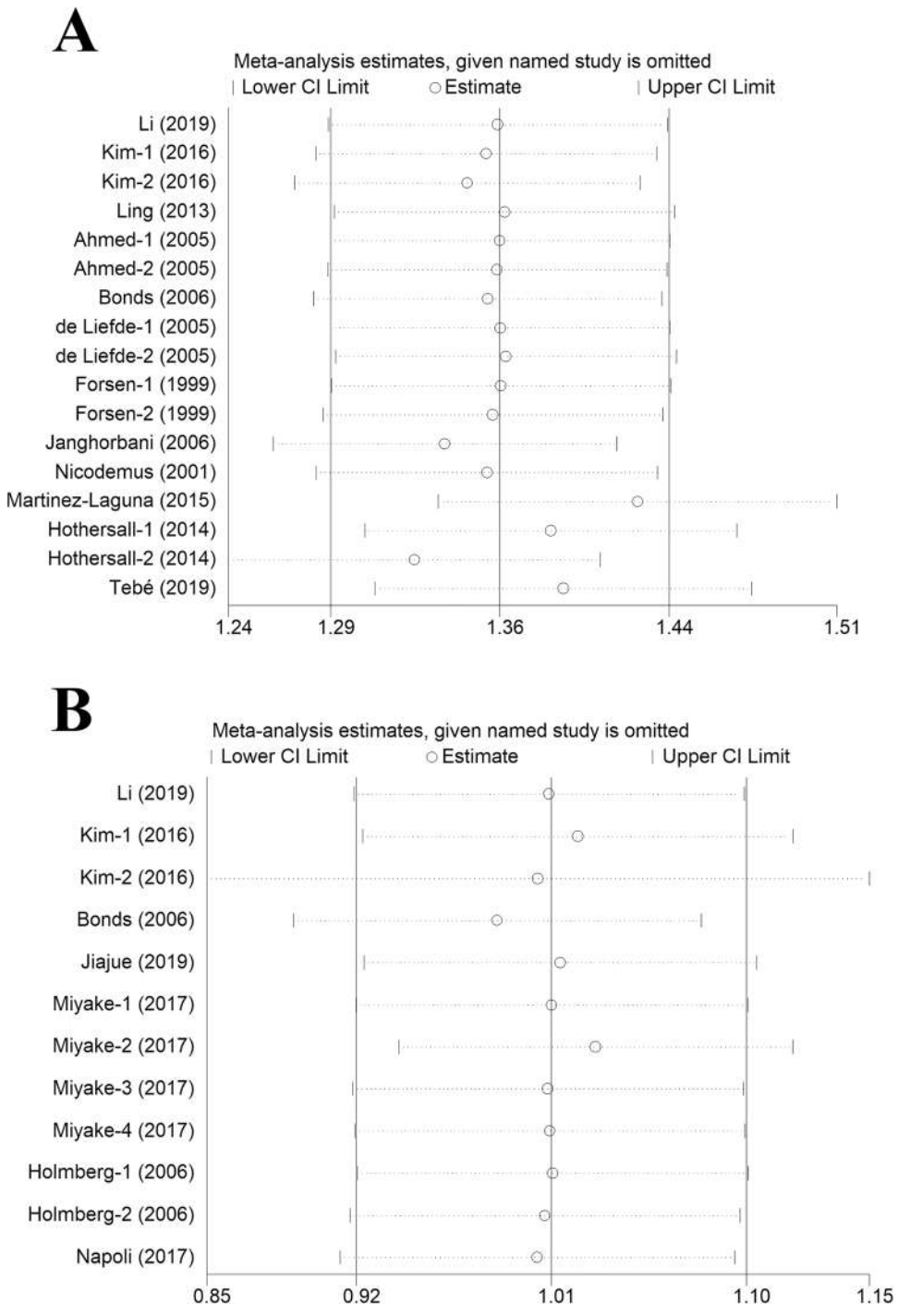


Fig. 3 Meta-analysis estimates, given named study is omitted

(1.20–1.40) for North America, Asia, and Europe, respectively. However, region-based subgroup analysis showed no significant difference in the RR for all regions, with the exception of North America (RR = 1.28; 95% CI 1.01–1.55).

Publication bias

For these two types of fractures, the results of Egger and Begg tests showed no evidence of publication bias (Begg = 0.54 and 0.21, Egger = 0.08 and 0.67), respectively. Furthermore, we conducted trim and fill analysis (Fig. 4A), and found no significant publication bias.

Discussion

The high incidence of T2DM and osteoporotic fractures poses a very serious health challenge worldwide. Our study showed that T2DM was significantly associated with osteoporotic hip fractures (RR = 1.50; 95% CI 1.33–1.67) but not with vertebral fractures (RR = 1.00; 95% CI 0.81–1.18).

Previously, several meta-analyses of T2DM and osteoporotic fractures have been performed, but they were impaired by the diversity of the study population and the type of literature included. Therefore, some of these studies' conclusions are controversial. A 2007 study by Vestergard et al. [44] showed that both men and women with T2DM are at higher risk of sustaining hip and wrist fractures but not vertebral fractures. In 2016, a meta-analysis of hip fracture risk in postmenopausal women with T2DM by Joanna et al. [45] showed an increased risk for hip fractures but not vertebral fractures (OR 1.296 and 1.134; 95% CI 1.069–1.571 and 0.936–1.374, respectively). In 2017, Moayeri et al. [5] performed a meta-analysis of studies among T2DM patients with various types of fractures and showed that T2DM was positively associated with hip, vertebral, and foot fractures but not with wrist, proximal humeral, or ankle fractures. Schwartz et al. [46] reported RR of 1.06–1.09 for vertebral fractures in elderly women with diabetes. Several meta-analyses investigated whether sex is a factor that affects fracture risk in patients with T2DM. The findings of the present study show that, among patients with T2DM, the RR of hip fractures in women was significantly higher than in men despite no significant sex-based specificity in the RR of vertebral fractures.

The BMD of patients with T2DM is, on an average, higher than the BMD of individuals without T2DM. Paradoxically, the risk of fragility fractures is significantly higher in patients with T2DM than in people without T2DM, which may be related to several factors. (1) There exists a well-known, significant correlation between BMD and obesity, body mass index (BMI) [44], serum insulin levels, and drugs [47]. However, the distribution of weight, BMI, treatment, and serum insulin levels of patients with T2DM in the study population was often uneven, leading to a heterogeneous research sample. (2) Most of the BMD evaluations in the included studies were completed by measuring bone density with dual-energy X-ray absorptiometry (DEXA). However, the size of the body or bone will affect the results of DEXA, which evaluates the area rather than the "real" volume BMD (vBMD). (3) Chronic hyperglycemia, coupled with oxidative stress, can induce AGE accumulation in various bone proteins, and higher levels of AGE will lead to increased porosity of cortical bone, which consequently reduces bone quality and increases the fracture risk. (4) Change in bone turnover is another important factor, and levels of biochemical markers of bone turnover (e.g., bone-specific alkaline phosphatase and type 1 collagen carboxy terminal peptide) tend to decrease in patients with T2DM [48]. (5) Several antidiabetic drugs, such as thiazolidinediones, can affect bone metabolism [49, 50]. Peroxisome proliferator-activated receptor-gamma agonists improve the insulin sensitivity of muscle and adipose tissue and promote preferential differentiation of mesenchymal stem cells into adipocytes instead of osteoblasts. Some studies have shown that it can significantly increase the incidence of fractures [51–53].

Table 2 Subgroup analysis for hip and vertebral fractures by gender and geographical area

			No. of studies	RR value	95% CI	*P* value	I^2(%)
Gender	Hip fracture	Male	9	1.24	1.14–1.33	0.82	0
		Female	12	1.50	1.31–1.68	0.001	65
	Vertebral fracture	Male	5	1.0	0.81–1.18	0.92	0
		Female	6	0.92	0.54–1.30	0.01	69
Geographical area	Hip fracture	N.A	3	1.53	1.24–1.82	0.58	0
		Europe	7	1.30	1.20–1.40	0.32	12.9
		Asia	2	1.92	1.62–2.23	0.29	18.5
	Vertebral fracture	N.A	3	1.28	1.01–1.55	0.93	0
		Europe	1	1.91	−0.68–4.51	0.08	68.4
		Asia	3	0.89	0.69–1.10	0.13	39.5

N.A, North American

In addition, the duration of diabetes and different antidiabetic treatments are related to the risk of fractures [54–56], wherein the duration of diabetes shows a positive correlation [54]. The main reason is diabetes-related complications such as diabetic peripheral neuropathy and retinopathy, and lower extremity muscle weakness, which increase the risk of falls [11]. Some drugs prescribed for the treatment of diabetes can increase the risk of fracture. For example, insulin treatment increases the risk of falls and fractures in patients with HbA1c < 6.5%. In postmenopausal women, thiazolidinedione is closely associated with a higher risk of fracture [51].

In summary, T2DM patients have decreased bone quality and an increased risk of falls. The combined effect of these two factors leads to increased fracture risk in T2DM

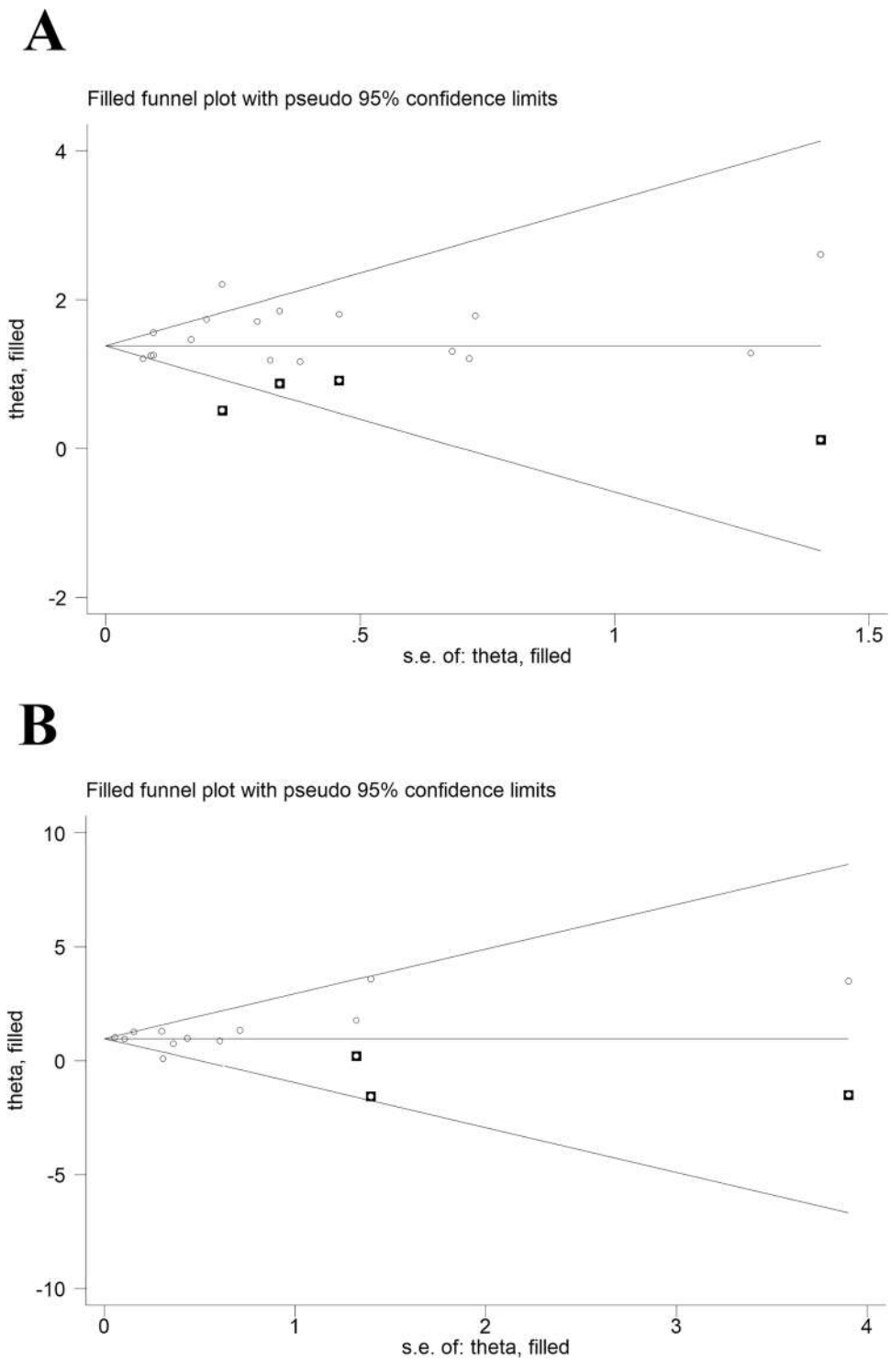


Fig. 4 Filled funnel plot with pseudo 95% confidence limits

patients. Our study further confirmed a higher risk of hip fractures in patients with T2DM than in individuals without T2DM. However, the risk of vertebral fractures is not significantly increased in this population; this, in part, explains the contradiction between high BMD and increased fracture risk in most T2DM patients.

The strengths of the present study are as follows: First, the total sample size and fragile fracture events are quite large; this not only greatly enhances the statistical power of the analysis but also reduces the impact of small probability events on the final result. Second, compared to other types of research, the characteristics of prospective research determine that it has the smallest impact on our final results. Third, data processing was undertaken by two authors, whereas the data were checked by another author, and this ensured data accuracy in our research. Finally, the low publication bias has a positive impact on the objectivity of the final results.

Nonetheless, the shortcomings of this study must also be acknowledged. The quality of the included studies was unequal, and the included studies undertook different adjustments for potential confounders; moreover, not all studies reported the value of each adjusted RR. Inevitably, our research results are affected by confounding factors within the included cohort. Because the research focus of the initial study was not the same, we were not able to conduct a subgroup analysis of more risk factors. The majority of patients included in these studies were from European countries and America. Therefore, our results may not be generalizable to patients in other regions. The sample size of patients with vertebral fractures was smaller than that of patients with hip fracture, and this potentially influenced the final outcomes. Finally, the included studies had significant differences in follow-up duration, which inevitably affects the final outcome of osteoporotic fractures in the elderly.

Conclusions

Patients with T2DM older than 50 years had a higher risk of hip fracture than patients without diabetes, but there was no risk of vertebral fracture. The risk of any fracture is increased in older patients with T2DM due to decreased bone mass and increased risk of falls. More research is needed to reduce the risk of fracture in older patients with T2DM.

Authors' contributions NW, JM, and MZ conceived of the design of this meta-analysis. MZ, NW, and LYH performed the literature retrieval and article writing. JDJ and DL contributed to the data extraction. All authors read and approved the final manuscript.

Funding This work was financially supported through grants from the Natural Science Foundation of Shandong Province (ZR2016HM43), the Medical and Health Science and Technology Development Project of Shandong Province (2016WS0582), the Clinical Medical Science and Technology Innovation Program of the Jinan Science and Technology Bureau (201602303), and the grant from the Traditional Chinese Medicine Technology Development Plan of Shandong Province (2019–0154).

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

Declarations

Competing interests The authors declare that they have no competing interests.

References

1. Jackuliak P, Payer J. Osteoporosis, fractures, and diabetes [published correction appears in Int J Endocrinol. 2017;2017:2846080]. Int J Endocrinol. 2014;2014:820615
2. de Waard EA, van Geel TA, Savelberg HH, Koster A, Geusens PP, van den Bergh JP. Increased fracture risk in patients with type 2 diabetes mellitus: an overview of the underlying mechanisms and the usefulness of imaging modalities and fracture risk assessment tools. Maturitas. 2014;79(3):265–74.
3. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;94(3):311–21.
4. Vilaca T, Schini M, Harnan S, Sutton A, Poku E, Allen IE, Cummings SR, Eastell R. The risk of hip and non-vertebral fractures in type 1 and type 2 diabetes: a systematic review and meta-analysis update. Bone. 2020;137:115457.
5. Moayeri A, Mohamadpour M, Mousavi SF, Shirzadpour E, Mohamadpour S, Amraei M. Fracture risk in patients with type 2 diabetes mellitus and possible risk factors: a systematic review and meta-analysis. Ther Clin Risk Manag. 2017;13:455–468. Published 2017 Apr 11.
6. Carnevale V, Romagnoli E, D'Erasmo E. Skeletal involvement in patients with diabetes mellitus. Diabetes Metab Res Rev. 2004;20(3):196–204.
7. Merlotti D, Gennari L, Dotta F, Lauro D, Nuti R. Mechanisms of impaired bone strength in type 1 and 2 diabetes. Nutr Metab Cardiovasc Dis. 2010;20(9):683–90.
8. Leslie WD, Rubin MR, Schwartz AV, Kanis JA. Type 2 diabetes and bone [published correction appears in J Bone Miner Res. 2017 Nov;32(11):2319]. J Bone Miner Res. 2012;27(11):2231–2237.
9. Ma L, Oei L, Jiang L, et al. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. Eur J Epidemiol. 2012;27(5):319–32.
10. Tang SY, Vashishth D. The relative contributions of non-enzymatic glycation and cortical porosity on the fracture toughness of aging bone. J Biomech. 2011;44(2):330–6.
11. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int. 2010;21(2):195–214.

12. Schwartz AV, Garnero P, Hillier TA, et al. Pentosidine and increased fracture risk in older adults with type 2 diabetes. J Clin Endocrinol Metab. 2009;94(7):2380–6.
13. Schwartz AV, Vittinghoff E, Sellmeyer DE, et al. Diabetes-related complications, glycemic control, and falls in older adults [published correction appears in Diabetes Care. 2008 May;31(5):1089]. Diabetes Care. 2008;31(3):391–396.
14. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ; Blue Mountains Eye Study. Diabetes and risk of fracture: the Blue Mountains Eye Study. Diabetes Care. 2001;24(7):1198–1203.
15. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol. 2007;166(5):495–505.
16. Nicodemus KK, Folsom AR; Iowa Women's Health Study. Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. Diabetes Care. 2001;24(7):1192–1197.
17. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: the Nurses' Health Study. Diabetes Care. 2006;29(7):1573–8.
18. Ahmed LA, Joakimsen RM, Berntsen GK, Fønnebø V, Schirmer H. Diabetes mellitus and the risk of non-vertebral fractures: the Tromsø study [published correction appears in Osteoporos Int. 2009 May;20(5):841]. Osteoporos Int. 2006;17(4):495–500.
19. Meyer HE, Tverdal A, Falch JA. Risk factors for hip fracture in middle-aged Norwegian women and men. Am J Epidemiol. 1993;137(11):1203–11.
20. Vestergaard P, Rejnmark L, Mosekilde L. Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. Diabetologia. 2005;48(7):1292–9.
21. Miao J, Brismar K, Nyrén O, Ugarph-Morawski A, Ye W. Elevated hip fracture risk in type 1 diabetic patients: a population-based cohort study in Sweden. Diabetes Care. 2005;28(12):2850–5.
22. Strotmeyer ES, Kamineni A, Cauley JA, et al. Potential explanatory factors for higher incident hip fracture risk in older diabetic adults. Curr Gerontol Geriatr Res. 2011;2011:979270.
23. Giangregorio LM, Leslie WD, Lix LM, et al. FRAX underestimates fracture risk in patients with diabetes [published correction appears in J Bone Miner Res. 2017 Nov;32(11):2319]. J Bone Miner Res. 2012;27(2):301–308.
24. Oei L, Zillikens MC, Dehghan A, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. Diabetes Care. 2013;36(6):1619–28.
25. Jia P, Bao L, Chen H, Yuan J, Liu W, Feng F, Li J, Tang H. Risk of low-energy fracture in type 2 diabetes patients: a meta-analysis of observational studies. Osteoporos Int. 2017;28:3113–21.
26. Napoli N, Schwartz AV, Schafer AL, Vittinghoff E, Cawthon PM, Parimi N, Orwoll E, Strotmeyer ES, Hoffman AR, Barrett-Connor E, Black DM, Osteoporotic Fractures in Men (MrOS) Study Research Group. Vertebral fracture risk in diabetic elderly men: the MrOS Study. J Bone Miner Res. 2018;33:63–9.
27. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA. 1998;280:1690–1.
28. McNutt LA, Wu C, Xue X, Hafner JP. Estimating the relative risk in cohort studies and clinical trials of common outcomes. Am J Epidemiol. 2003;157:940–3.
29. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ. 2011;342:d671.
30. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177–88.
31. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557–60.
32. Martinez-Laguna D, Tebe C, Javaid MK, et al. Incident type 2 diabetes and hip fracture risk: a population-based matched cohort study. Osteoporos Int. 2015;26(2):827–33.
33. Li G, Prior JC, Leslie WD, et al. Frailty and risk of fractures in patients with type 2 diabetes. Diabetes Care. 2019;42(4):507–13.
34. Wallander M, Axelsson KF, Nilsson AG, Lundh D, Lorentzon M. Type 2 diabetes and risk of hip fractures and non-skeletal fall injuries in the elderly: a study from the fractures and fall injuries in the elderly cohort (FRAILCO). J Bone Miner Res. 2017;32(3):449–60.
35. Kim SH, Kim YM, Yoo JS, Choe EY, Kim TH, Won YJ. Increased risk of hip fractures in Korean patients with type 2 diabetes: a 6-year nationwide population-based study [published correction appears in J Bone Miner Metab. 2017 Jan 27;:]. J Bone Miner Metab. 2017;35(6):623–629.
36. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. J Clin Endocrinol Metab. 2006;91(9):3404–10.
37. de Liefde II, van der Klift M, de Laet CE, van Daele PL, Hofman A, Pols HA. Bone mineral density and fracture risk in type-2 diabetes mellitus: the Rotterdam Study. Osteoporos Int. 2005;16(12):1713–20.
38. Forsén L, Meyer HE, Midthjell K, Edna TH. Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trøndelag Health Survey. Diabetologia. 1999;42(8):920–5.
39. Hothersall EJ, Livingstone SJ, Looker HC, et al. Contemporary risk of hip fracture in type 1 and type 2 diabetes: a national registry study from Scotland. J Bone Miner Res. 2014;29(5):1054–60.
40. Jiajue R, Qi X, Jiang Y, et al. Incident fracture risk in type 2 diabetic postmenopausal women in mainland China: Peking Vertebral Fracture Study. Calcif Tissue Int. 2019;105(5):466–75.
41. Miyake H, Kanazawa I, Sugimoto T. Association of bone mineral density, bone turnover markers, and vertebral fractures with all-cause mortality in type 2 diabetes mellitus. Calcif Tissue Int. 2018;102(1):1–13.
42. Holmberg AH, Johnell O, Nilsson PM, Nilsson J, Berglund G, Akesson K. Risk factors for fragility fracture in middle age. A prospective population-based study of 33,000 men and women [published correction appears in Osteoporos Int. 2006;17(11):1704]. Osteoporos Int. 2006;17(7):1065–1077.
43. Tebé C, Martínez-Laguna D, Carbonell-Abella C, Reyes C, Moreno V, Diez-Perez A, Collins GS, Prieto-Alhambra D. The association between type 2 diabetes mellitus, hip fracture, and post-hip fracture mortality: a multi-state cohort analysis. Osteoporos Int. 2019 Dec;30(12):2407–15.
44. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis. Osteoporos Int. 2007;18(4):427–44.
45. Dytfeld J, Michalak M. Type 2 diabetes and risk of low-energy fractures in postmenopausal women: meta-analysis of observational studies. Aging Clin Exp Res. 2017;29(2):301–9.
46. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab. 2001;86(1):32–8.
47. Yan W, Li X. Impact of diabetes and its treatment in skeletal diseases. Front Med 2013;7:81e90.
48. Naot D, Cornish J. Cytokines and hormones that contribute to the positive association between fat and bone. Front Endocrinol (Lausanne). 2014;5:70. Published 2014 May 9.
49. Clemens TL, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. J Bone Miner Res. 2011;26(4):677–80.
50. Lipscombe LL, Jamal SA, Booth GL, Hawker GA. The risk of hip fractures in older individuals with diabetes: a population-based study. Diabetes Care. 2007;30(4):835–41.

51. Montagnani A, Gonnelli S. Antidiabetic therapy effects on bone metabolism and fracture risk. Diabetes Obes Metab. 2013;15(9):784–91.
52. Loke YK, Singh S, Furberg CD. Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. CMAJ. 2009;180(1):32–9.
53. Meier C, Kraenzlin ME, Bodmer M, Jick SS, Jick H, Meier CR. Use of thiazolidinediones and fracture risk. Arch Intern Med. 2008;168(8):820–5.
54. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL. Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol. 2017;13(4):208–19.
55. Poiana C, Capatina C. Fracture risk assessment in patients with diabetes mellitus. J Clin Densitom. 2017;20(3):432–43.
56. Conway BN, Long DM, Figaro MK, May ME. Glycemic control and fracture risk in elderly patients with diabetes. Diabetes Res Clin Pract. 2016 May;115:47–53.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):40–48
https://doi.org/10.1007/s13410-021-00950-8

REVIEW ARTICLE

The impact of different modes of exercise training on GLP-1: a systematic review and meta-analysis research

Reihaneh Nejati[1,2] · Nahid Bijeh[1] · Morteza Motahari Rad[1] · Seyyed Reza Attarzadeh Hosseini[1]

Received: 7 June 2020 / Accepted: 5 April 2021 / Published online: 22 April 2021

Abstract

Background The impact of exercise training on glucagon-like peptide 1 (GLP-1) of people with type 2 diabetes has been investigated and it has been found that it can improve their levels of blood glucose; however, the effect of exercise intervention mode on GLP-1 levels is still controversial.

Objective The purpose of this study was to investigate the duration, mode, and intensity of exercise intervention effect on the levels of GLP-1 by a systematic review and meta-analysis.

Data sources By March 29, 2020, Google Scholar, PubMed, Medline, Scopus database, Science Direct, and reference lists of articles had been randomly dealing with the subject matter with the purpose of investigating the effect of different variables of duration and short-term and long-term exercise training on GLP-1 through pre-test and post-tests. Thus, to strengthen the outcome of the present study, sixteen studies with 1562 subjects were included.

Results In the present study, we found a significant change on GLP-1 levels in both types of duration exercise intervention groups (MD: − 1.60 pmol/l; 95% CI [− 2.20, − 1.01]; $p < 0.00001$). Separately investigated, the level of GLP-1 in short-term training was MD − 1.26 pmol/l, 95% CI (− 1.79, − 0.73), $p < 0.00001$, and in long-term training, it was −2.76 pmol/l, 95% CI (− 5.10, − 0.43), $p = 0.02$. The intensity of short-term training was between 55 and 65% max HR, and for the long-term-training, it was 65–85% max HR.

Conclusion In this meta-analysis, it was found that the levels of GLP-1 could be affected by short-term and long-term training with different modes and intensity. As a result, current evidence shows that it may be a good choice for patients with type 2 diabetes to control their blood glucose. The mechanism of this GLP-1 increase has not yet been fully discovered. Further longitudinal studies and exploration into mechanisms of action are required in order to determine the precise role of GLP-1 in insulin responses to an exercise intervention.

Keywords GLP-1 · Exercise training · Short-term training · Long-term training · Meta-analysis

Introduction

Over the past three decades, the number of people with type 2 diabetes (T2DM) has more than doubled globally, making it one of the most important public health challenges for all nations [1]. The progress of T2DM is characterized by insulin resistance and insulin secretion from beta cells in the pancreas [2–4]. On the one hand, the cell membranes via the glucose transport (GLUT) allow insulin to bring glucose into the cells; on the other hand, insulin by the Akt substrate of 160 kDa (AS160) pathway has increased the regulation of GLUTs [5]. It has been observed that the other receptors can act like insulin receptors and change the blood glucose levels [6]. In this regard, glucagon-like peptide-1 (GLP-1) is a gastric hormone and plays an important role in responding to the increase of blood glucose after meal ingestion [4, 7], and GLP-1 receptors (GLP-1Rs) represent a unique approach to the treatment of diabetes with benefits extending outside

✉ Reihaneh Nejati
reihaneh.nejati@gmail.com

[1] Department of Sport Physiology, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Faculty of Sports Sciences, Ferdowsi University of Mashhad, Po BOX: 1574, 91779-48979, Azadi Square, Mashhad, Iran

glucose control and including positive effects on weight, blood pressure, cholesterol levels, and beta cell functions [8]. A little amplification of GLP-1 can improve beta cells and their function to increase insulin secretion and glucagon suppression [9] where binding GLP-1 to GLP-1Rs can bring about cell proliferation via distinct intracellular signaling pathway and can improve glycemic control with similar mechanisms of glucose-dependent insulin secretion from the beta cells. GLP-1Rs act like the insulin receptor and GLP-1 binds the specific G-proteins, increasing intracellular Ca2+ and adenylate cyclase. It activates PKC (protein kinase c) and PI3k (phosphoinositide 3-kinase) and conveyors GLUT towards the membranes to reduce the blood glucose [2, 10]. It has also been shown that GLP-1Rs preserve and improve the markers of beta cell function [11]. Thus, it is suggested that therapy with the addition of a short-acting GLP-1Rs be incorporated into the programs to bring about some advantages such as the effects on slowing gastric emptying [11]. Slower gastric delivery of meal contents leads to smaller glucose response excursion [12] since glucose and energy intakes are closely related [13]. Therefore, the effect of exercise training on energy intake to reduce the glucose levels can be impressive [4]. In line with this, there is plenty of research on exercise which shows that it reduces the blood glucose and it has proved to be healthier than the other ways in controlling diabetes. Thus, it is becoming increasingly clear that exercise and any kind of physical activity can be a therapeutic tool in a variety of ways for patients with or at the risk of diabetes, though the regulation of GLP-1 and insulin secretion through an exercise program for T2DM patients is still under investigation and question [4, 14, 15]. Nevertheless, according to some articles, the expression of GLP-1 from L-distal ileum has been proven to increase by an exercise program and it can improve pancreatic beta cell function [2, 7]. In the same way, some systematic reviews have measured the efficacy of duration of the exercise training [16], and the mechanism pathways to influence glucose uptake in short-term training versus long-term training have been shown to be different [17].

Scientifically speaking, it has been observed that the blood glucose plays an important role in acute training and decreases rapidly after 15–45 min, depending on the workload. These feedback signals can affect the levels of GLP-1 [18], and in patients with T2DM, for example, it is characterized by a reduced incretin effect. Seemingly, a single bout of exercise can bring about a remarkable development in the plasma levels of GLP-1 to reduce energy intake through AS160 pathway, so it can be the cornerstone as the diabetes management [7, 19]. A study recommended a 90-min free weight lifting session followed by a 6.5-h rest period in a 12-repetition round of resistance training and a 60-min running speed required to elicit 70% maximum heart rate. It should be followed by a 7-h rest period with aerobic exercise that can eventually regulate GLP-1 and increase GLUTs in the cell membrane [20]. How long this effect can last has not yet been examined [17].

In the same line, it was observed that weekly exercise volume was positively related with the improvement of T2DM status [21]. The findings showed statistically and clinically significant improvement of glycemic control on the diabetic patients [22]. Respectively, research studies have compared either type of exercise with the control group [19]. On the other hand, not much research has been devoted to the effect of long-term or short-term training on GLP-1 [20]. Although previous findings from aerobic training studies indicated the exercise intensity, they found that structured exercise duration of more than 150 min/week was associated with a decrease in blood glucose and increase in GLP-1 in type 2 diabetes patients [19, 23]. Thus, it seems that the short-term training can bring about more advantages, but since the research of long-term training on GLP-1 is limited, the aim of this study was set to systematically review the literature on the effect of exercise and find out about the best methods that are used in exercise training on GLP-1 and insulin sensitivity in people with T2DM.

Methods

Data sources and searches

We searched and utilized the database in English language on PubMed, CINAHL, Google Scholar, Medline, and Scopus. Pre-specified search terms were GLP-1, incretin, insulin and insulin resistance, blood glucose, aerobic training, long-terms, and acute training. We precisely searched titles, abstracts, subjects, headings, and the contents, and employed the Boolean search terms (AND, OR, or NOT) to create the search strategy. Meta-analyses, systematic reviews, and all references were included. This thorough search was conducted in a time limit of March 29, 2020.

Study selection

The long-term group of exercise training in these analyses was randomly assigned to pre-test and post-test of ≥ 12-week duration and one session of acute training. In our meta-analyses, exercise training included resistance exercise (including full-body training with machine or weight-bearing including at least 6 movements in the upper and lower body), aerobic exercise (including walking, running, and aerobic training), and concurrent exercise (resistance + aerobic). Two authors validated the studies, treatment guidelines, titles, summaries, and full-texts of the appropriate articles to gain qualified analyses.

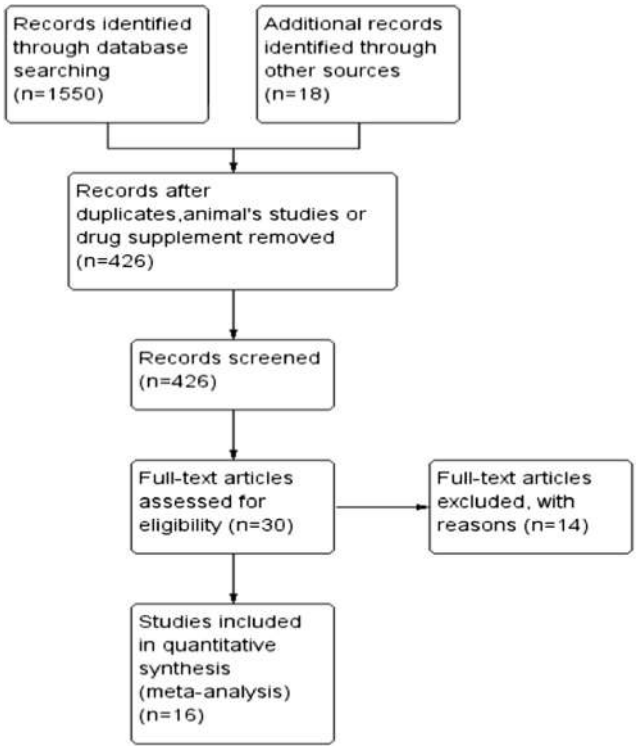


Fig. 1 PRISMA flow diagram

Inclusion/exclusion criteria

In these studies, the following criteria were employed for identification and selection: average age between 35 and 60 years old, T2DM for more than 1 year (fasting blood glucose greater than 126 mg/dl or 7 mmol/l, 2-h plasma glucose equal to or greater than 200 Mg/dl, glycosylated hemoglobin 6.5% or higher). The subjects did not take insulin; rather, they only took their daily requirements like metformin during the treatment period. In this review research, the study protocols used were aerobic, resistance, and concurrent training with an intervention period of ≥ 6 months by a pre-post-test design compared to the control group. Some systematic review articles, conferences, abstracts, and study protocols, as well as studies in which the subjects took part in an exercise regimen during the last 6 months, were excluded.

Data extraction

Three authors collected the data from the articles included in the review. The data were inclusive of subjects' characteristics (age, gender, body mass index [BMI]), the number of subjects, exercise intervention features (frequency, intensity, duration, and mode of exercise), methods and procedures of measuring the levels of GLP-1, authors, year of publication, study design, mean, standard deviation (SD) of continuous outcomes, and details of the biomarker evaluation methodology.

Data synthesis

In contrast to all studies, we extracted the effect size for any findings by measuring the mean difference between the pre- and post-tests. All the results were reported separately and were analyzed by using the same methods of reporting techniques for the findings. The mean difference for GLP-1 (pmol L^{-1}) in pre- and post-test conditions, sample size, participant characteristics, blood analytical methods, and exercise treatment information were the

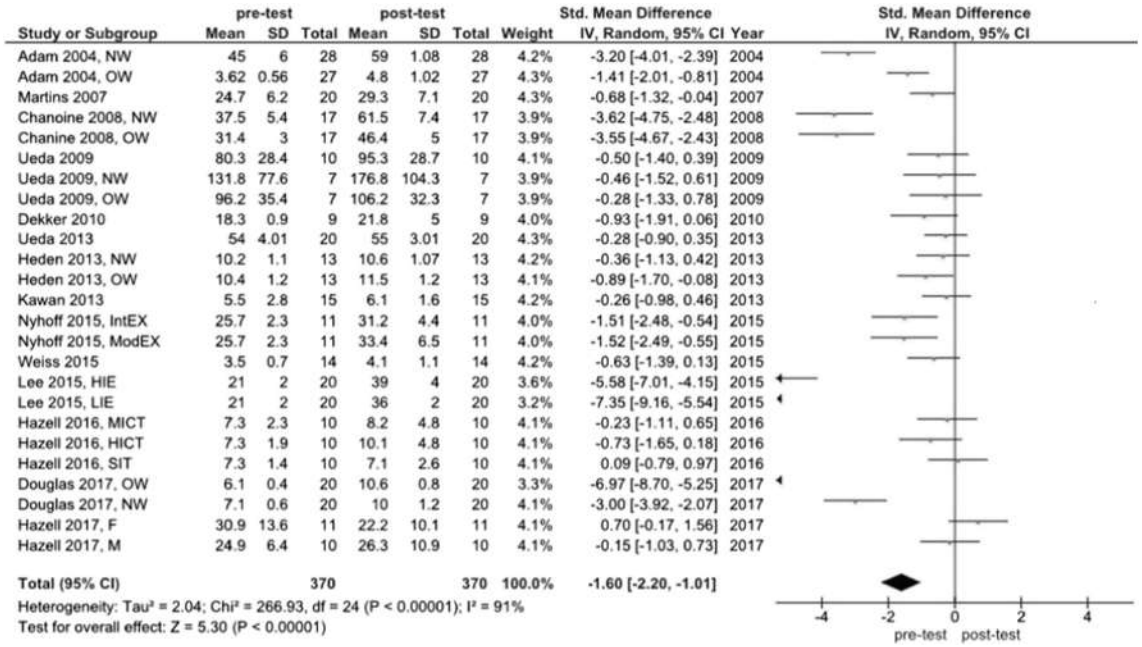


Fig. 2 Forest plot on levels of GLP-1 in exercise intervention

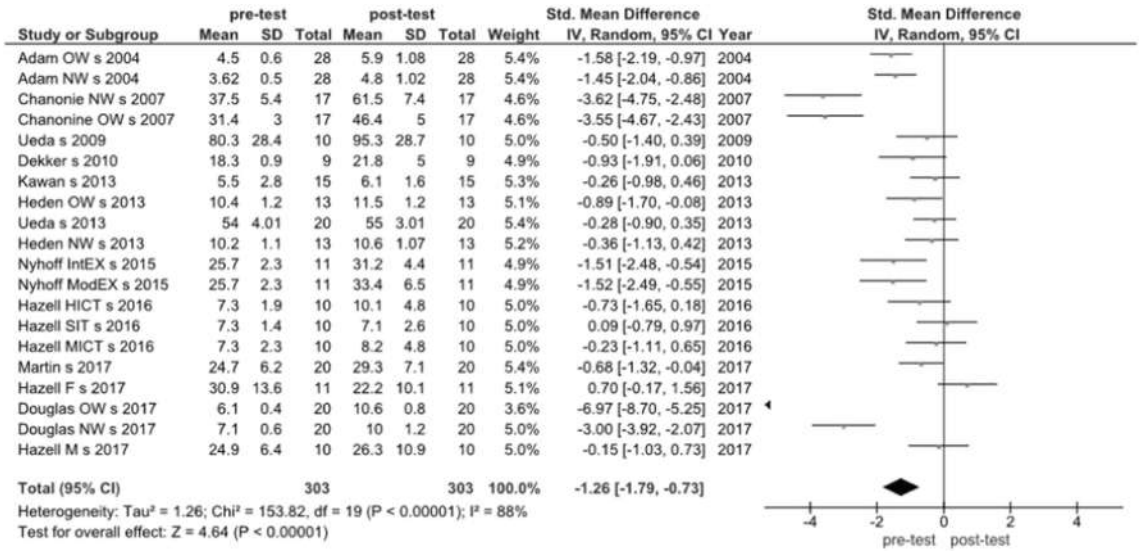


Fig. 3 Forest plot on levels of GLP-1 in short-term training

measures applied. The analysis was done by using the Review Manager 5.3 (The Nordic Cochrane Center, Copenhagen, Denmark). The post-test mean was subtracted from the pre-test mean, and the standard error of means (SEM) value was changed to standard deviation values. If any data was not shown in the texts or tables and we were unable to reach the authors, the data displayed in figures was extracted by employing the TA TechTip and GetData Graph Digitizer software. Where a subject was included in the control group or in more than one intervention group, we reported each group separately and fitted the sample size to the number of other groups. Therefore, heterogeneity was calculated as Cochrane's Q and I^2 index and it was > 50%. Eventually, we presented a 5% level of significance for the forest plot to describe the results.

Study quality

To describe the quality of the studies, we evaluated the data by using the fifteen-point tool in exercise reporting (TESTEX) scales. Two reviewers (RN and MMR) performed the quality control of the studies and reported the assessment.

Results

Study and subject characteristics

One thousand five hundred sixty-two articles were investigated having been searched in the major databases (Google Scholar, PubMed, Scopus, Science Direct, and hand searching). We eliminated animal studies, drug intervention, and duplicate titles. Four hundred twenty-six full-text articles were screened, and after eliminating the irrelevant records, excluded through reading titles and abstracts, we first chose 30 studies and finally 16 articles were selected for the moderator variables through the inclusion and exclusion criteria (PRISMA flow diagram; Fig. 1). Through these 16 studies, 370 subjects had been investigated through a pre-test/post-test design.

Intervention details

The time period during which the selected studies had been conducted ranged from 24 h to 12 weeks. Accordingly, the short-term training interventions ranged from 30 to 60 min at an intensity of 60–85% VO_{2max}, and in the long-term training, it ranged from 45 to 80% VO_{2max}. These findings came out

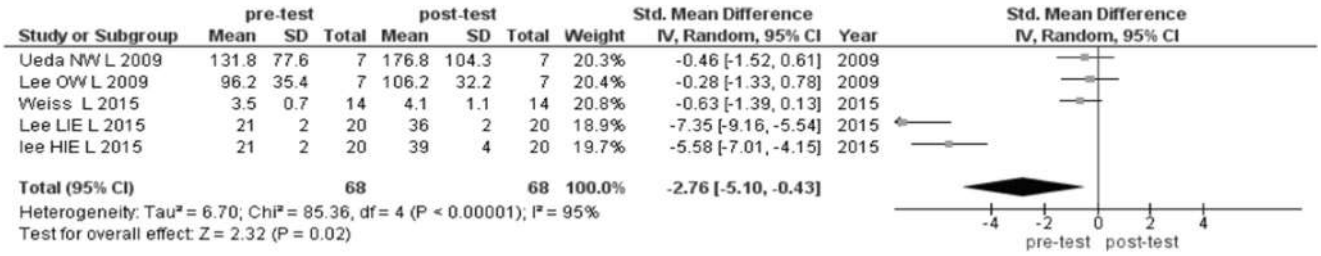


Fig. 4 Forest plot on levels of GLP-1 in long-term training

Table 1 Characteristic of included studies in the meta-analysis

Study	Age mean ± SD	BMI mean ± SD	Disease	Gender	*N* E X (CON)	Modes of exercise	Intervention group: frequency and duration	Assessment measure/units
Martins et al. [24]	25.9±4.6	22.0±3.2	None	6 males, 6 females	12 (8)	Short-term aerobic	65% max HR, 60-min interval exercise	GLP-1
Chanoine et al. [15]	15.3±0.2	NW (20.7±0.5) OW (32.4±1.7)	None obese	36 boys	36	Short-term aerobic	5 days aerobic training (1 h/day)	GLP-1
Ueda et al. [4]	23.4±4.3	22.5±1.0	None	10 males	10	Short-term aerobic	3 sessions (75% VO_{2max}) (50% VO_{2max}) and resting session	GLP-1
Lee et al. [25]	15.3±2.2	24.0±3.8	T2DM	Not mention	20	Long-term aerobic	12 weeks (HIE group: ≥ 80% HR, LIE group: ≤ 45% HR)	GLP-1
Ueda et al. [26]	NW (22.4±4.2) OW (22.9±3.4)	NW (22.4±2.4) OW (30.0±3.1)	None obese	Male	7 (7)	Short-term aerobic	2 sessions (50% VO_{2max} for 60 min)	GLP-1
Hazell et al. [27]	30.5±7.9	23.5±2.8	None	27 female	18 (9)	Short-term aerobic	3 sessions (MICT; 65% VO_{2max}), (SIT)	GLP-1
Hazell et al. [28]	M (28.6±5.9) F (30.5 ±7.9)	M (23.7±2.2) F (23.5±2.8)	None	11 female, 10 male	21	Short-term aerobic	3 sessions (MICT: 30 min cycling at 65% VO_{2max}), (SIT; 6×30 s with 4-min recovery	GLP-1
Ueda et al. [29]	49.1±0.8	27.6±0.4	None	28 female	20	Long-term aerobic	12 weeks, 3 times per week(10-m warm-up, 60-m jogging) 65%HR	GLP-1
Heden et al. [30]	NM (26.0±2) OW (25.4±1)	NM (23.0±0.5) OW (34.6±1)	None obese	NM (7 M, 6F) OW (6 M, 7F)	26	Short-term aerobic	1 h of treadmill walking (55–60% $VO_{2\ peak}$)	GLP-1
Hazell et al. [31]	29±6	23.7±2.2	None	Male	10	Short-term aerobic	4 sessions (MICT; 30-m cycling at 65% VO_{2max}), (HICT; 30-m cycling at 85% VO_{2max}), (SIT; 6×30-s cycling)	GLP-1
Adam et al. [32]	NW (F: 35±12.7) OW (47.1±11.9)	NW (22.9±1.4) OW (30.9±2.7)	None obese	NW (F: 16, M: 12) OW (F: 6, M: 21)	NW= 28 OW= 27	Short-term aerobic	60-min cycling at 25% maximal power output	GLP-1
Weiss et al. [33]	EX (56±9) CON (57± 9)	EX (23.1±1.6) CON (25.3±2.3)	None	EX (M: 13, F: 1) CON (M: 13, F: 1)	14 (14)	Long-term aerobic	Balk treadmill test	GLP-1
Dekker et al. [34]	59±2	33.8±1.5	Hypertriacylglycerolemic	Male	9	Short-term aerobic	60 min of treadmill walking (55% VO_2peak)	GLP-1
Nyhoff et al. [35]	24.3±4.6	37.3±7.0	Obese	Female	11	Short-term aerobic	ModEX (55% VO_{2max}), IntEX (4 min (80% VO_{2max})/3 min (50%VO_{2max}))	GLP-1
Douglas et al. [36]	L (37.5±15.2) O (45.0 ±12.4)	L (22.4±1.5) O (29.2±2.9)	Lean obese	Female	40	Short-term aerobic	60-min treadmill (59.4% peak oxygen uptake)	GLP-1
Kawano et al. [37]	24.4±1.7	22.1±2.0	None	Male	15	Short-term aerobic	Rope skipping (3 sets × 10 m with 5-m interval), bicycle ergometer (3 sets × 10 m with 5-m interval)	GLP-1

from the aerobic training investigations and there is no study for resistance training on GLP-1 concentration.

GLP-1 assessment

All the studies reported hormone values in pmol/l. If any study reported them otherwise, we converted them to pmol/l.

Outcome measures

Change in GLP-1

Sixteen studies in which a total of 370 subjects had been investigated through 25 pre-test- and post-test-reported changes in GLP-1 levels. We mixed the outcomes to make use of random-effect model and revealed a significant change in GLP-1 after post-test exercise intervention (MD: − 1.60 pmol/l; 95% CI [− 2.20, − 1.01]; $p < 0.00001$); Fig. 2).

Analysis of the mode of exercise training

We examined the duration of short-term and long-term training intervention for the levels of GLP-1. These analyses revealed that GLP-1 increased significantly in both types of interventions compared to the values gained through the pre-tests. The results obtained were MD − 1.26 pmol/l, 95% CI (− 1.79, − 0.73), $p < 0.00001$, for the short-term intervention training, and MD −2.76 pmol/l, 95% CI (− 5.10, − 0.43), $p = 0.02$, for the long- term intervention; however, the levels of GLP-1 grew in the two types (Figs. 3 and 4).

Analysis of intensity of exercise training

In all the studies that examined the intensity of short-term training, the value was between 55 and 65% max HR, and for the long-term training, it was 65–85% max HR (Table 1).

Heterogeneity and publication bias

Publication bias was utilized by the funnel plot as described in the subgroup analysis; however, we distributed the mean ES cause of random sampling error if there was no study bias (Fig. 5).

Study quality

The quality of all the studies was judged to be moderate to good, with an average TESTEX score of 10 (ranging between 7 and 12) of a maximum score of 15 (Table 2). Each one of the criteria of monitoring the physical activity was met in all the studies, with the intention-to-treat analysis in 5 studies and relative exercise intensity in 11 studies. The criteria of assessor blinding were also met in 4 studies; however, the criteria of allocation concealment were met in only 3 studies. The other TESTEX criteria were each met in at least 50% of trials.

Discussion

As exercise intolerance is well recognized in patients with type 2 diabetes, the main purpose of this review was to perform a meta-analysis to investigate the impact of duration, mode, and intensity of exercise intervention on the levels of GLP-1 of the subjects.

Our primary analysis shows that the levels of GLP-1 were affected by two types of exercise duration (long-term and short-term training), mode, and intensity. Yet, the effect of short-term training with 55–65% max HR intensity protocol and long-term training with 65–85% max HR might also change GLP-1 concentration.

As the overall analysis of short-term training and GLP-1 shows, the GLP-1 concentration can be enhanced following a bout of exercise session. According to the overall analysis in participants, our short-term training findings differ from the

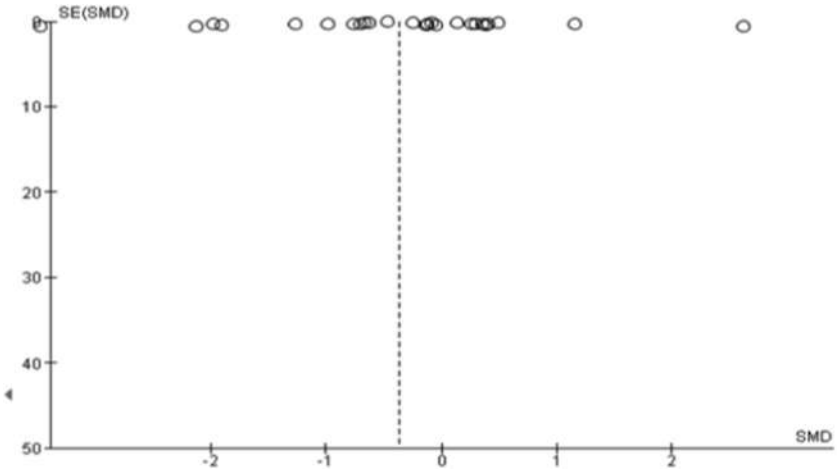


Fig. 5 Funnel plot aerobic and resistance

Table 2 (TESTEX)

Study	Eligibility criteria specified	Randomization details specified	Allocation concealed	Groups similar at baseline	Assessors blinded	Outcome measures assessed > 85% participants[#]	Intention to treat analysis	Reporting between group statistical comparison	Point measures and measures of variability	Activity monitoring in control group	Relative exercise intensity constant	Exercise volume and energy expenditure	Overall TESTEX [15]
Martin et al.	1	1	0	1	1	3	1	2	1	0	1	0	12
Chanoine et al.	1	1	0	1	0	2	0	2	1	0	1	1	10
Ueda et al.	1	1	0	1	0	2	0	2	1	0	1	1	10
Lee et al.	1	0	0	0	0	1	0	2	1	0	1	1	7
Ueda et al.	1	1	0	1	0	3	0	2	1	0	1	1	11
Hazell et al.	1	1	0	1	0	2	0	2	1	0	1	1	10
Hazell et al.	1	1	1	1	1	2	0	2	1	1	0	1	12
Ueda et al.	1	0	0	1	0	1	0	2	1	1	0	1	8
Heden et al.	1	0	0	1	1	3	1	2	1	0	1	1	12
Hazell et al.	1	1	1	1	0	2	0	2	1	0	0	1	10
Adam et al.	1	1	0	1	0	3	1	2	1	0	0	0	10
Weiss et al.	1	0	0	0	0	2	0	2	1	0	1	0	7
Dekker et al.	1	1	0	1	0	1	0	2	1	0	1	1	9
Nyhoff et al.	1	0	0	1	0	2	0	2	1	1	1	1	10
Douglas et al.	1	1	1	1	0	2	1	2	1	0	1	1	12
Kawan et al.	1	1	0	0	1	2	1	2	1	1	0	0	10

findings of Heden et al. (2013) who found that 1 h of treadmill walking did not change the GLP-1 concentration between trials [30]. In addition, Ueda et al. (2009) reported that a session of cycling exercise at 50% VO_{2max} significantly did not change the GLP-1 levels [26], which differ from our findings. Our short-term training group analysis indicated a positive effect of exercise training on GLP-1 concentration. According to the findings of Heden et al., exercise training may decrease postprandial insulin levels via reduced pancreatic β cell insulin secretion. Interestingly, the reduction in insulin secretion in the exercise training happened in the face of similar plasma glucose and GLP-1 levels as compared to the control group [30]. They also suggested that GLP-1 concentration is not only modulated by blood glucose, but it is as well impressed by other hormones and nervous system. It is possible that these levels are masked by alterations in the other variables. Yet, care shall be taken in explaining the findings because the short-term training group analysis only contained a small number of studies.

From the clinical research in short-term training group, our findings are close to the findings of Martin et al. (2007) who found that GLP-1 concentration is related with 1 h of 65% HR cycling and it can significantly increase the GLP-1 concentration [24]. In addition, Adams et al. (2004) reported that a 60-min cycling at 25% maximum power output significantly changes the GLP-1 concentration compared with that of the pre-test group [32].

Although other articles had reported the positive change in GLP-1 concentration that increases immediately after an acute session, these studies investigated only one session of exercise training effects. In this regard, Chanoine et al. (2008) reported that 5 days of aerobic exercise training increases the GLP-1 concentration [15]. Hazell et al. (2017) reported the sessions of MICT and SIT only increased the GLP-1 concentration in females following MICT training compared to the CTRL group [28].

Compared to the pre-test results, the long-term training group's GLP-1 concentration was highly affected and increased as the post-test results indicated. To explain this, Lee et al. (2015) found that 12 weeks of exercise training with an intensity of ≤ 45 to ≥ 80% significantly increased the GLP-1 concentration in patients with type 2 diabetes [25]. In the same line, Ueda et al. (2013) reported that 12 weeks of exercise training with 65% max HR significantly increased the GLP-1 concentration compared with the findings in the pre-test [29]. However, a cross-sectional study reported that post-exercise GLP-1 levels do not differ between the control group and non-obese control subjects who had higher insulin levels. These findings suggest that the lower levels of insulin in the control group are not mediated by the reduction in GLP-1 concentration. It also reported that lower blood glucose might provide less β cell stimulus for insulin. This stimulus to GLP-1 might be reduced in exercise-trained individuals.

Regarding the GLP-1 function on pancreas, the mechanism pathway by exercise is still unclear. Exercise training including short-term or long-term training has been prescribed for increasing GLP-1 concentration and appears to be one of the safest treatments for the patients with type 2 diabetes. Furthermore, some investigations suggested that the exercise training can prevent blood glucose in patients with type 2 diabetes from increasing [38]. As far as our knowledge allows, this is the first systematic review and meta-analysis to investigate the effect of long-term and short-term training with the mode and intensity suggestions on the GLP-1 concentration. There are, of course, some limitations in our meta-analysis that need to be reported. First of all, some studies have been conducted only on animals and few studies on humans; secondly, some studies have used several types of medicines which limited us; thirdly, the number of the articles that worked on different types of training was limited; and finally, the studies were limited to English language, so we could not extract all the data to obtain all potentially relevant studies.

Conclusion

Through this meta-analysis, we found that short-term and long-term training with different modes and intensities could influence the levels of GLP-1. The mechanism of this increase has not yet fully been discovered and many questions still exist.

Abbreviations GLP-1, Glucagon-like peptide-1; GLP-1Rs, Glucagon-like peptide-1 receptors; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; Vo_{2max}, Maximal oxygen uptake; 1RM, One repetition maximum; Max HR, Maximum heart rate; HIE, High-intensity interval exercise; LIE, Low-intensity interval exercise; MICT, Moderate-intensity continuous training; SIT, Sprint interval training; HICT, High-intensity continuous training; NW, No overweight; OW, Overweight; ModEX, Moderate-intensity aerobic continuous exercise; IntEX, High-intensity aerobic interval exercise; NOEX, No exercise; Vs, Versus; GLUT, Glucose transport; AS160, Akt substrate of 160 kDa; AMPK, 5′ AMP-activated protein kinase; MD, Mean difference; PKC, Protein kinase C; EI, Exercise intensity; PI3K, Phosphoinositide 3-kinase

Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol. 2012;8(4):228–36.

2. Furtado LM, Somwar R, Sweeney G, Niu W, Klip A. Activation of the glucose transporter GLUT4 by insulin. Biochem Cell Biol. 2002;80(5):569–78.
3. Wollheim CB. Beta-cell mitochondria in the regulation of insulin secretion: a new culprit in type II diabetes. Diabetologia. 2000;43(3):265–77.
4. Ueda S-y, Yoshikawa T, Katsura Y, Usui T, Fujimoto S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. J Endocrinol. 2009;203(3):357–64.
5. Kramer HF, Witczak CA, Fujii N, Jessen N, Taylor EB, Arnolds DE, et al. Distinct signals regulate AS160 phosphorylation in response to insulin, AICAR, and contraction in mouse skeletal muscle. Diabetes. 2006;55(7):2067–76.
6. Sheikh A. Direct cardiovascular effects of glucagon like peptide-1. Diabetol Metab Syndr. 2013;5(1):47.
7. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet. 1987;2(8571):1300–4.
8. Prasad-Reddy L, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. Drugs Context. 2015;4:212283.
9. Gupta V. Glucagon-like peptide-1 analogues: an overview. Indian J Endocrinol Metab. 2013;17(3):413–21.
10. Atlas D. International diabetes federation. IDF Diabetes Atlas, 7th edn Brussels, Belgium: International Diabetes Federation. 2015.
11. Balena R, Hensley IE, Miller S, Barnett AH. Combination therapy with GLP-1 receptor agonists and basal insulin: a systematic review of the literature. Diabetes Obes Metab. 2013;15(6):485–502.
12. Chaikomin R, Doran S, Jones KL, Feinle-Bisset C, O'Donovan D, Rayner CK, et al. Initially more rapid small intestinal glucose delivery increases plasma insulin, GIP, and GLP-1 but does not improve overall glycemia in healthy subjects. Am J Physiol Endocrinol Metab. 2005;289(3):E504–E7.
13. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, et al. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. Am J Physiol Endocrinol Metab. 2007;293(3):E743–E53.
14. Martins C, Morgan L, Truby H. A review of the effects of exercise on appetite regulation: an obesity perspective. Int J Obes. 2008;32(9):1337–47.
15. Chanoine J-P, Mackelvie KJ, Barr SI, Wong ACK, Meneilly GS, Elahi DH. GLP-1 and appetite responses to a meal in lean and overweight adolescents following exercise. Obesity. 2008;16(1):202–4.
16. Gordon BA, Benson AC, Bird SR, Fraser SF. Resistance training improves metabolic health in type 2 diabetes: a systematic review. Diabetes Res Clin Pract. 2009;83(2):157–75.
17. Kristiansen S, Hargreaves M, Richter E. Exercise-induced increase in glucose transport, GLUT-4, and VAMP-2 in plasma membrane from human muscle. Am J Physiol Endocrinol Metab. 1996;270(1):E197–201.
18. Huda MSB, Wilding JPH, Pinkney JH. Gut peptides and the regulation of appetite. Obes Rev. 2006;7(2):163–82.
19. Umpierre D, Ribeiro PAB, Kramer CK, Leitão CB, Zucatti ATN, Azevedo MJ, et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. JAMA. 2011;305(17):1790–9.
20. Broom DR, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. Am J Physiol Regul Integr Comp Physiol. 2009;296(1):R29–35.
21. Umpierre D, Ribeiro P, Schaan B, Ribeiro J. Volume of supervised exercise training impacts glycaemic control in patients with type 2 diabetes: a systematic review with meta-regression analysis. Diabetologia. 2013;56(2):242–51.
22. Zanuso S, Jimenez A, Pugliese G, Corigliano G, Balducci S. Exercise for the management of type 2 diabetes: a review of the evidence. Acta Diabetol. 2010;47(1):15–22.
23. Lee SS, Yoo JH, So YS. Effect of the low- versus high-intensity exercise training on endoplasmic reticulum stress and GLP-1 in adolescents with type 2 diabetes mellitus. J Phys Ther Sci. 2015;27(10):8–3063.
24. Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. J Endocrinol. 2007;193(2):251–8.
25. Lee SS, Yoo JH, So YS. Effect of the low-versus high-intensity exercise training on endoplasmic reticulum stress and GLP-1 in adolescents with type 2 diabetes mellitus. J Phys Ther Sci. 2015;27(10):3063–8.
26. Ueda S-y, Yoshikawa T, Katsura Y, Usui T, Nakao H, Fujimoto S. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. J Endocrinol. 2009;201(1):151.
27. Hallworth JR, Copeland JL, Doan J, Hazell TJ. The effect of exercise intensity on total PYY and GLP-1 in healthy females: a pilot study. J Nutr Metab. 2017;2017:1–7.
28. Hazell TJ, Townsend LK, Hallworth JR, Doan J, Copeland JL. Sex differences in the response of total PYY and GLP-1 to moderate-intensity continuous and sprint interval cycling exercise. Eur J Appl Physiol. 2017;117(3):431–40.
29. Ueda S-y, Miyamoto T, Nakahara H, Shishido T, Usui T, Katsura Y, et al. Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. Springerplus. 2013;2(1):83.
30. Heden TD, Liu Y, Kearney ML, Park Y, Dellsperger KC, Thomas TR, et al. Prior exercise and postprandial incretin responses in lean and obese individuals. Med Sci Sports Exerc. 2013;45(10):1897–905.
31. Hazell TJ, Islam H, Hallworth JR, Copeland JL. Total PYY and GLP-1 responses to submaximal continuous and supramaximal sprint interval cycling in men. Appetite. 2017;108:238–44.
32. Adam TCM, Westerterp-Plantenga MS. Activity-induced GLP-1 release in lean and obese subjects. Physiol Behav. 2004;83(3):459–66.
33. Weiss EP, Royer NK, Fisher JS, Holloszy JO, Fontana L. Postprandial plasma incretin hormones in exercise-trained versus untrained subjects. Med Sci Sports Exerc. 2014;46(6):1098–103.
34. Dekker MJ, Graham TE, Ooi TC, Robinson LE. Exercise prior to fat ingestion lowers fasting and postprandial VLDL and decreases adipose tissue IL-6 and GIP receptor mRNA in hypertriacylglycerolemic men. J Nutr Biochem. 2010;21(10):983–90.
35. Nyhoff LM, Heden TD, Leidy HJ, Winn NC, Park Y-M, Thyfault JP, et al. Prior exercise does not alter the incretin response to a subsequent meal in obese women. Peptides. 2015;71:94–9.
36. Douglas JA, King JA, Clayton DJ, Jackson AP, Sargeant JA, Thackray AE, et al. Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. Int J Obes. 2017;41(12):1737–44.
37. Kawano H, Mineta M, Asaka M, Miyashita M, Numao S, Gando Y, et al. Effects of different modes of exercise on appetite and appetite-regulating hormones. Appetite. 2013;66:26–33.
38. Motahari Rad M, Bijeh N, Attarzadeh Hosseini SR, Raouf SA. The effect of two concurrent exercise modalities on serum concentrations of FGF21, irisin, follistatin, and myostatin in men with type 2 diabetes mellitus. Arch Physiol Biochem. 2020:1–10.

International Journal of Diabetes in Developing Countries (January–March) 42(1):49–52
https://doi.org/10.1007/s13410-021-00994-w

SHORT ARTICLE

Long-term persistent symptoms of COVID-19 infection in patients with diabetes mellitus

Ahmed Mechi[1] · Alhan Al-Khalidi[2] · Rasha AL-Darraji[3] · Mohammed Noori Al-Dujaili[1] · Karrar Al-Buthabhak[1] · Mohammed Alareedh[1] · Foaad Shaghee[2] · Hussein Nafakhi[1]

Received: 18 June 2021 / Accepted: 12 August 2021 / Published online: 24 August 2021

Abstract

Objective The study aimed to assess the long-term persistent symptoms of patients with diabetes mellitus (DM) and COVID-19 infection at 9 months after acute infection.

Methods This single-center cross-sectional study was conducted from May 20 to June 1, 2021.

Results A total of 112 patients were included in the present study. The most frequently reported persistent symptoms among DM group were fatigue ($p = 0.01$), shortness of breath ($p = 0.01$), and chest pain ($p = 0.02$) compared to non-DM group. Sulfonylurea use was associated with persistent cough ($p = 0.04$).

Conclusion Long-term persistent symptoms of COVID-19 infection are common among patients with DM.

Keywords DM · COVID-19 · Long term · Symptoms

Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) primarily involves the respiratory tract leading to flu-like symptoms and coronavirus disease-2019 (COVID-19). COVID-19 is recently recognized as a multi-organ disease often causing extra-pulmonary manifestations after the acute phase, even among those who experience mild infection [1–3].

Diabetes mellitus (DM) is major comorbidity of COVID-19 infection, and patients with DM have a high prevalence of disease severity and adverse outcome during COVID-19 infection [2]. Thus, patients with COVID-19 and DM may be particularly at risk for long-term persistent symptoms or health consequences beyond the acute phase [2].

In the literature, little is known about the long-term symptoms of COVID-19 infection in patients with DM after 9 months of the acute phase. We aimed to assess the long-term persistent symptoms of patients with DM and laboratory-confirmed SARS-CoV-2 infection at 9 months after acute infection.

Methods

This is single-center cross-sectional study of patients with laboratory-confirmed SARS-CoV-2 infection who presented to the outpatient clinic of Al-Sader Teaching

✉ Hussein Nafakhi
husseinaf.alnaffakh@uokufa.edu.iq

Ahmed Mechi
ahmedm.mechi@uokufa.edu.iq

Alhan Al-Khalidi
alhan.abbas@jmu.edu.iq

Rasha AL-Darraji
husseinalinafak@yahoo.com

Mohammed Noori Al-Dujaili
mohammedn.aldujaili@uokufa.edu.iq

Karrar Al-Buthabhak
kararm.zwain@uokufa.edu.iq

Mohammed Alareedh
mohammed.alareedh@uokufa.edu.iq

Foaad Shaghee
f.fertossy@jmu.edu.iq

[1] Internal Medicine Department, Medicine College, University of Kufa, P.O. 21, Kufa, Najaf, Iraq

[2] Internal Medicine Department, Medicine College, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Najaf, Iraq

[3] Allergy and Asthma Center, Al-Sader Teaching Hospital, Najaf Health Bureau, Ministry of Health, Najaf, Iraq

Hospital after 9 months of acute infection from May 20 to June 1, 2021.

Patients were interviewed by a trained physician in the outpatient clinic and asked to report symptoms from a pre-defined list of symptoms, including shortness of breath interfere with routine daily activities; easy fatigue; cough; chest pain; palpitation; joint pain; and neurocognitive dysfunction which includes dizziness, headache, concentration abnormalities, memory disturbances, smell loss, and taste loss. If there was a symptom that was not mentioned in the symptom questionnaire list, patients were asked to describe it. The baseline clinical comorbidities and demographic data included age, sex, hypertension, glucose-lowering drugs or insulin, smoking, body mass index (BMI), and hospital admission and duration. DM was defined as any established diagnosis prior to acute COVID-19 infection.

Statistical analysis

Statistical analysis was performed using SPSS ver. 23.0 (SPSS Inc., Chicago, IL, USA). Demographic, comorbidities, and long-term symptoms were expressed as mean ± standard deviation for continuous variables or as numbers with percentages for categorical data. Continuous variables were compared using the Student *t*-test. The Chi-square test was used for comparisons of categorical data. *p* Value of < 0.05 was chosen for statistical significance.

Results

A total of 112 patients with laboratory-confirmed SARS-CoV-2 at 9 months after the acute phase of COVID-19 infection were included in the present study. The patients were divided into the DM group [42 patients with age (years) 60 ± 10, 26 (62%) were males] and the non-DM group [70 patients with age (years) 45 ± 12, 48(68%) were males). The prevalence of old age, hypertension, and increased BMI was higher among the DM group than the non-DM group. Patients, characteristics are shown in Table 1.

The most frequently reported persistent symptoms among DM group compared to non-DM group were fatigue (76% vs. 53%, $p = 0.01$), shortness of breath (45% vs. 21%, $p = 0.01$), chest pain (31% vs. 13%, $p = 0.02$), cough (26% vs. 13%, $p = 0.07$), palpitation (21% vs. 13%, $p = 0.23$), dizziness (24% vs. 10%, $p = 0.73$), concentration disturbances (19% vs. 13%, $p = 0.37$), and memory disturbances (17% vs. 13%, $p = 0.57$) (Table 1).

Table 1 Patients' characteristics

Variables	DM $n = 42$	Non-DM $n = 70$	*p* Value
Age (years), mean ± SD	60 ± 10	45 ± 12	< 0.01
Male, *n* (%)	26 (62)	48 (68)	0.55
BMI, mean ± SD	32 ± 6	28 ± 4	< 0.01
Smoking, *n* (%)	6 (14)	10 (14)	0.12
Hypertension, *n* (%)	29 (69)	18 (26)	< 0.01
Hospital stay (days), mean ± SD	7 ± 1	5 ± 1	0.31
Hospital admission, *n* (%)	24 (57)	28 (40)	0.07
Persistent symptoms			
At least one symptom, *n* (%)	37 (88)	55 (79)	0.05
Fatigue, *n* (%)	32 (76)	37 (53)	0.01
Shortness of breath, *n* (%)	19 (45)	15 (21)	0.01
Chest pain, *n* (%)	13 (31)	9 (13)	0.02
Cough, *n* (%)	11 (26)	9 (13)	0.07
Joint pain, *n* (%)	10 (24)	21 (30)	0.45
Palpitation, *n* (%)	9 (21)	9 (13)	0.23
Neurocognitive dysfunction symptoms			
Dizziness, *n* (%)	10 (24)	7 (10)	0.73
Concentration abnormalities, *n* (%)	8 (19)	9 (13)	0.37
Memory disturbances, *n* (%)	7 (17)	9 (13)	0.57
Headache, *n* (%)	6 (14)	14 (20)	0.44
Smell loss, *n* (%)	2 (5)	7 (10)	0.48
Taste loss, *n*(%)	1 (2)	5 (7)	0.15

Regarding the relationship of diabetes lowering drugs and long-term post-COVID-19 infection symptoms, sulfonylurea use was associated with cough ($p = 0.04$) and fatigue ($p = 0.05$). Although not statistically significant, insulin use was associated with neurocognitive dysfunction, shortness of breath, and fatigue. On the other hand, metformin use was associated with decreased prevalence of shortness of breath, fatigue, and neurocognitive dysfunction compared to patients who did not use metformin, although the difference was not statistically significant (Table 2).

Discussion

We found that at 9 months after the acute COVID-19 infection, 88% of patients with DM reported at least one long-term persistent symptom, especially fatigue, neurocognitive dysfunction, and shortness of breath.

Long-term persistent musculoskeletal, respiratory, and neurocognitive symptoms have been reported for other coronaviruses (SARS and MERS) in long-term follow-up studies. Persistent viremia, relapse or reinfection, immune dysfunction, deconditioning, and post-traumatic stress have

Table 2 Diabetes lowering drugs and post-COVID-19 persistent symptoms

Symptoms	Metformin			Sulfonylurea			Insulin		
	With $n=29$	Without $n=13$	p	With $n=14$	Without $n=28$	p	With $n=10$	Without $n=32$	p
Shortness of breath, n (%)	11 (38)	7 (54)	0.23	7 (50)	11 (39)	0.57	6 (60)	12 (38)	0.23
Fatigue, n(%)	20 (69)	11 (85)	0.12	13 (93)	18 (64)	0.05	9 (90)	22 (69)	0.22
Cough, n (%)	8 (28)	2 (15)	0.45	6 (43)	4 (14)	0.04	1 (10)	9 (28)	0.22
Neurocognitive dysfunction*, n (%)	11 (38)	8 (62)	0.09	8 (57)	11 (39)	0.31	7 (70)	12 (38)	0.07
Chest pain, n (%)	10 (34)	3 (23)	0.55	6 (43)	7 (25)	0.26	3 (30)	10 (31)	0.89

*Neurocognitive dysfunction which include dizziness, headache, concentration abnormalities, memory disturbances, smell, and taste loss

been suggested as potential mechanisms for long-term health consequences following acute viral infection [2, 4].

Based on recently available data, the coexistence of comorbid conditions, including older age and obesity, among patients with DM and COVID-19 infection, would predispose patients to adverse clinical outcomes and persistent symptoms [2, 3]. It has been suggested that COVID-19 infection may exhibit shared pathologies with DM and other comorbidities via immune dysfunction, chronic low-grade inflammation, high prevalence of preexisting comorbidities, and endothelial dysfunction [2].

According to available data, results related to DM lowering drugs and COVID-19 severity and prognosis have been inconsistent among clinical studies. Ethnic disparity, access to medical care, study design, number of enrolled patients, and frequency of adverse events in some studies populations may have influenced the inconsistent results of the literature [5].

The present study has several limitations. It is single-center study with a relatively small size population. This limitation did not allow us to eliminate the potential impacts of other confounders such as hypertension, older age, and obesity. Data regarding DM severity or duration before COVID-19 were not reported. Although symptoms were collected by a well-trained physician, we did not assess the severity of symptoms or perform quality of life or cognitive function assessments. There could be a possibility of selection bias as patients who attended the outpatient clinic were more likely to be more symptomatic.

Further studies are required to assess the pathogenesis and prognosis of long-term persistent symptoms following COVID-19 infection in patients with DM.

In conclusion, long-term persistent symptoms of COVID-19 infection are common among patients with DM. Fatigue, neurocognitive dysfunction, shortness of breath, and chest pain are the most common symptoms among patients with DM.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Graham EL, Clark JR, Orban ZS, Lim PH, Szymanski AL, Taylor C, et al. Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers." Ann Clin Transl Neurol. 2021;8(5):1073–85.
2. Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and diabetes: a collision and collusion of two diseases. Diabetes. 2020;69(12):2549–65.

3. Logue JK, Franko NM, McCulloch DJ, McDonald D, Magedson A, Wolf CR, et al. Sequelae in adults at 6 months after COVID-19 infection. JAMA Netw Open. 2021;4(2):e210830.
4. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021;397(10270):220–32.
5. Nafakhi H, Alareedh M, Al-Buthabhak K, Shaghee F, Nafakhi A, Kasim S. Predictors of adverse in-hospital outcome and recovery in patients with diabetes mellitus and COVID-19 pneumonia in Iraq. Diabetes Metab Syndr. 2021;15(1):33–8.

International Journal of Diabetes in Developing Countries (January–March) 42(1):53–61
https://doi.org/10.1007/s13410-021-00992-y

ORIGINAL ARTICLE

How the COVID-19 outbreak affected patients with diabetes mellitus?

Pinar Sisman[1] · Irmak Polat[2] · Ensar Aydemir[3] · Remzi Karsi[4] · Ozen Oz Gul[3] · Soner Cander[3] · Canan Ersoy[3] · Erdinc Erturk[3]

Received: 27 June 2021 / Accepted: 28 July 2021 /Published online: 10 September 2021

Abstract

Background Global COVID-19 outbreak has been such a stressful experience for most of the people. Using a web-based cross-sectional study, we aimed to evaluate the acute stress response, depression, and anxiety in patients with diabetes mellitus (DM) during the COVID-19 pandemic, and to examine the effect of these psychiatric problems on diet habits and glycemic controls of patients.

Methods This web-based survey of COVID-19 was sent to the patients through the Whatsapp platform. All participants reported their demographic data, diabetes-related information, changes in self-monitoring blood glucose measurements, physical parameters, and eating habits after COVID-19, then completed Hospital Anxiety and Depression Scale (HADS) and the Impact of Event Scale, Revised (IES-R) questionnaires which assessed acute stress sypmtoms, anxiety, and depression.

Results Three hundred and four patients with DM [(141 type 1 DM (T1D) and 163 type 2 (T2D)] were included in the study. In our study, female gender, higher BMI and weight, decreased in financial income after outbreak, presence of diabetic complications and comorbid diseases (i.e., retinopathy, neuropathy, diabetic foot, hypertension, dyslipidemia), worsened glycemic levels, increased carbohydrate consumption, and snacking were associated with higher anxiety and depression scores. Depression was higher in patients with T2D and duration of illness was correlated with acute stress level.

Conclusions It is important to be aware of the possibility of acute stress, depression, and anxiety after pandemic in patients with DM whose glycemic control is impaired. Psychological problems should not be ignored beyond physical inactivity and worsening eating habits.

Keywords Diabetes mellitus · COVID-19 · Pandemic · Anxiety · Depression

Introductıon

The World Health Organization (WHO) declared the new Coronavirus Disease 2019 (COVID-19) outbreak as an International Public Health Emergency of International Concern on January 2020 and as a pandemic on March 2020. The pandemic has disrupted the lives of people across the world by its rapid spread and high mortality. Information about the physical effects of the COVID-19 on organs and systems has been increased overtime [1]. Yet, the contagiousness of the disease and the high mortality rates, combined with mass hysteria, economic burden, and financial losses, caused mass fear, which was called "coronaphobia" [2].

Diabetes mellitus (DM) is a chronic, progressive disease considered the largest global health problem of the twenty-first century, according to the International Diabetes Federation [3]. Patients with DM need to perform daily self-care, develop new eating habits, and adopt specific behaviors to properly manage the disease. All these changes and being aware of problems related to the disease can be stressful for diabetics. [4].

Diabetes mellitus is associated with many neuropsychiatric diseases, especially depression. It is known that the frequency of depression is 2–3 times higher in diabetic patients than in the general population. However, depression is

✉ Pinar Sisman
drpinarsisman@gmail.com

1 Medicana Hospital, Endocrinology and Metabolism Clinic, Bursa, Turkey

2 Istanbul Faculty of Medicine Department of Psychiatry, Istanbul University, Istanbul, Turkey

3 Medical School Department of Endocrinology and Metabolism, Uludag University, Bursa, Turkey

4 School of Health Science, KTO Karatay University, Konya, Turkey

considered one of the most overlooked symptoms in diabetics [5–7]. Severity of depressive attacks in diabetic patients is associated with decreased quality of life, increased diabetic comorbidities, and impaired glycemic control [8]. Anxiety prevalence is also higher in people with DM [9]. Apart from depression, the emotional response seen in diabetic patients and also defined as "diabetic distress" contributes to the worsening of diabetes in the event of a perceived lack of support from family and healthcare professionals [10]. Diabetic symptoms, complications, increased disease burden, inadequate self-care, disability, and decreased quality of life are observed more in patients with comorbid depression and/or anxiety [11, 12]. Stress is a major risk factor for diabetic patients which worsens glycemic control and thus results in more symptoms and complications [13, 14].

Table 1 Sociodemographic and clinical characteristics of the patients with diabetes included to the study

	Type 1 diabetes (*n*=141)	Type 2 diabetes (*n*=163)	Diabetes (*n*=304)
Age (year)	30.6±11.4	52.0±11.1	42.1±15.5
Duration of diabetes (year)	12.0±9.7	8.9±7.1	10.3±8.5
Gender			
Female (%)	87 (61.7%)	82 (50.3%)	
Male (%)	54 (38.3%)	81 (49.7%)	
BMI (kg/m^2)	23.4±4.1	29.9±5.4	26.9±5.8
Alcohol			
No	104 (73.8%)	130 (79.8%)	234 (77.0%)
Yes	27 (19.1%)	15 (9.2%)	42 (13.8%)
Stop dinking during pandemic	5 (3.5%)	15 (9.2%)	20 (6.6%)
Stop drinking before pandemic	5 (3.5%)	3 (1.8%)	8 (2.6%)
Smoking			
No	81 (57.4%)	76 (46.6%)	157 (51.6%)
Yes	45 (31.9%)	57 (35.0%)	102 (33.6%)
Stop smoking during pandemic	10 (7.1%)	27 (16.6%)	32 (12.2%)
Stop smoking during pandemic	5 (3.5%)	3 (1.8%)	8 (2.6%)
Education			
Higher or postgraduate	88 (62.4%)	71 (43.6%)	159 (52.3%)
High school	44 (31.2%)	52 (31.9%)	96 (31.6%)
Elementary school	9 (6.4%)	40 (24.5%)	49 (16.1%)
Marital status			
Single	76 (53.9%)	14 (8.6%)	90 (29.6%)
Married	59 (41.8%)	133 (81.6%)	192 (63.2%)
Divorced/widowed	6 (4.3%)	16 (9.8%)	22 (7.2%)
Social status			
Living with family	129 (91.5%)	155 (95.1%)	284 (93.4%)
Living alone	12 (8.5%)	8 (4.9%)	20 (6.6%)
Financial income			
High	6 (4.3%)	11 (6.7%)	17 (73.8%)
Middle	106 (75.2%)	130 (79.8%)	236 (77.6%)
Low	29 (20.6%)	22 (13.5%)	51 (16.8%)
Diabetic complications			
Retinopathy (%)	25 (17.7)	45 (27.6%)	70 (23.0%)
Nephropathy (%)	15 (10.6)	18 (11.0%)	33 (10.9%)
Neuropathy (%)	40 (28.4%)	77 (47.2%)	117 (38.5)
Diabetic ulcers (%)	10 (7.1%)	20 (12.3%)	30 (9.9%)
Treatment			
OADs (%)	-	97 (59.5%)	
Insulin (%)	141 (100%)	10 (6.1%)	
OADs and insulin (%)	-	56 (34.4%)	

BMI body mass index, *OADs* oral antidiabetics

Table 2 Scores of Hospital Anxiety and Depression Scale and Impact of Event Scale, Revised and frequency of anxiety and depression of the patients

	Diabetes (*n*=304)	Type 1 diabetes (*n*=141)	Type 2 diabetes (*n*=163)	*p**
HADS Total	13.6±7.4	12.9±6.6	14.1±8.0	0.15
Anxiety	7.3±4.0	7.1±3.6	7.5±4.3	0.37
Depression	6.2±4.0	5.8±3.7	6.6±4.2	**0.08**
Frequency of anxiety (%)	45.7	44.7	46.6	0.73
Frequency of depression (%)	33.9	27.7	39.3	**0.03**
IES,R	23.5±13.6	23.5±13.4	23.5±13.8	0.99
Intrusion	7.2±5.8	6.8±5.5	7.5±6.0	0.25
Avoidance	10.0±5.2	10.5±.5.5	9.6±4.9	0.13
Hyperarousal	6.3±4.6	6.2±4.3	6.3±4.8	0.79

HADS Hospital Anxiety and Depression Scale, *IES,R* Impact of Event Scale, Revised

*Statistical analysis between type 1 and type 2 diabetes

Global COVID-19 outbreak has been such a stressful experience for most of the people. Therefore, using a web-based cross-sectional study, we aimed to evaluate the acute stress response, depression, and anxiety in diabetic patients during the COVID-19 pandemic, and to examine the effect of these psychiatric problems on diet habits and glycemic controls of patients.

Material and methods

In a cross-sectional study, we evaluated patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) receiving outpatient care at Uludag University Medical School and Medicana Hospital Endocrinology and Metabolism Clinic in Bursa, Turkey. Inclusion criteria were as follows: (i) being age 18 years old and older, (ii) having been diagnosed with type 1 and type 2 DM for at least 6 months, (iii) being followed for at least 3 months in the same centers, and (iv) volunteering to fill in the questionnaire. This study was approved by the Institutional Review Board of the Uludag University of Turkey. All procedures in this clinical trial were carried out in accordance with the ethical principles and standards in the recently revised Declaration of Helsinki.

This web-based survey of COVID-19 was sent to the patients through the Whatsapp platform. All participants reported voluntarily their demographic data, diabetes-related information, and completed two standardized questionnaires which assessed acute stress, anxiety, and depression. In our study, the sociodemographic and behavioral data of our diabetic patients were obtained through the questionnaire which is developed for this study. The following data are obtained in our study: antropometric data of the patients, duration of diabetes, treatment protocols, presence of comorbid diseases and diabetic complications, and educational, marital, employment, and social status. Also, during the COVID-19 pandemic, patients' blood glucose measurements, appetite, eating habits, weight, and physical activities were compared to those in the past. All participants completed Hospital Anxiety and Depression Scale (HADS) and the Impact of Event Scale, Revised (IES-R) questionnaires which assessed acute stress sypmtoms, anxiety, and depression.

- HADS: This self-report scale is frequently used for anxiety and depression screening in people with physical illness. It was developed by Zigmond and Snaith in 1983 and consists of 14 questions that are specific for depression and anxiety, seperately. Items are scored between 0 and 3. The cut-off score for the subscales is 8 [15]. Turkish validity and reliability were made by Aydemir et al. in 1997 [16].
- IES-R: Developed by Weiss and Marmar in 1997, IES-R is one of the widely used scales used to evaluate the distress and other traumatic symptoms within the past week that are associated with a traumatic life event [17]. Turkish validity and reliability was made by Çorapçıoğlu et al. in 2006 [18]. This self- report scale consists of 22 questions that are scored between 0 to 4 and three subscales i.e. intrusion, avoidance and hyperarousal. In our study, the word "event" in the original scale was changed to "COVID-19 disease" in order to make it easier for the participants to understand.

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows version 23 (IBM Corp. Armonk, NY, USA). Normal distribution was assessed in numerical variables using the Shapiro–Wilk test. Results were displayed in the form of mean ± standard deviation (SD) for normal distributed data or median for skewed distributed data. Categorical variables are summarized as number and percentage. Correlation analysis was performed to evaluate the relationship between

Table 3 Factors related to anxiety, depression, and acute stress during the pandemic period in our study

	Variable	HAD-D	HAD-T	IES,R	Gender	BMI	Weight	Alcohol	MS	FI	L-DA
HAD anxiety	*p*	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.07	0.04	0.01	<0.01
	r	0.60	0.89	0.60	−0.18	0.15	0.14	−0.10	0.11	−0.14	0.44
	Variable	HAD-A	HAD-T	IES,R	BMI	Weight	Alcohol	L-DA			
HAD depression	*p*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01			
	r	0.60	0.89	0.46	0.20	0.18	−0.12	0.33			
	Variable	HAD-A	HAD-D	IES,R	Gender	BMI	Weight	Alcohol	Medeni	FI	L-DA
HAD total	*P*	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.02	0.04	0.01	<0.01
	r	0.89	0.88	0.59	−0.14	0.20	0.17	−0.12	0.11	−0.13	0.43
	Variable	HAD-A	HAD-D	HAD-T	Gender	DM years	L-DA				
IES,R	*p*	<0.01	<0.01	<0.01	0.03	0.04	<0.01				
	r	0.60	0.46	0.59	−0.12	0.11	0.40				

HAD, Hospital Anxiety and Depression Scale; *HAD-A: HAD*, Hospital Anxiety and Depression Scale, anxiety scores; *HAD-D: HAD*, Hospital Anxiety and Depression Scale, depression scores; *HAD-T HAD*, Hospital Anxiety and Depression Scale, total scores; *IES,R*, Impact of Event Scale, Revised; *BMI*, body mass index; *MS*, marital status; *FI*, financial income; *L-DA*, limitation of daily activities; *DM years*, duration of diabetes

anxiety, depression, event impact scores, variables related to post-covid changes, and baseline characteristics of the patients. Variables with significance values below 0.01 in Spearman coefficient were considered to be correlated.

Results

Three hundred and four diabetic patients (169 female and 135 male) were included in the study. Mean age of the patients was 42.1±15.5 years and mean illness duration was 10.3±8.5 years. 30.9% of the participants were obese and the mean BMI was 26.9±5.8 kg/m^2. Smoking and alcohol usage rates were 13.8% and 33.6%, respectively. Almost half of the population had high school or postgraduate education, 63.2% was married, 93.4% was living with their family, and most of them had modarete financial income. Sociodemographic and clinical characteristics of the participants are shown in Table 1.

Patients with T1D (*n*=141) were younger and had lower BMI than patients with T2D (*n*=163). Alcohol usage rate was higher in individuals with T1D and smoking rate was higher in individuals with T2D. Patients with T1D had higher education level, lower marriage rates, and lower financial income. Ninety-seven of the patients with T2D were receiving only oral antidiabetic drugs (OADs) while 56 were receiving OADs and insulin therapy. Ten patients with T2D were using only insulin due to kidney failure.

When the rates of anxiety and depression in patients with T1D and T2D were compared, there was no significant difference in anxiety between the groups ($p = 0.73$), while the frequency of depression was found to be higher in individuals with T2D (p=0.03). Scores of Hospital Anxiety and Depression Scale and Impact of Event Scale, Revised in the patients included to the study, are shown in Table 2. Among the patients included in our study, anxiety and acute stress were more common in female gender. A positive correlation was found between high body mass index (BMI)

Table 4 Post-pandemic changes in eating habits, physical activity, weight, and SMBD

	Type 1 diabetes (*n*=141)	Type 2 diabetes (*n*=163)	Diabetes (*n*=304)
Appetite			
Decreased	27 (19.1%)	16 (9.8%)	43 (14.1%)
Not changed	71 (50.4%)	101 (62.0%)	172 (56.6%)
Increased	43 (30.5%)	46 (28.2%)	89 (29.3%)
Physical activity			
Decreased	94 (66.7%)	96 (58.9%)	190 (62.5%)
Not changed	27 (19.1%)	59 (36.2%)	86 (28.3%)
Increased	20 (14.2%)	8 (4.9%)	28 (9.2%)
Weight			
Decreased	22 (15.6%)	23 (14.1%)	45 (14.8%)
Not changed	58 (41.1%)	85 (52.1%)	143 (47.0%)
Increased	61 (43.3%)	55 (33.7%)	116 (38.2%)
SMBG			
Decreased	22 (15.6%)	12 (7.4%)	34 (11.2%)
Not changed	63 (44.7%)	119 (73%)	182 (59.9%)
Increased	56 (39.7%)	32 (19.6%)	88 (28.9%)
Carbohydrate consumption			
Increased	67 (47.5%)	59 (36.2%)	126 (41.4%)
Not increased	74 (52.5%)	104 (63.8%)	178 (58.6%)
Snacking			
Increased	76 (53.9%)	84 (51.5%)	160 (52.6%)
Not increased	65 (45.1%)	79 (48.5%)	144 (47.4%)

SMBG self-monitoring blood glocose

Table 5 Factors affecting post-pandemic changes in eating habits, physical activity, weight, and SMBD in the study

	Variable	HAD-D	HAD-T	IES,R	DM type	FI	Appetite	PA	CH-C	Snacking			
Change in SMBG	*p*	0.01	0.01	0.01	0.03	0.01	<0.01	<0.01	<0.01	<0.01			
	r	0.14	0.13	0.13	−0.11	−0.14	0.17	−0.17	0.23	0.20			
	Variable	Age	Weight	Appetide	PA	CH-C	Snacking						
Weight	*p*	0.04	0.04	<0.01	0.02	<0.01	<0.01						
	r	−0.11	0.11	0.57	−0.13	0.41	0.42						
	Variable	Gender	Weight	BMI	Weight-C	SMBG-C	CH-C	Snacking					
Appetite	*p*	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01					
	r	0.13	0.23	0.19	0.57	0.17	0.42	0.42					
	Variable	IES,R	BMI	Work-C	SMBG-C	Weight-C	CH-C	Snacking					
Physical activity	*p*	<0.01	0.03	0.03	<0.01	0.02	<0.01	<0.01					
	r	−0.15	−0.12	−0.12	−0.17	−0.13	−0.29	−0.19					
	Variable	HAD-A	HAD-D	HAD-T	IES,R	Age	DM type	Education	Work-C	SMBG-C	Appetite	PA	Snacking
Carbohydrate concumption	*p*	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	r	0.21	0.23	0.24	0.33	−0.22	−0.11	0.16	0.20	0.23	0.42	−0.29	0.57
	Variable	HAD-A	HAD-D	HAD-T	IES,R	Age	Education	Weight-C	Work-C	SMBG-C	Appetite	PA	CH-C
Snacking	*p*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	r	0.17	0.20	0.20	0.22	−0.17	0.19	0.42	0.18	0.20	0.42	−0.19	0.57

HAD, Hospital Anxiety and Depression Scale; *HAD-A: HAD*, Hospital Anxiety and Depression Scale, anxiety scores; *HAD-D: HAD*, Hospital Anxiety and Depression Scale, depression scores; *HAD-T HAD*, Hospital Anxiety and Depression Scale, total scores; *IES,R*, Impact of Event Scale, Revised; *FI*, financial income; *PA*, physical activity; *CH-C*, carbohydrate consumption; *Weight-C*, change of weight; *SMBG-C*, change of self-monitoring blood glocose; *Work-C*, change of work

and anxiety and depression. HAD-Anxiety (HAD-A) and HAD-Total (HAD-T) scores were higher in patients whose financial income decreased during the pandemic. While the duration of illness was positively correlated with acute stress level, it was observed that the duration of illness did not pose a risk for anxiety or depression. A positive correlation was found between limitation of daily activities due to diabetes and anxiety, depression, and acute stress (Table 3)

Carbohydrate consumption (p=0.04) and worsening in glycemic values (p<0.001) were significantly higher in patients with T1D. Weight gain during the pandemic was similar in both groups (p=0.11). Post-pandemic changes in eating habits, physical activity, weight, and self-monitoring blood glucose (SMBD) are shown in Table 4.

In diabetic patients included in our study, decreased financial income, stress, and depression were found to be associated with poor glycemic control during the pandemic (Table 5). Worsening in glycemic values was higher in patients with T1D compared to those with T2D. Increased appetite, increased carbohydrate consumption, more frequent snacking, and decreased physical activity were observed to worsen glycemic control.

A negative correlation was found between age and weight gain in patients included in our study. Weight gain was also lower in individuals with lower body weight before the pandemic. There was no significant correlation between weight gain and anxiety, depression, and acute stress. Increased appetite, increased carbohydrate consumption, more frequent snacking, and decreased physical activity were found to be associated with weight gain.

Discussion

The risk of developing depression is 24% higher in type 2 diabetics than non-diabetics [19]. Depression causes poor glycemic control and increased risk of developing diabetic complications and comorbidities by activating hypothalamic-pituitary-adrenal axis and sympathetic nervous system leading to an increase in peripheral glucocorticoid and catecholamine levels, decreasing insulin sensitivity, increasing inflammation, and platelet aggregation [20]. Stress may have either a direct effect on blood glucose level via disrupting the hypothalamo- pituitary- adrenal axis hormones, or an indirect effect on patients' health behaviors such as worsening their adherence to treatment and diet [21, 22]. Emotional distress has been also found to be related with developing cardiovascular disease [20]. Major depression causes a 2.3-fold increase in mortality in patients with DM, while minor depression causes a 1.7-fold increase [22].

Khuwaja et al. found that the prevalence of anxiety and depression in patients with DM is 58% and 44%, respectively, in Pakistan. The results were about 2 times higher than patients with DM living in developed countries [14]. In a study conducted by Jarso et al., the prevalence of depression was found 37.8% in patients with DM and it was accociated with female gender, type 2 DM, duration of illness >6 years, and high fear of complications [23]. In our sample, anxiety rates were similar between patients with T1D and T2D; however, depression was higher in patients with T2D.

Our results show that duration of illness was correlated with acute stress level but did not show significant relation with anxiety or depression. Duration of illness might be a factor for this finding such as burden of disease for a longer period of time is a risk factor for depression [24]. Acute stress is an earlier psychological problem and could be considered as an adjustment reaction to a compelling situation [25]. Not all the individuals that show acute stress progress to anxiety or depressive disorders [26]. On the other hand, having a chronic disease for a longer period of time may strengthen one's ability to adjust to life changes or other possible medical conditions; thus, one may cope with stress better and not preceed to anxiety or depressive disorders [27].

Among developed and developing countries, presence of depression and anxiety differs due to social factors such as differences in education and income levels. Collins et al. estimated that one-third of individuals with DM have depression and one-fourth of those have anxiety in the UK. The study also showed that poor glycemic control and presence of diabetic complications are independent risk factors for anxiety and depression [28]. As a result of our study, women were found to be more prone to acute stress and anxiety. Anxiety and total HAD scores were higher in individuals whose income decreased due to the pandemic restrictions.

Khuwaja et al. reported BMI as an independent risk factor for depression and fasting blood glucose as an independent risk factor for anxiety and depression in type 2 DM [14]. In a research during COVID-19 pandemic, there was a significant correlation between anxiety levels and the gender, BMI, and HbA1c values [29]. In our sample, blood glucose level before COVID-19 correlated positively with depression and anxiety scores. This result is consistent with previous research. The psychological parameters of our study such as HAD-D and IES were correlated with change in blood glucose level after the outbreak and BMI, but not weight gain

Female gender, younger and/ or older age, physical inactivity, having a comorbid chronic disease such as hypertension or ischemic heart disease, living alone, inadequate social support, and low economic status are frequently reported risk factors for developing depression and anxiety in diabetic patients [30]. In a study conducted by Lin et al. after adjustment for prior complications and demographic and clinical factors, major depression was found to be associated with significantly higher risks of microvascular and macrovascular outcomes [31]. In a population-based matched cohort study, including a total of 38,537 incident

patients with diabetes who had depressive disorders, depression was found to be associated with macrovascular complications and all-cause mortality. But there was no relationship between depression and microvascular diabetic complications. The effect of depression on diabetic complications and mortality was more prominent among young adults than among middle-aged and older adults [32]. In our study, female gender, higher BMI and weight, decrease in financial income after outbreak, presence of diabetic complications and comorbid diseases (ie. retinopathy, neuropathy, diabetic foot, hypertension, dyslipidemia), worsened glycemic levels, increased carbohydrate consumption, and snacking were associated with higher anxiety and depression scores. In our study, high rates of anxiety, depression, and acute stress were found in patients who stated that diabetes disease limits their daily activities.

Increased released of β-endorphine during exercise has a protective effect on psychiatric diseases. In the literature, many studies suggested a relationship between physical inactivity and anxiety depression in different populations, including diabetic patients [14, 33, 34]. In our study, although there was no significant relationship between anxiety/depression and physical activity, a negative correlation was found between stress and physical activity.

The COVID-19 outbreak is thought to have many causes for developing psychiatric problems. Some of these reasons are financial difficulties, physical distance measures, and quarantine. As well as infected people, non-infected people are prone to the development of anxiety and depression [35–37]. In a study of 1210 participants in China, 29% reported moderate to severe anxiety symptoms; and 17% reported moderate to severe depressive symptoms during the outbreak. Female gender and student status were found to be associated with a greater higher levels of stress, anxiety, and depression [38]. Ozdin found that anxiety and depression were higher in women, individuals with chronic disease, and those have previous psychiatric illness history in Turkish society [39]. In another study, Huang et al. reported that people younger than 35 years and those who spent too much time focusing on the outbreak were more likely to suffer from anxiety symptoms [40].

Health authorities around the world reported that people with diabetes are in the high-risk group for COVID infection and if infected, their prognosis is worse compared to the non-diabetics. Given the scarcity of information on the disease, it may be considered that these reports may increase feelings of depression, anxiety, and stress in this spesific group [41]. Similarly, encouraging people not to visit the hospital except in an emergency situation may have made it difficult for diabetics to cope with diabetes [42].

In their research with 202 healthcare professionals over 47 countries, Chudasama et al. investigated the impact of COVID-19 on routine care of chronic diseases. Eighty percent of the healthcare professionals reported that the mental health of their patients worsened during the pandemic and diabetes was the most impacted condition due to the reduction of healthcare resources [43]. The study with 1396 diabetic people showed that patients frequently worried about being overly affected by COVID-10, due to their chronic disease state (56%), being characterized as high-risk group (39%) and not being able to manage their healthy state if infected (28%) [44]. It has been observed that among all diabetic patients, those with type 1 diabetes, those had diabetic complications, and females experience more concerns related to COVID-19 [41]. Similarly, our study showed that female gender and the presence of comorbidity are risk factors for depression.

Our study has some limitations. First, using a web-based survey method due to the self-isolation and quarantine led us to choose sampling of volunteers who would respond to the survey via the web system. Data was collected from two centers, so it can not be generalized to whole population. Second, since there are many factors that affect glycemic control, it is difficult to make a causal conclusion that anxiety and depression are the only factors that worsen glycemic control. Third, absence of a control group without diabetes is also a limitation of the study. In addition, the fact that the patients' HAD and IES scores belonging to the pre-pandemic period were unknown, prevented us from making comments about their mental changes. In our study, a mental evaluation based on objective criteria was not made by a psychiatrist. People may have shown themselves in a better or worse state than actual. This may raise questions on the accuracy or degree of mental symptoms.

As a result of our study, it was concluded that anxiety, depression, and acute stress in diabetic individuals led to carbohydrate consumption and more snacking. During the pandemic, changing in working patterns of the patients and staying much more time at home were also associated with more carbohydrate consumption and snacking. These changes in the patients' eating habits were associated with the worsening of glycemic values.

As a conclusion, we investigated the indirect effects of COVID-19 pandemic on the psycological health such as those mediated through physical distancing measures such as self-isolation or quarantine in diabetic patiens who are not infected. Based on the results, it could be concluded that COVID-19 pandemic has a serious impact on the mental health among patients with DM. Considering that approximately one-third of the depressive diabetic patients received adequate medical treatment and only 6.7% received adequate psychotherapy sessions [45], it is important to evaluate the effects of this pandemic, in patients with DM that are prone to the development of psychological problems. Clinicians must be aware of the possibility of acute stress, depression, and anxiety after pandemic in diabetic patients. Especially

in those whose glycemic control is impaired, psychological problems should not be ignored beyond physical inactivity and worsening eating habits.

Declarations

Ethical approval This study was approved by the Institutional Review Board of the Uludag University of the Turkey. All procedures in this clinical trial were carried out in accordance with the ethical principles and standards in the recently revised Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. The authors have no relevant financial or non-financial interests to disclose.

References

1. Wang C, Pan R, Wan X, et al. A longitudidal study on the mental health of general population during the COVID-19 epidemic in China. Brain Behav Immun 2020;13: doi: https://doi.org/10.1016/j.bbi.2020.04.028.
2. Dubey S, Biswas P, Ghosh R, et al. Physichosocial impact of COVID-19. Diabetes Metab Syndr. 2020;14(5):779–88.
3. International Diabetes Federation. IDF Diabetes, 7 ed., Brussels, Belgium: International Diabetes Federation, 2015. http:/www.diabetesatlas.org.
4. Buin E, Pavin EJ, Silverira MSVM. High anxiety and depressive symptoms in partners of type 1 diabetes persons in a sample of the Brazilian population. Diabetol Metab Syndr. 2020;12:23.
5. Briganti CP, Silva MT, Almeida JV, Bergamaschi CC. Association between diabetes mellitus and depressive symptoms in the Brazilian population. rev Saude Publica 2018;20;53: 5.
6. Barnard KD, Skinner TC, Peveler. The prevalence of co-morbid depression in adults with type 1 diabetes: systematic literature review. Diabet Med 2006;23(4): 445-448.
7. Zanoveli JM, Morais H, Dias ICS, Schreiber AK, Souza CP, Cunha JM. Depression associated with diabetes: from pathophysiology to treatment. Curr Diabetes rev. 2016;12(3):165–78.
8. Ceretta B, Reus GZ, Abelaira HM, et al. Increased prevalence of mood disorders and suicidal ideation in type 2 diabetic patients. Acta diabetol. 2012;49(1):227–34.
9. Grigsby AB, Anderson RJ, Freedland KE, et al. Prevalence of anxiety in adults with diabetes: a systemic review. J Psychosom Res. 2002;53(6):1053–60.
10. Berry E, Lockhart S, Davies M, et al. Diabetes distress: understanding the hidden struggles of living with diabetes and exploring intervention strategies. Postgrad Med. 2015;91(1075):278–83.
11. de Groot M, Anderson R, Freedland KE, et al (2001). Association of depression and diabetes complications: a meta-analysis. Psychosom Med 63(4): 619. (E.J. Ludman et al. / General Hospital Psychiatry 2004;26: 430 – 436.
12. Smith KJ, Beland M, Clyde M, et al. Association of diabetes with anxiety: a systemic review and meta-analysis. J Psychosom Res. 2013;74(2):89–99.
13. Lloyd CE, Dyer PH, Lancashire RJ, et al. Association between stress and glycemic control in adults with type 1 (insulin-dependent) diabetes. DiabeFtes Care. 1999;22:1278–83.
14. Khuwaja AK, Lalani S, Dhanani R, et al. Anxiety and depression among outpatients with type 2 diabetes: a multi-centre study of prevalence and associated factors. Diabetol Metab Synd. 2010;2:72.
15. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand. 1983;67(6):361–70.
16. Aydemir Ö. Hastane Anksiyete ve Depresyon Ölçeği Türkçe formunun geçerlilik ve güvenilirliği. Türk Psikiyatri Dergisi. 1997;8(4):280–7.
17. Weiss DS, Marmar CR. The ımpact of event scale-revised. In: Wilson JP, Keane TM, editors. Assessing psychological trauma and PTSD: a handbook for practitioners. New York, NY: Guilford Press pp; 1997. p. 399–411.
18. Corapcioglu A, Yargic I, Geyran P, Kocabasoglu N. Olayların Etkisi Ölçeği Türkçe versiyonunun geçerlilik ve güvenilirliği. New Symposium Journal. 2006;44(1):14–22.
19. Nouwen A, Winkley K, Twisk J, et al. Type two diabetes mellitus as a risk factor for the onset of depression: a systemic review and meta-analysis. Diabetologia. 2010;53:2480–6.
20. Pah AM, Bucuras P, Buleu F, et al. The importance of DS-14 and HADS questionnaires in quantifying psychological stress in type 2 diabetes mellitus. Medicina. 2019;55:569.
21. Marcovecchio ML, Chiarelli F. The effects of acute and chronic stress on diabetes control. Sci Signal 2012;5(247): pt 10.
22. Gonzalez JS, Safren SA, Cagliero E, et al. Depression, self-care, and medication adherence in type 2 diabetes: relationship across the full range of symptom severity. Diabetes Care. 2007;30:2222–7.
23. Jarso MH, Likasa DD. Prevalence and associated factors of depression among diabetic outpatiens Ethiopia. Prim Care Companion CNS Disord b 2020;22(2): doi: 10. 4088/PCC. 19m02479.
24. Ludman EJ, Katon W, Russo J, et al. Depression and diabetes symptom burden. Gen Hosp Psychiatry. 2004;26(6):430–6.
25. Bryant RA. Acute stress disorder Curr Opin Psychol. 2017;14:127–31.
26. Maercker A, Brewin CR, Bryant RA, et al. Diagnosis and classification of disorders specifically associated with stress: proposal for ICD-11. World Phychiatry. 2013;12(3):198–206.
27. Kim GM, Lim JY, Kim EJ, Prk SM. Healyh Soc Care Community. 2019;27(4):797–807.
28. Collins MM, Corcorant P, Perry IJ. Anxiety and depression symptoms in patients with diabetes. Diabet Med. 2009;26:153–61.
29. Ruiz-Roso MB, Knott-Torcal C, Matilla-Escalante DC, et al. COVID-19 lockdown and changes of the dietary pattern and physical activity habits in a cohort of patients with type 2 diabetes mellitus. Nutrients. 2020;12(8):2327.
30. Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord. 2012;142:S8-21.
31. Lin EH, Rutter CM, Katon W, et al. Depression and advanced complications of diabetes: a prospective cohort study. Diabetes Care. 2010;33:264–9.
32. Wu CS, Hsu LY, Wang SH. Association on depression and diabetes complications and mortality: a population based cohort study. Epidemiol Psychiatr Sci 2020;29: e96.
33. Khuwaja AK, Qureshi R, Azam SI. Prevalence and factors associated with anxiety and depression among family practitioners in Karachi. Pakistan J Pak Med Assoc. 2004;54:45–9.
34. Hong X, Li J, Xu F, et al. Physical activity inversely associated with the presence of depression among urban adolescents in regional China. BMC Public Health. 2009;9:148.
35. Lewnard JA, Lo NC (2020). Scientific and ethical basis for socialdistancing interventions against COVID-19. Lancet Infect Dis published online March 23. https://doi.org/10.1016/S1473- 3099(20)30190-0.
36. Brooks SK, Webster RK, Smith LE, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet. 2020;395:912–20.

37. Rogers JP, Chesney E, Oliver D, et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. Lancet Psychiatry Published Online May 18, 2020. https://doi.org/10.1016/S2215-0366(20)30203-0.
38. Wang C, Pan R, Wan X, et al. Immediate psychological responses and associated factors during the initial stage of the 2019 Coronavirus Disease (COVID-19) epidemic among the general population in China. Int J Environ Public Health. 2020;17(5):1729. https://doi.org/10.3390/ijerph17051729.
39. Ozdin S, Ozdin SB. Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: the importance of gender. Int J Soc Psychiatry 2020.20764020927051. https://doi.org/10.1177/0020764020927051.
40. Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 outbreak in China: a web-based cross-sectional survey. Psychiatry Res. 2020. https://doi.org/10.1016/j.psychres.2020.112954.
41. Joensen LE, Madsen KP, Holm L, et al. Research: educational and psychological aspects diabetes and COVID-19 pandemic in people with diabetes in Denmark-what characterizes people with high levels of COVID-19-releated worries? Diabet Med. 2020;00:1–9.
42. Nachimuthu S, Vijayalakshmi R, Sudha M, Viswanathan V. Coping with diabetes during the COVID-19 lockdown in India: results of an online pilot survey. Diabetes Metab Syndr. 2020;14(4):579–82.
43. Chudasama YV, Gillies CL, Zaccardi F, et al. Impact of COVID-19 on routine care for chronic disease: a global survey of views from healthcare professionals. Diabetes Metab Syndr. 2020;14(5):965–7.
44. Joensen LE, Madsen KP, Holm L, et al. Diabetes and COVID-19: psychosocial consequences of the COVI(D-19 pandemic in people with diabetes in Denmark-what cfharacterizes people with high levels of COVID-19-releated worries? Diabet Med. 2020;37(7):1146–54.
45. Katon WJ, Simon G, Russo J, et al. Quality of depression care in a population-based sample of patients with diabetes and major depression. Med Care. 2004;42:1222–9.

International Journal of Diabetes in Developing Countries (January–March) 42(1):62–69
https://doi.org/10.1007/s13410-021-00966-0

ORIGINAL ARTICLE

Resistin, TNF-α, and microRNA 124-3p expressions in peripheral blood mononuclear cells are associated with diabetic nephropathy

Amin Monjezi[1] · Azam Khedri[2] · Mehrnoosh Zakerkish[3] · Ghorban Mohammadzadeh[4]

Received: 15 June 2020 / Accepted: 9 June 2021/Published online: 21 July 2021

Abstract

Introduction Diabetic nephropathy causes chronic kidney disease and renal failure globally. We investigated the mRNA expression of resistin, TNF-α, and microRNA 124-3p (miR-124-3p) in peripheral blood mononuclear cells (PBMCs) of diabetic patients with and without nephropathy.

Methods The mRNA expression of target genes in isolated PBMCs from whole blood was determined using real-time quantitative reverse transcription (RT) polymerase chain reaction (qRT-PCR). Demographic and clinical characteristics of study patients were also measured by standard methods.

Results Relative expression of resistin and TNF-α was significantly increased (approximately 10-fold, and 1.5-fold respectively) in patients with nephropathy compared to that in patients without nephropathy ($p < 0.001$). However, the miR-124-3p expression was significantly decreased (approximately 10-fold) in patients with nephropathy in comparison to that in patients without nephropathy ($p < 0.05$). The expression of resistin was significantly associated with inflammatory markers such as creatinine, BUN, TNF-α, and miR-124-3p. The high expression of resistin was significantly associated with diabetic nephropathy (OR = 1.145; 95% C = 1.1–18.289, $p = 0.024$). The diagnostic performance of resistin, TNF-α, and miR-124-3p was significant based on ROC curve analysis (0.803, 95% CI: 0.723–0.926; 0.705, %95 CI: 0.559–0.851; and 0.707, 95% CI: 0.559–0.855, respectively). Thus, they can represent a diabetic biomarker for reflecting an advanced stage of the disease and inflammatory condition.

Conclusions Our findings indicated that high expression of resistin in PBMCs is associated with diabetic nephropathy. Additionally, we suggested that the expression of resistin, TNF-α, and miR-124-3p in PBMCs of diabetic patients may be represented as predictive biomarkers for the diagnosis of nephropathy.

Keywords Resistin · TNF-α · MicroRNA 124-3p · Diabetic nephropathy

Introduction

Diabetic nephropathy, the main unfavorable micro-vascular complication of diabetes, is characterized by pathological urine albumin excretion, glomerular lesions, and reduction of the glomerular filtration rate [1]. Resistin, an adipose-derived pro-inflammatory cytokine, was first detected in mouse adipocytes and has been associated with insulin resistance and the development of type 2 diabetes mellitus [2]. Unlike in rodents, where adipocytes are the major source of blood resistin [2], in humans, the expression and secretion of resistin are mainly reported in peripheral blood mononuclear cells (PBMCs), macrophages, and skeletal muscle cells [3, 4]. Although the inflammatory role of resistin in humans has been well-demonstrated [5], its role in obesity and insulin resistance is controversial.

✉ Ghorban Mohammadzadeh
mohammadzadeh@ajums.ac.ir

[1] Department of Clinical Biochemistry, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Department of Clinical Biochemistry, Faculty of Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran

[3] Health Research Institute, Diabetes Research Center, Department of Internal Medicine, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[4] Hyperlipidemia Research Center, Department of Clinical Biochemistry, Faculty of Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran

Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory adipocytokine, was originally identified in macrophages that infiltrated in adipose tissue, and its serum level was positively associated with BMI [6]. TNF-α not only participates in systemic inflammation but also stimulates the acute phase reaction [7]. In humans, TNF-α is predominantly produced not only by macrophages but also by a wide variety of other cells such as adipocytes [8].

microRNAs (miRNAs), endogenous noncoding small RNAs, have been reported to have a key role in several cellular processes by regulating gene expression [9, 10]. It has been evidenced that miRNAs can modulate the expression of nearly 60% of protein-coding genes, and changes in miRNA expression profiles have been detected in numerous pathological processes [11, 12]. The role of some miRNA overexpression in the pathogenesis of diabetic nephropathy and some of the other miRNA downregulation for renal protection has been reviewed recently [13, 14]. In comparison to the other traditional biomarkers in human blood, miRNAs are very stable and usually considered to be major regulators of two key factors in the pathogenesis of type 2 diabetes including pancreatic β-cell-active mass and function [15, 16]. Therefore, miRNAs are recently being considered favorable biomarkers for both diagnoses of type 2 diabetes and investigating its response to treatment [17, 18]. Moreover, miRNAs may be considered renal biomarkers and presented a good perspective for the clinical monitoring and management of diabetic kidney disease in addition to the determination of glomerular filtration rate (GFR) and albuminuria [19]. It has been demonstrated that the miR-124 may be implicated in the pathogenesis of diabetes and diabetic nephropathy in animal models and in vitro conditions [16, 20, 21]. However, there is no report regarding the association between miR-124-3p expression in human PBMCs and diabetic nephropathy; thus, in the current study, we aimed to investigate the level of resistin, TNF-α, and miR-124-3p expression in PBMCs of diabetic patients with and without nephropathy.

Materials and methods

Study population

In the current case–control study, 30 diabetic patients with nephropathy and 31 diabetic patients without nephropathy were assessed. The patients were enrolled at Ahvaz Golestan Hospital, Ahvaz, Iran. Diabetes was diagnosed according to the American Diabetes Association (ADA) criteria by an expert endocrinologist. Subjects who had fasting blood sugar (FBS) > 126 mg/dL or use of hypoglycemic drugs were considered with diabetes. To diagnose diabetic nephropathy, blood urea nitrogen (BUN), serum creatinine (Cr), and albuminuria were measured. The study protocol was approved by the local ethics committee at the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.MEDICINE.REC.1398.047). Ten milliliters of venous blood samples was collected after obtaining informed consent from all study subjects and divided into two tubes. Three milliliters of the sample without anticoagulant was used for the separation of serum and biochemical measurements. The 7 mL remaining was used for PBMC isolation. All samples were stored at − 80 °C until the final analysis. PBMC isolation was performed immediately after sample collection. Moreover, 24-h urine samples were collected from all individuals to measure albuminuria. The control group consisted of type 2 diabetic patients aged over 35 years, who had a negative history of nephropathy. Smokers, alcoholism, pregnant individuals, subjects under insulin therapy, and those with chronic liver and renal disease as well as acute infectious diseases, including active diabetic foot, were not included. Demographic indices of the subjects, including age, gender, height, weight, BMI, and systolic and diastolic pressure, were measured by standard methods. Full-fasted lipid profile contains total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C), and, also, BUN, creatinine, plasma glucose concentration, and urine albumin were measured with a biochemistry analyzer (BT3000, Italy) using Pars Azmun kits based on manufacturer instruction (Tehran, Iran). Body mass index was calculated as body weight (in kg) divided by square height (m^2). The body weight and height of the entire participants were measured by standard methods while they were wearing light clothing and not wearing shoes.

PBMC isolation

Venous blood was drawn from subjects who had at least 10 to 12 h of fasting. The PBMCs were isolated from 7 mL of peripheral blood by Ficoll-Paque (Pharmacia, Freiburg, Germany) density gradient centrifugation. In brief, 3.5 mL of Ficoll was pipetted into 15-mL centrifuge tubes. Heparinized blood samples were diluted with a 1:1 ratio in phosphate-buffered saline (PBS, pH = 7.4), and carefully suspended over a Ficoll-Paque gradient (7 mL/tube). Samples were centrifuged at 600 × *g* for 20 min at room temperature. The PBMC-containing layer was aspirated and the cells were washed twice with PBS, pH = 7.4 (10 min, 270 × *g*, 4 °C, and 10 min, 220 × *g*, 4 °C), followed by a final wash step in PBS. Finally, the extracted PBMCs were stored at − 80 °C until use for RNA isolation.

eGFR calculation

Based on the recommendation of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), we used the creatinine-based CKD-EPI equation ($CKD\text{-}EPI_{creat}$) for estimation of the GFR as follows:

$$\begin{aligned} GFR = & 141 \times \min(Scr/\kappa, 1)\,\alpha \\ & \times \max(Scr/\kappa, 1) \\ & - 1.209 \times 0.993 Age \\ & \times 1.018\ [\text{if female}] \\ & \times 1.159\ [\text{if black}] \end{aligned}$$

Abbreviations/units are as follows:

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2
S_{Cr} (serum creatinine) = mg/dL
$\kappa = 0.7$ (females) or 0.9 (males)
$\alpha = -0.329$ (females) or -0.411 (males)
min = indicates the minimum of S_{Cr}/κ or 1
max = indicates the maximum of S_{Cr}/κ or 1
age = years

RNA isolation, c.DNA synthesis, and real-time PCR amplification

Total RNA was extracted from PBMC using a Favor Prep™ Tissue Total RNA Mini Kit (Favorgen Biotech Corp., Wembley, Australia), according to the manufacturer's instructions. The quantity of RNA was determined by measuring absorbance at 260 nm, using the NanoDrop ND-2000 spectrophotometer (Thermo Scientific, USA). The integrity of RNA was assessed on 1% agarose gel electrophoresis with visualization of 18S and 28S rRNA bands by the Gel Documentation System (Bio-Rad Hercules, CA, USA). The complementary DNA (cDNA) was synthesized using the Yakta Tajhiz Azma (YTA) cDNA synthesis kit (Cat No. YT 4500, Tehran, Iran) based on manufacturer's instructions. The mRNA expression was determined by ABI Step One Plus Real-time PCR (Applied Biosystems, USA), using RealQ Plus 2 × Master Mix Green High ROX™ (Amplicon, Odense, Denmark) with specific primers for target genes (resistin, TNF-α, and hsa-miR-124), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and small nuclear RNA U6 as an internal control gene. The miR-124-3P cDNA was synthesized using the microRNA kit Human-mouse-rate100 reactions kit (Zist Poyesh, Tehran, Iran), based on the manufacturer's protocol. The synthesized cDNA was used immediately or stored at − 70 °C for later use. Real-time PCR of the miR-124-3p was performed using SYBR green fluorescence quantitative PCR reagent on a Step One Plus Real-time Quantitative PCR instrument (ABI, USA), and each sample was analyzed in duplicate. Twenty microliters of PCR mixture reaction was prepared as follows: 1 µL c.DNA, 10 µL of qPCR Master Mix SYBR Green, and 0.8 µL of primer (forward and reverse, 10 µM each), and distilled water up to 20 µL. The thermal cycling condition was included one step at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, and 62 °C for 30 s, and one step at (55–95 °C) for 30 min to assess the melting curve. The expression of target genes was quantified according to the ΔΔCt method and normalized to housekeeping GAPDH and small nuclear RNA U6 expression. The efficiency of primer and PCR products was evaluated using the LinRegPCR software. The Relative Expression Software Tool (REST, 2009 v2.0.13) was used for the analysis of fold expression based on the $2^{-\Delta\Delta Ct}$ method. The specific primers for human resistin, TNF-α, miR-124-3p, and GAPDH (NM_001193374, NM_000594, MIMAT0000422, and NM_001289745.3, respectively), were designed using Primer-Blast (NCBI) as shown in Table 1. The PCR product size and their specificity were determined on agarose

Table 1 The primers sequence of target genes for real-time qRT-PCR amplification

Primers	Primer length	Sequence (5′→3′)	PCR product size(bp)
GAPDH forward	21	GGTCGGAGTCAACGGATTTGG	194
GAPDH reverse	21	TGATGACAAGCTTCCCGTTCT	
Resistin forward	18	TACTTGCCCCCGAGGCTT	119
Resistin reverse	18	CTCCGGTCCAGTCCATGC	
TNF-α forward	21	CCCATGTTGTAGCAAACCCTC	102
TNF-α reverse	22	GCTGGTTATCTCTCAGCTCCAC	
U6 forward	25	GCTTCGGCAGCACATATACTAAAAT	100
U6 reverse	23	CGCTTCACGAATTTGCGTGTCAT	

qRT-PCR quantitative reverse transcription polymerase chain reaction, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *TNF-α* tumor necrosis factor-alpha, *U6* U6 small nuclear RNA, *bp* base pair

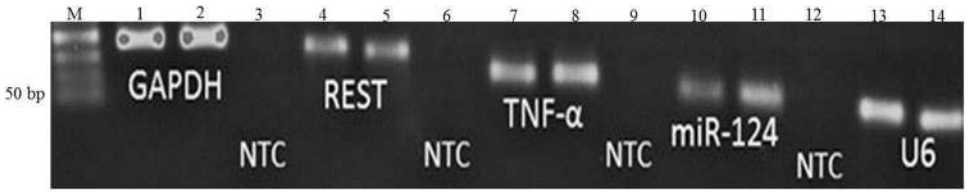


Fig. 1 The amplified cDNA pattern of target genes. Lane M shows 50-bp ladder (Fermentas, Germany); lanes 1 and 2 show PCR products of GAPDH (194 bp); lane 3 shows the negative control; lanes 4 and 5 show PCR products of resistin (119 bp); lane 6 shows negative control; lanes 7 and 8 show PCR products of TNF-α (102 bp); lane 9 shows negative control; lanes 10 and 11 show PCR products of miRNA-124-3p; lane 12 shows negative control; and lanes 13 and 14 show PCR products of U6 (100 bp)

gel electrophoresis and melting curve analysis, respectively, as shown in Fig. 1.

Statistical analysis

Statistical analysis of the data was performed using the commercially available IBM Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software. Quantitative data of mRNA expression were presented as mean ± standard error of the mean (SEM). Data were analyzed by using the chi-square test for categorical variables, t-test for quantitative normally distributed variables, and Mann–Whitney U test for not normally distributed variables. The normal distribution of data was assessed by the Kolmogorov–Smirnov test. Association between the gene expression and diabetic nephropathy was determined by a logistic regression analysis and results presented as the odds ratio (OR) (95% confidence intervals (CIs)). The relative expression of genes was determined using the $2^{-\Delta\Delta Ct}$ method and was normalized to the level of reference gene. Receiver operator characteristic (ROC) curve analysis and calculation of the area under the curve (AUC) were carried out to assess the power of resistin, TNF-α, and miR-124-3p mRNA expression in PBMCs to properly discriminate diabetic patients with nephropathy from those without nephropathy. A two-sided p value of less than 0.05 was considered significant for all tests performed.

Results

Demographic indices and clinical characteristics of the study patients

Enrolled diabetic patients with nephropathy had a mean age of 55.27 years, and 46.7% were male, whereas diabetic patients without nephropathy had a mean age of 50.23 years, and 54.8% were male, respectively. Details of the patient's demographic and clinical characteristics are presented in

Table 2 Demographic and clinical characteristics of the study patients

Variables	Patients without nephropathy	Patients with nephropathy	p-value
Sex (males/females) (%)	17/14	14/16	0.351
Age (year)	50.23 ± 9.68	55.27 ± 7.59	0.028
SBP (mmHg)	131.47 ± 22.67	161.26 ± 24.01	< 0.001
DBP (mmHg)	78.43 ± 17.01	95.63 ± 13.30	< 0.001
BMI (kg/m^2)	27.5 ± 3.47	26.60 ± 2.75	0.309
Total cholesterol (mg/dL)	188.92 ± 62.95	183.49 ± 52.03	0.525
Triglyceride (mg/dL)	209.17 ± 114.46	164.4 ± 76.39	0.173
HDL-C (mg/dL)	39.72 ± 12.15	39.46 ± 8.08	0.924
LDL-C (mg/dL)	126.08 ± 37.7	130.1 ± 44.90	0.655
FBS (mg/dL)	185.40 ± 68.40	193.2 ± 72.4	0.722
BUN (mg/dL)	15.03 ± 6.66	51.1 ± 18.74	< 0.001
Cr (mg/dL)	0.9 ± 022	4.83 ± 2.12	< 0.001
BUN/Cr	16.4 ± 3.96	11.90 ± 5.35	0.219
Albuminuria (mg/24 h)	10.83 ± 3.60	452.82 ± 169	< 0.001
eGFR (mL/min/1.73 m^2)	89.72 ± 18.18	14.26 ± 8.49	< 0.001

Data are mean ± SD. *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *FBS* fasting blood sugar, *BUN* blood urea nitrogen, *Cr* creatinine, *eGFR* estimated glomerular filtration rate

Table 2. Patients with or without nephropathy were not significantly different in terms of gender distribution, BMI, total cholesterol, triglyceride, HDL, and FBS. However, there was a significant difference between the two groups in terms of age, systolic, and diastolic blood pressure, BUN, creatinine, and albuminuria. Patients with nephropathy had significantly higher BUN, creatinine, and albuminuria, and lower HDL-cholesterol level and estimated glomerular filtration rate (eGFR) compared to patients without nephropathy.

Table 3 The association between resistin, TNF-α, and miRNA 124-3p expression and diabetic nephropathy

	Without DN/ with DN	OR (95% CI)	p value
Resistin	30/31	1.145 (1.018–1.289)	0.024
TNF-α	30/31	1.012 (0.989–1.035)	0.323
miRNA 124-3p	30/31	0.563 (0.248–1.287)	0.170

DN diabetic nephropathy, *OR* odds ratio, *CI* confidence interval

mRNA expression of resistin, TNF-α, and miR-124-3p in PBMCs

The levels of mRNA expression were studied in freshly ready RNA from PBMCs. Results from analysis of resistin, TNF-α, and miR-124-3p mRNA expression are presented as the fold change of each target gene in patients with nephropathy compared to those in patients without nephropathy. The levels of resistin and TNF-α expression were higher by 10.0-fold and 1.5-fold ($p < 0.05$) in PBMCs of diabetic patients with nephropathy compared with diabetic patients without nephropathy (Fig. 2). The level of miR-124-3p expression was significantly decreased by 10.0-fold ($p < 0.05$), in PBMCs of diabetic patients with nephropathy compared with that of diabetic patients without nephropathy (Fig. 2). Also, logistic regression analysis indicated that high expression of resistin was associated with diabetic nephropathy (OR = 1.145; 95% C = 1.1–18.289, p = 0.024) (Table 3).

Diagnostic value of resistin, TNF-α, and miR-124-3p expression in PBMCs for diabetic nephropathy

The ROC curves were analyzed to assess the diagnostic value of resistin, TNF-α, and miR-124-3p expression in PBMCs for diabetic nephropathy (Fig. 3). The diagnostic performance of resistin, TNF-α, and miR-124-3p in PBMCs to differentiate diabetic patients with nephropathy from diabetic patients without nephropathy is summarized in Table 4. The increased expression of resistin and TNF-α, and decreased expression of miR-124-3p in PBMCs can differentiate diabetic patients with nephropathy from diabetic patients without nephropathy with area under curve (AUC) = [0.803, 95% CI: 0.726–0.926, 0.705,

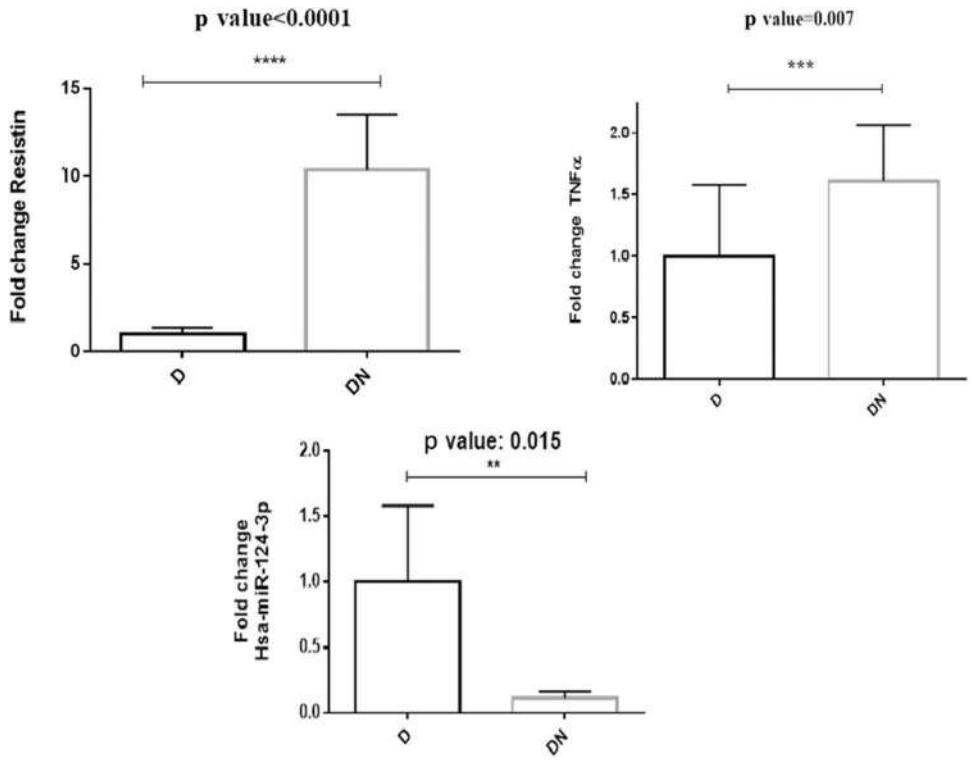


Fig. 2 The relative mRNA expression of resistin, TNF-α, and miRNA124-3p in PBMCs of study patients. The difference in target gene expression between the two groups is reported as fold change. Error bars represent the standard error of the mean. D diabetic patients without nephropathy; *DN* diabetic patients with nephropathy

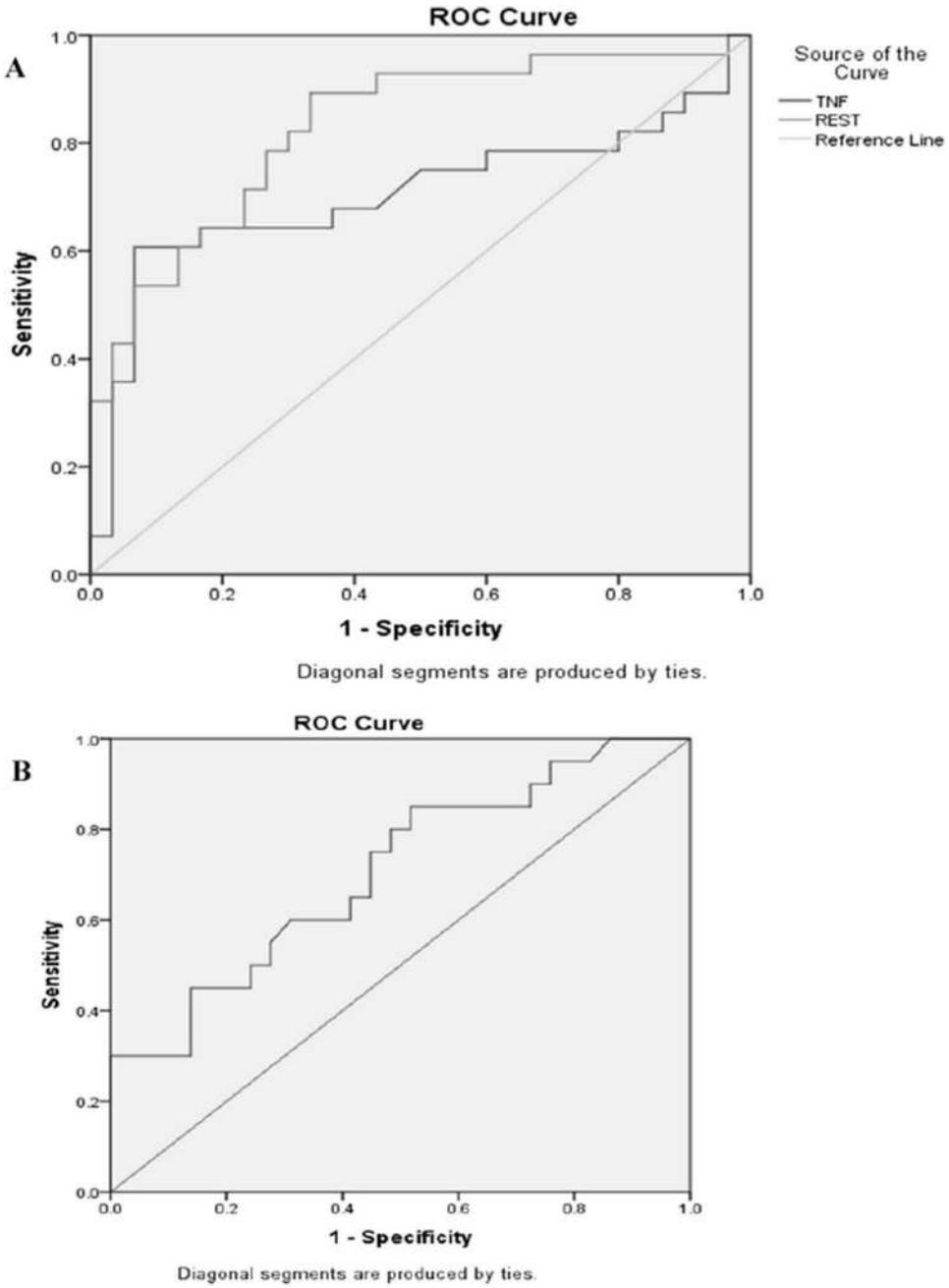


Fig. 3 The ROC curve was analyzed to assess the diagnostic performance of resistin, TNF-α (**A**), and miRNA 124-3p (**B**) in PBMCs of diabetic patients. The yellow and green lines represent the ROC curves for distinguishing diabetic patients with nephropathy from those without nephropathy. TNF TNF-α, REST resistin, ROC receiver operator characteristic

%95 CI: 0.559–0.851, and 0.707, 95% CI: 0.559–0.850, respectively], (Fig. 3 A and B). Therefore, the expression of resistin, TNF-α, and miR-124-3p may represent a diabetic biomarker reflecting the advanced stage of the disease and inflammatory state.

Table 4 Diagnostic value of PBMCs biomarkers for diabetic nephropathy

Variables	AUC	95% CI	p-value
Resistin	0.830	0.723–0.936	< 0.001
TNF-α	0.705	0.559–0.851	0.007
miRNA 124-3p	0.707	0.559–0.855	0.015

PBMCs peripheral blood mononuclear cells, *AUC* area under the curve, *CI* confidence interval

Discussion

Clinically, micro-albuminuria measurement is the base of current methods for diagnosis and monitoring diabetic nephropathy. However, this method is not sensitive as other processes including inflammation and tissue damage also cause micro-albuminuria. Moreover,

micro-albuminuria is one of the many characteristics of glomerular damages. The gold standard method for diagnosis of diabetic nephropathy is the biopsy of renal tissue, which is a very expensive procedure and may have the risk of biopsy bleeding side effects. Therefore, recently, the use of a sensitive and reliable biomarker for diagnosis of diabetic nephropathy is an interesting subject among researchers. Additionally, PBMCs are recently being considered a valuable source of biomarkers including interleukins, adipokines, and miRNAs in clinical and experimental studies because they are readily available and can easily be obtained from patients.

We have found that the mRNA expression of resistin in PBMCs of type 2 diabetic patients with nephropathy was significantly increased in comparison to that of patients without nephropathy accompanied by a significant increase in mRNA expression of TNF-α. Resistin, an adipose-derived adipocytokine, which has an important role in insulin resistance and glucose homeostasis, is mostly expressed in white adipose tissue in rodents [2]. But, in humans, resistin is rarely expressed in the adipose tissue [4, 5, 16], whereas it is abundantly expressed in human peripheral blood mononuclear cells [4, 22, 23], as well as in endothelial [22] and muscular cells [4, 22]. Therefore, it is suggested that PBMCs can be the main source of blood resistin in humans. Unlike in rodents where resistin primarily implicates in glucose homeostasis and insulin resistance, in humans, resistin seems to have a strong pro-inflammatory characteristic. In humans, several inflammatory factors could induce hyper-resistinemia [5, 24], whereas the expression of the pro-inflammatory adipocytokines including TNF-α and IL-6 in both human and murine macrophages can stimulate by human resistin through activation of NF-κB-dependent pathways [5]. Results obtained from patients with severe inflammatory and patients with myocardial infarction, where serum resistin levels significantly increased, could further verify the pro-inflammatory potent of human resistin [25].

TNF-a, a strong pro-inflammatory adipocytokine, is mainly expressed by infiltrated macrophages and lymphocytes into adipose tissue as well as adipocytes. In humans, the serum levels of TNF-a were significantly and positively associated with the BMI and the degree of hyperinsulinemia [6]. TNF-a can downregulate the expression of glucose transporter 4 in adipocyte and myocyte [26]. In a study conducted by Kayser et al., TNF-α increased the expression of resistin mRNA in the human PBMCs, thus suggesting that the increase of serum resistin level might be another mechanism that causes TNF-α-induced insulin sensitivity impairments [27], whereas Shojima et al. [28] found that TNF-α inhibits the expression of resistin. These inconsistent findings might be due to the use of the different methods, although Shojima et al. assessed the effects of TNF-α on the expression of resistin in murine 3T3-L1 adipocytes, whereas Kayser et al. used human PBMCs.

Diabetic nephropathy, a major chronic and multifactorial complication of diabetes can cause chronic kidney disease and renal failure globally. However, the pathogenesis of this disease has been yet completely determined. Recently, research on the miRNAs has become an attractive topic, since they have a vital role in the regulation of several coding mRNA expressions that may involve in the pathogenesis of several diseases. A large number of miRNAs were reported to participate in the pathogenesis of diabetic nephropathy, whereas others had renal protective activity [11, 12]. miRNAs belong to small noncoding RNAs that have a key role in the regulation of numerous genes in animals and plants. The miRNAs can regulate the target mRNA expression at the post-transcriptional level in a sequence-specific manner either by blocking their translation or by promoting their cleavage and degradation. Numerous miRNAs have recently been involved in the regulation of both glucose metabolism and insulin signal transduction [9–16]. Additionally, in the current study, for the first time, we found that the expression of miR-124-3p to be downregulated in PBMCs of diabetic patients with nephropathy in comparison to that of patients without nephropathy. miR-124-3p, as a tumor suppressor, has been established to be downregulated in several cancers and its role in the regulation of cell proliferation and apoptosis by targeting the specific mRNA of several genes has been reported [29]. However, the role of this miRNA in glucose-induced nephropathy has not been completely understood. Li et al., in a study conducted on the male Wistar rats, found that miR-124 participated in the kidney damage induced by a high capacity of podocyte adhesion and may participate in the pathogenesis of diabetic nephropathy [20]. Regmi et al. in a nested case-controlled study found that serum levels of miR-99b and miR-122 were significantly increased, and mir-20a and miR-486 decreased in the diabetic kidney disease compared to those in healthy controls [30]. Sebastiani et al., in a study, found that miR-124a is overexpressed in isolated islets from the human pancreas and could negatively regulate insulin secretion [17]. Finally, our findings showed that the low level of miR-124-3p expression in PBMCs of diabetic patients was significantly associated with the high level of resistin expression ($r=0.307$, $p=0.034$). It is hypothesized that the downregulation of miR-124-3p expression in PBMCs of diabetic patients may cause upregulation of resistin expression. However, further interventional studies are required to verify this recommended hypothesis.

Conclusion

Our results indicated that diabetic nephropathy is associated with increased mRNA expression of resistin and TNF-α and decreased mRNA expression of miR-124-3p in the PBMCs.

Although, the mRNA expression of resistin, TNF-a, and miR-124-3p in PBMCs maybe use for distinguishing patients with nephropathy from patients without nephropathy. However, further prospective and longitudinal studies are needed to evaluate whether resistin, TNF-α, and miR-124-3p in PBMCs can be used as a predictive biomarker for monitoring and management of diabetic nephropathy.

Funding This paper is issued from the thesis of Amin Monjezi and financial support was provided by Hyperlipidemia Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Grant No. HLRC-9808).

Declarations

Participants The current research involves human participants.

Informed consent Informed consent was obtained from all study subjects before sampling.

Conflict of interest The authors declare no competing interests.

References

1. Gheith O, Farouk N, Nampoory N, Halim MA, al-Otaibi T. Diabetic kidney disease: worldwide difference of prevalence and risk factors. J Nephropharmacol. 2016;5(1):49–56.
2. Steppan CM, Bailey ST, Bhat S, Elizabeth J, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature. 2001;409(6818):307–12.
3. Patel L, Buckels AC, IKinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun. 2003;300:472–6.
4. Dietze D, Koenen M, Rohrig K, Horikoshi H, Hauner H, Eckel J. Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes. Diabetes. 2002;51:2369–76.
5. Park HK, Ahima RS. Resistin in rodents and humans. Diabetes Metab J. 2013;37(6):404–14.
6. Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, Gedrich K, Pollmächer T. TNF-alpha, soluble TNF receptor and interleukin-6 plasma levels in the general population. Eur Cytokine Netw. 2006;17:196–201.
7. Moller DE. Potential role of TNF alpha in the pathogenesis of insulin resistance and type 2 diabetes. Ternds Endocrinol Metab. 2000;11:212–7.
8. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. 2013;2013:139239.
9. Bhatt K, Mi QS, Dong Z. MicroRNAs in kidneys: biogenesis, regulation, and pathophysiological roles. Am J Physiol Renal Physiol. 2011;300:nF602-F610.
10. Fernandez-Valverde SL, Taft RJ, Mattick JS. MicroRNAs in β-cell biology, insulin resistance, diabetes and its complications. Diabetes. 2011;60:1825–31.
11. Shantikumar S, Caporali A, Emanueli C. Role of microRNAs in diabetes and its cardiovascular complications. Cardiovasc Res. 2012;93(4):583–93.
12. Kato M, Park JT, Natarajan R. MicroRNAs and the glomerulus. Exp Cell Res. 2012;318:993–1000.
13. Kato M, Natarajan R. MicroRNAs in diabetic nephropathy: functions, biomarkers, and therapeutic targets. Ann N Y Acad Sci. 2015;1353:72–88.
14. Bhatt K, Kato M, Natarajan R. Minireview: emerging roles of microRNAs in the pathophysiology of renal diseases. Am J Physiol Renal Physiol. 2016;310:F109–18.
15. Guay C, Regazzi R. New emerging tasks for microRNAs in the control of β-cell activities. Biochimica et Biophysica Acta. 2016;1861:2121–9.
16. Sebastiani G, Po A, Miele E, et al. MicroRNA-124a is hyper expressed in type 2 diabetic human pancreatic islets and negatively regulates insulin secretion. Acta Diabetologica. 2015;52:523–30.
17. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol. 2013;9:513–21.
18. Yang Z, Chen H, Si H, et al. Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes. Acta Diabetologica. 2014;51:823–31.
19. Nassirpour R, Raj D, Townsend R, Argyropoulos C. MicroRNA biomarkers in clinical renal disease: from diabetic nephropathy, renal transplantation and beyond. Food Chem Toxicol. 2016;98:73–88.
20. Li D, Lub Z, Jiaa J, Zhenga Z, Lina S. MiR-124 is related to podocytic adhesive capacity damage in STZ-induced uninephrectomized diabetic rats. Kidney Blood Press Res. 2013;37:422–31.
21. Li D, Lu Z, Jia J, Zheng Z, Lin S. Changes in microRNAs associated with podocytic adhesion damage under mechanical stress. J Renin Angiotensin Aldosterone Syst. 2013;14(2):97–102.
22. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes. 2001;50:2199–202.
23. McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker J, Barnett AH, Kumar S. Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab. 2002;87:2407.
24. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA. An inflammatory cascade leading to hyperresistinemia in humans. PLoS Med. 2004;1:161–8.
25. Anderson PD, Mehta NN, Wolfe ML, et al. Innate immunity modulates adipokines in humans. J Clin Endocrinol Metab. 2007;92:2272–9.
26. Peraldi P, Hotamisligil GS, Buurman WA, White MF. Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem. 1996;271:13018–22.
27. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun. 2003;309(2):286–90.
28. Shojima N, Sakoda H, Ogihara T, Fujishiro M, Katagiri H, Anai M, Onishi Y, Ono H, Inukai K, Abe M, Fukushima Y, Kikuchi M, Oka Y, Asano T. Humoral regulation of resistin expression in 3T3-L1 and mouse adipose cells. Diabetes. 2002;51:1737–44.
29. Xinqi Jia Xu, Wang XG, Ji J, Lou Ge, Zhao J, et al. MicroRNA-124: an emerging therapeutic target in cancer. Cancer Med. 2019;8(12):5638–50.
30. Regmi A, Liu G, Zhong X, Hu S, Ma R, Gou L, et al. Evaluation of serum microRNAs in patients with diabetic kidney disease: a nested case-controlled study and bioinformatics analysis. Med Sci Monit. 2019;25:1699–708.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):70–76
https://doi.org/10.1007/s13410-021-00945-5

ORIGINAL ARTICLE

High expression of lncRNA MALAT1 is associated with decreased insulin secretion under hyperglycemic stress in patients with type 2 diabetes mellitus

Da-wei Yang[1,2] · Lianji Zhou[2] · Qing Huang[2] · Linan Ou[2] · Biao-liang Wu[1,2]

Received: 28 October 2020 / Accepted: 24 March 2021 / Published online: 21 April 2021

Abstract

Objective This study aimed to explore the expression of lncRNA MALAT1 in patients with T2DM and its clinical significance. A total of 25 normal controls and 69 patients with T2DM were selected.

Methods Real-time polymerase chain reaction was used to determine the expression level of lncRNA MALAT1 in blood leukocytes of the two groups.

Results The expression level of lncRNA MALAT1 in patients with T2DM was significantly higher than that in the control group ($p < 0.001$). The binary regression analysis revealed that lncRNA MALAT1 ($p < 0.001$, OR = 11.667) and superoxide dismutase (SOD) ($p = 0.018$, OR = 0.958) were the risk factors for the onset of T2DM. Spearman correlation analysis showed that the expression level of lncRNA MALAT1 correlated positively with cortisol (8 AM), hemoglobin, and blood glucose levels (60, 120, and 180 min) and negatively with SOD and insulin levels after 60 min in the oral glucose tolerance test. The receiver operating characteristic (ROC) results demonstrated that the area under the curve of ROC was 0.804, sensitivity was 78.3%, and specificity was 84% ($p < 0.001$).

Conclusion LncRNA MALAT1 was highly expressed in patients with T2DM, and high expression of lncRNA MALAT1 is associated with decreased insulin secretion under hyperglycemic stress.

Keywords Diabetes · LncRNA MALAT1 · Clinical research · Disease association studies · Gene expression

Introduction

Type 2 diabetes mellitus (T2DM) is the most common type of diabetes. The related chronic complications, such as diabetic nephropathy and diabetic foot, pose a huge threat to people's health and also exert a great economic burden on society [1]. However, its pathogenesis is still inconclusive [2–4]. LncRNA has gradually attracted the attention of researchers with the development of human genomics in recent years. It has been shown as an active participant in the pathogenesis of diabetes mellitus [5, 6].

Long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a classic lncRNA widely studied by researchers. MALAT1 is expressed in a variety of tissues. The initial studies mainly focused on the relationship of MALAT1 with tumors [7, 8]. However, subsequent studies showed that lncRNA MALAT1 was closely associated with diabetes mellitus and its complications [9]. A study in China examined 50 patients with gestational diabetes and 47 healthy pregnant women. The findings revealed that the lncRNA MALAT1 level in serum increased in patients with gestational diabetes [10]. Liu used sh-MALAT1 to silence the expression of MALAT1 in human umbilical vein endothelial cells and found that the expression levels of resistin, Ang II, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and soluble intercellular adhesion molecule-1 (sICAM-1) decreased and insulin resistance was alleviated after MALAT1 was silenced. At the same time, they observed that exercise could downregulate the MALAT1 level and reduce the incidence of insulin resistance in a mouse model [11]. Yan et al. found that hyperglycemia could increase the expression level of

Da-wei Yang and Lianji Zhou contributed equally to this work.

✉ Biao-liang Wu
mucun889@163.com

[1] JiNan University, Guangzhou, China

[2] Affiliated Hospital of Youjiang Medical University For Nationalities, Zhongshan No 2 Road 18, Baise 533000, China

MALAT1 in retinal endothelial cells and retinas of rats with diabetes, while silencing the expression of MALAT1 could significantly relieve diabetes-induced retinal vascularization, vascular leakage, and retinal inflammation [12]. The down-regulation of lncRNA MALAT1 could protect cardiomyocytes and improve heart function in cardiomyocytes of rats with diabetes [13]. Another study reported that MALAT1 was highly expressed in the renal cortex of mice with diabetic nephropathy, which could cause β-catenin to translocate to the nucleus and also enhance the expression of serine/arginine-rich splicing factor 1 simultaneously, thereby causing damage to renal podocytes. The podocyte function could be partially restored after the early silencing of MALAT1 [14].

The aforementioned studies demonstrated that lncRNA MALAT1 was closely associated with the occurrence and development of diabetes mellitus and its complications. However, studies on the expression of lncRNA MALAT1 in patients with T2DM are still lacking. This study was performed to further explore the expression of lncRNA MALAT1 in a Chinese population with T2DM and also its clinical significance, aiming to provide new biomarkers for the diagnosis and treatment of T2DM.

Materials and methods

Participants

A total of 94 participants treated in the inpatient department of the Affiliated Hospital of Youjiang Medical University For Nationalities in Guangxi Zhuang Autonomous Region from January 2017 to July 2017 were selected. Of the participants, 69 had diabetes mellitus, including 44 men and 25 women, with an average age of 55.54 ± 11.93 years. The remaining 25 participants were healthy controls, including 14 men and 11 women, with an average age of 52.12 ± 12.82 years. No significant differences in sex and age were found between the two groups (Table 1).

The inclusion criteria for the diabetes mellitus group were as follows: (1) patients aged 35–65 years; (2) patients with no tumors, infection, infectious diseases, and other major diseases; and (3) patients diagnosed with T2DM in accordance with the diagnostic criteria issued by the American Diabetes Association in 2018 [15] (Table 2).

(4) All the enrolled patients had undergone the OGTT test

The inclusion criteria for the control group were the same as the inclusion criteria (1) and (2) of the diabetes mellitus group, but the participants were not diagnosed with diabetes mellitus using the glucose tolerance test.

Anthropometrical and biochemical parameter measurements

All participants underwent a physical examination upon admission, during which basic data, such as weight, height, blood pressure, and heart rate, were collected. The body mass index (BMI) was calculated as BMI = weight/height2 (kg/m^2). The levels of fasting blood glucose, hemoglobin, blood lipid, and other biochemical indicators were measured using Mindray BS-2000M automatic biochemical analyzer

Table 1 Comparison of clinical characteristics between the two groups

	Control group (*N*=25)	Diabetes group (*N*=69)	*t* or $\chi 2$	*p* value
Sex	Male 14 Female 11	Male 44 Female 25	0.198	0.656
Age (year)	52.12±12.82	55.54±11.93	−1.203	0.232
Smoke (*n*/%)	5/20%	11/15.94%	0.023	0.879
BMI (kg/m2)	24.63±4.80	24.03±4.17	1.986	0.592
Cortisol (mmol/L)	377.61±81.26	398.60±53.26	−1.454	0.149
HbA1C (%)	5.59±0.95	10.90±3.08	−8.500	≤0.001**
Cr (umol/L)	69.92±16.97	76.29±18.52	−1.505	0.136
TG (mmol/L)	1.94±0.38	2.17±0.54	−1.958	0.053
LDL-C (mmol/L)	2.54±0.49	2.68±0.51	−1.188	0.238
FBG (mmol/L)	4.32±1.06	7.66±3.40	−4.813	≤0.001**
Fasting insulin (pmol/L)	61.54±21	53.55±24.03	1.986	0.145
SOD (u/ml)	132.64±18.50	116.32±22.32	3.269	0.002*

*p<0.05

**p<0.001

Abbreviations: *BMI* body mass index, *HbA1c* glycated hemoglobin, *Cr* creatinine, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *FBG* fasting blood glucose, *FINS* fasting insulin, *HOMA-IR* homeostasis model assessment insulin resistance, *SOD* superoxide dismutase

Table 2 Criteria for the diagnosis of diabetes

FPG≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h
OR
2-h PG≥200mg/dL (11.1mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75g of anhydrous glucose dissolved in water
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose level≥200 mg/dL (11.1 mmol/L).
In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing

(Mindray, Shenzhen, China). The insulin and cortisol levels were tested using a chemiluminescence immunoassay (CLIA) A2000 (Antu Experimental Instrument (Zhengzhou) Co., Ltd. Zhengzhou, China). All tests were performed by technicians trained in the hospital.

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was started at 8 o'clock the next day under fasting conditions. Further, 75 g of anhydrous glucose was dissolved in 200–300 mL of boiling water within 5 min. Blood samples were collected at 30 min, 60 min, 120 min, and 180 min after administration of glucose powder to detect serum glucose and insulin levels.

Total RNA extraction and reverse transcription

The blood sample was collected from all patients at 8 o'clock in the morning. For this, 5 mL of venous blood was collected, mixed with 9 mL of erythrocyte lysate, and centrifuged at 2500 rpm and 4C for 5 min. The supernatant was discarded, and the precipitate was rinsed with 1 mL of phosphate-buffered saline. The extracted leukocytes were stored at –80C. The total RNA in leukocytes was extracted using a TRIzol kit (Pufei Biotechnology Co., Ltd., Shanghai, China) and then reverse transcribed using a Promega Reverse Transcriptase Kit (M-MLV) (Ribo Biotechnology Co., Ltd., Guangzhou, China), for which the reverse transcription primers were purchased from Ribo Biotechnology Co., Ltd. These operations were performed strictly in accordance with the instructions.

Table 3 Primer sequences for RT-qPCR

Primer	Sequence
MALAT1 upstream	5′- CAGACCACCACAGGTTTACAG-3′
MALAT1 downstream	5′-AGACCATCCCAAAATGCTTCA-3′
GADPH upstream	5′-TGACTTCAACAGCGACACCCA-3′
GADPH downstream	5′-CACCCTGTTGCTGTAGCCAAA- 3′

Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) analysis was performed using the SYBR Green (Tiangen Bio., Beijing, China). The PCR conditions were as follows: 95°C for 15 min, 95°C for 10 s, and 60°C for 20 s, a total of 40 cycles. The PCR reaction was evaluated using a dissolution curve. For each sample, three parallel samples were set, and the average value was adopted. The expression level of lncRNA MALAT1 was represented using $2–\Delta\Delta Ct$ value; $\Delta\Delta Ct$ = (CTMALAT1 – CTGADPH)DM – (CTMALAT1 – CTGADPH)con. The primer sequences are listed in Table 3.

Statistical analysis

Statistical analyses were performed using the SPSS17.0 software. Measurement data were expressed as mean ± standard deviation (x ± s). Data in accordance with normal distribution were compared using the *t* test, while those with a heterogeneity of variance were compared using the nonparametric test. Spearman correlation was used to analyze the correlation between variables. A binary logistic regression model was used to analyze the risk factors for the development of T2DM. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to evaluate the efficacy of diagnostic indicators. A difference with $p < 0.05$ was considered statistically significant.

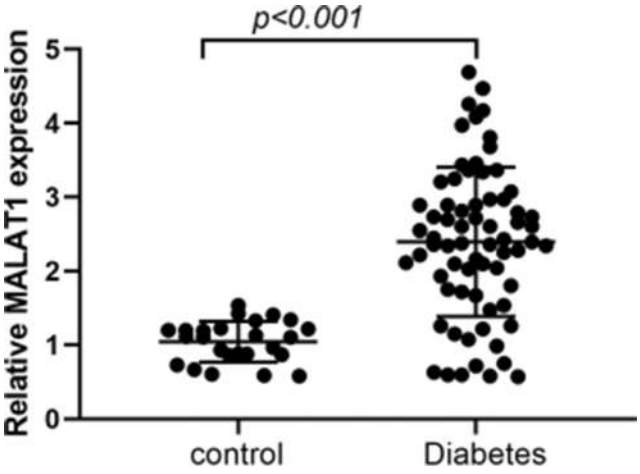
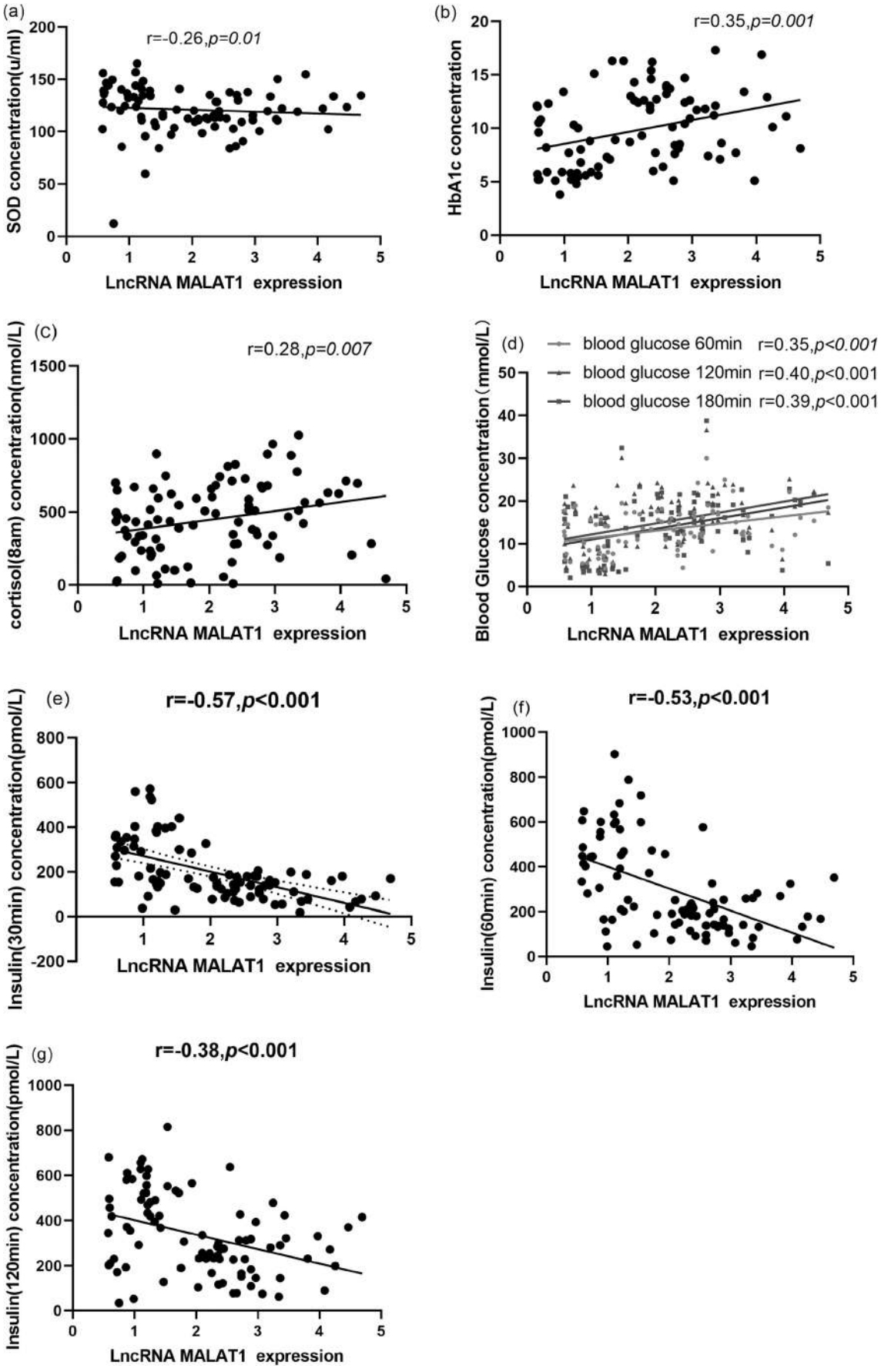


Fig. 1 Relative MALAT1 expression in two groups


(a)
r=-0.26,p=0.01
SOD concentration(u/ml)
LncRNA MALAT1 expression
(b)
r=0.35,p=0.001
HbA1c concentration
LncRNA MALAT1 expression
(c)
r=0.28,p=0.007
cortisol(8am) concentration(nmol/L)
LncRNA MALAT1 expression
(d)
blood glucose 60min r=0.35,p<0.001
blood glucose 120min r=0.40,p<0.001
blood glucose 180min r=0.39,p<0.001
Blood Glucose concentration（mmol/L）
LncRNA MALAT1 expression
(e)
r=-0.57,p<0.001
Insulin(30min) concentration(pmol/L)
LncRNA MALAT1 expression
(f)
r=-0.53,p<0.001
Insulin(60min) concentration(pmol/L)
LncRNA MALAT1 expression
(g)
r=-0.38,p<0.001
Insulin(120min) concentration(pmol/L)
LncRNA MALAT1 expression

◀ **Fig. 2** Correlation analysis of the expression LncRNA MALAT1 with the concentration of SOD, HbA1C, cortisol, insulin (30min, 60min, 120min), and blood glucose in OGTT test

Results

Comparison of general clinical data

No significant differences in age, sex, smoking history, BMI, hypertension, and triglyceride were found between the two groups ($p > 0.05$). Meanwhile, the levels of fasting plasma glucose (FPG), SOD, glycosylated hemoglobin (HbA1c) ($p < 0.01$), and fasting insulin (Fins) were significantly higher in the diabetes mellitus group than in the control group; the differences were statistically significant ($p < 0.05$).

Expression of lncRNA MALAT1 was higher in patients with T2DM than in healthy controls

The expression of lncRNA MALAT1 in leukocytes of 25 controls and 69 patients with T2DM was measured using RT-PCR. The difference between the two groups was analyzed using the *t* test. The results revealed that the expression level of lncRNA MALAT1 was significantly higher in the leukocytes of patients with T2DM than in those of the control group ($t = -6.584$, $p < 0.001$) (Fig. 1).

LncRNA MALAT1 was a risk factor for the onset of T2DM

Binary logistic regression analysis was used to assess the risk factors for T2DM. The results showed that lncRNA MALAT1 ($p < 0.001$, OR = 11.667, 95% CI: 3.181–42.792) and SOD ($p = 0.018$, OR = 0.958, 95% CI: 0.925–0.993) were the risk factors for the onset of T2DM.

Correlation analysis of the expression of lncRNA MALAT1 with each indicator in the two groups

Spearman correlation analysis was used to assess the correlation of the expression of lncRNA MALAT1 with various indicators in both groups. The results revealed that the expression level of lncRNA MALAT1 correlated with SOD, cortisol (8 AM), and hemoglobin levels; insulin secretion (60 min); and blood glucose level (60, 120, and 180 min) in OGTT, but did not obviously correlate with other indicators (Fig. 2).

Predictive significance of lncRNA MALAT1 in T2DM detected using ROC analysis

ROC was used to assess whether lncRNA MALAT1 could be a biomarker for predicting T2DM. The results showed that the AUC was 0.807 (95% CI: 0.708–0.906, $p < 0.001$), sensitivity was 78.3%, and specificity was 84%. Hence, the expression of lncRNA MALAT1 was of certain predictive value for T2DM (Fig. 3).

Comparison of insulin secretion between high and low lncRNA MALAT1 expression groups in OGTT TEST

We took the median value of LncRNA MALAT1 expression as the cut point (1.80) and divided it into high expression group (n=51) and low expression group (n=43). Next, the *t* test was used to compare the difference in insulin secretion between the two groups, and the results found that at 30 min, 120 min, and 180 min, the insulin secretion of the high expression group was significantly less than that of the low expression group ($p < 0.001$) (Fig. 4).

Discussion

T2DM is a metabolic disease characterized by insulin resistance and accompanied by inadequate insulin secretion. The prevalence of T2DM has increased rapidly worldwide with the development of human society and changes in lifestyles, including developed and developing countries. The International Diabetes Federation has indicated 415 million patients with diabetes mellitus worldwide currently, and the number will continue to increase. By 2040, China will have the largest number of patients with diabetes mellitus in the world due to its rapid economic development, accelerated urbanization, and lifestyle changes [16]. Diabetes mellitus has become a chronic disease that seriously affects social development and the healthy and happy life of people. The risk factors for diabetes mellitus include unhealthy lifestyles, such

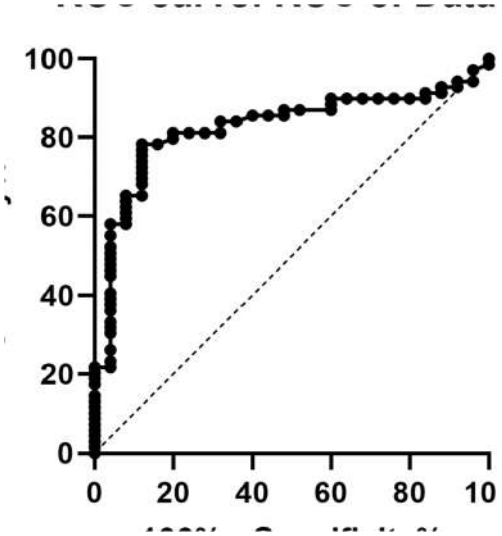


Fig. 3 ROC curve analysis of the diagnostic value of lncRNA MALAT1 for T2DM patients

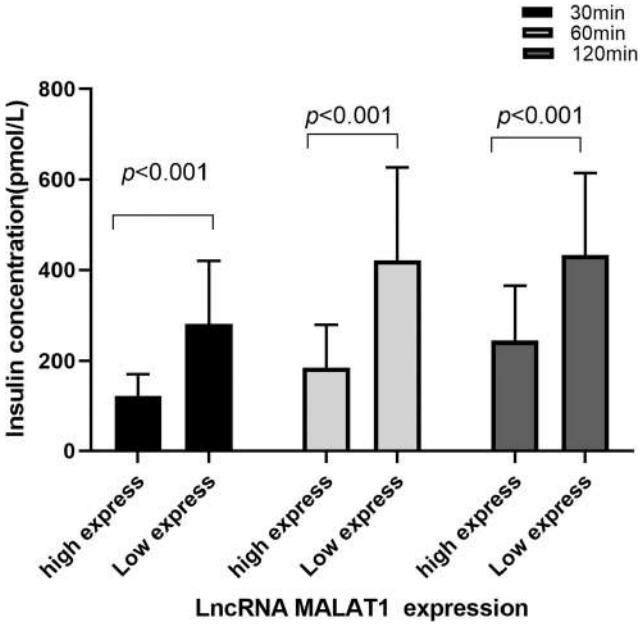


Fig. 4 The secretion of insulin under different level of the LncRNA MALAT1 expression in the OGTT test

as sedentariness and genetic factors, but early diagnosis is very difficult due to its latency [17].

LncRNA is a noncoding RNA in the human body. It is considered to be related to cell differentiation, proliferation, metabolism, and various vital activities and is involved in the occurrence and development of many diseases [18, 19]. Studies have pointed out that a variety of lncRNAs are closely associated with the occurrence and development of diabetes mellitus and its complications, including H19 [20], MEG3 [21], and uc.322 [22].

The present study found that the expression level of lncRNA MALAT1 was significantly higher in patients with T2DM than in the control group, which was consistent with the results reported by Zhang et al., who found that the expression level of lncRNA MALAT1 was higher in pregnant women with diabetes mellitus than in normal pregnant women [10]. In their follow-up studies, we found that GDM seems to upregulate the expression of lncRNA MALAT1 and may promote inflammation and proliferation, invasion, and migration of GDM placental trophoblast cells through the TGF-β/NF-κB signaling pathway [23]. The present study found that the expression level of lncRNA MALAT1 positively correlated with the hemoglobin level, indicating that lncRNA MALAT1 affected the blood glucose control in patients with diabetes mellitus. It also suggested that the increased expression level of lncRNA MALAT1 led to poor blood glucose control and accelerated the occurrence of complications in patients with diabetes mellitus. These results were consistent with those reported in recent years that lncRNA MALAT1 was involved in the pathogenesis of diabetic nephropathy [24] and diabetic retinopathy [25].

This study also found that the high expression level of lncRNA MALAT1 positively correlated with the increase in blood glucose level after 60, 120, and 180 min in the glucose tolerance test, indicating that lncRNA MALAT1 might affect the body's feedback regulation of hyperglycemia. The body's correct response to hyperglycemia was to secrete more insulin to reduce the excessive blood glucose level. Furthermore, this study found that the expression level of lncRNA MALAT1 negatively correlated with insulin secretion after 30 min, 60 min, and 120 min in OGTT, suggesting that lncRNA MALAT1 might affect the increase in blood glucose level by inhibiting hyperglycemia-induced active secretion of insulin. Feng et al. reported that lncRNA MALAT1 could inhibit breast cancer proliferation by downregulating miR124 expression to inhibit the CDK4/E2F1 signaling pathway, while the CDK4/E2F1 pathway was an important pathway affecting insulin secretion [26]. We observed the different expression levels of lncRNA MALAT1 in different groups and found that insulin secretion in the high expression group was significantly higher than in the low expression group at different time points in the OGTT test. Ding et al.'s study may give us some hints. In their study, they stimulated mouse pancreatic islet and β cell lines with IL-1β, followed by PCR to measure the expression of MALAT1 and PDX-1 and their promoters. The results show that MALAT1 induces the dysfunction of β cells via reducing the H3 histone acetylation of the PDX-1 promoter and subsequently inhibiting the expression of PDX-1, thus suppressing insulin secretion [27]. However, these findings need further verification.

In addition, the present study found that the expression level of lncRNA MALAT1 negatively correlated with the SOD level in the human body. SOD is the most important antioxidant in the human body, while oxidative stress is considered as an important cause of the occurrence and development of diabetes mellitus and many complications (2). Gong et al. showed that the expression level of lncRNA MALAT1 also negatively correlated with the SOD level in patients with diabetic cataract and speculated that it might induce oxidative stress through the p38MAPK signaling pathway [28]. Chen et al. revealed that the reactive oxygen species level significantly decreased in rats with downregulated lncRNA MALAT1 [29]. All these results indicated that lncRNA MALAT1 could induce an oxidative stress response in the body. Nonetheless, the specific molecular mechanism of lncRNA MALAT1–induced oxidative stress in the pathogenesis of T2DM needs further exploration.

Finally, ROC was used in this study to determine whether lncRNA MALAT1 had the potential as a biomarker to predict the onset of diabetes mellitus. The study found that the AUC was 0.852 (95% CI: 0.789–0.914, $p < 0.001$), sensitivity was 78.3%, and specificity was 100%. In short, lncRNA MALAT1 was of a certain value to predict T2DM.

In conclusion, LncRNA MALAT1 was highly expressed in patients with T2DM and may serve as a potential diagnostic biomarker for T2DM.

Funding This study was supported by the Natural Science Foundation of China (No. 81960875).

Declarations

Ethics approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the World Medical Association Declaration of Helsinki or comparable ethical standards. The study was approved by the ethics committee of the hospital, and all participants signed an informed consent form.

Conflict of interest The authors declare no competing interests.

References

1. American Diabetes A. Economic costs of diabetes in the U.S. in 2012. Diabetes Care. 2013;36(4):1033–46.
2. Halim M, Halim A. The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). Diabetes Metab Syndr. 2019;13(2):1165–72.
3. Gomes BF, Accardo CM. Immunoinflammatory mediators in the pathogenesis of diabetes mellitus. Einstein (Sao Paulo). 2019;17(1): eRB4596.
4. Dong G, Qu L, Gong X, Pang B, Yan W, Wei J. Effect of social factors and the natural environment on the etiology and pathogenesis of diabetes mellitus. Int J Endocrinol. 2019;2019:8749291.
5. Leung A, Natarajan R. Long noncoding RNAs in diabetes and diabetic complications. Antioxid Redox Signal. 2018;29(11): 1064–73.
6. Feng SD, Yang JH, Yao CH, Yang SS, Zhu ZM, Wu D, et al. Potential regulatory mechanisms of lncRNA in diabetes and its complications. Biochem Cell Biol. 2017;95(3):361–7.
7. Mei H, Liu Y, Zhou Q, Hu K, Liu Y. Long noncoding RNA MALAT1 acts as a potential biomarker in cancer diagnosis and detection: a meta-analysis. Biomark Med. 2019;13(1):45–54.
8. Cardamone G, Paraboschi EM, Solda G, Cantoni C, Supino D, Piccio L, et al. Not only cancer: the long non-coding RNA MALAT1 affects the repertoire of alternatively spliced transcripts and circular RNAs in multiple sclerosis. Hum Mol Genet. 2019;28(9):1414–28.
9. Abdulle LE, Hao JL, Pant OP, Liu XF, Zhou DD, Gao Y, et al. MALAT1 as a diagnostic and therapeutic target in diabetes-related complications: a promising long-noncoding RNA. Int J Med Sci. 2019;16(4):548–55.
10. Zhang Y, Wu H, Wang F, Ye M, Zhu H, Bu S. Long non-coding RNA MALAT1 expression in patients with gestational diabetes mellitus. Int J Gynaecol Obstet. 2018;140(2):164–9.
11. Liu SX, Zheng F, Xie KL, Xie MR, Jiang LJ, Cai Y. Exercise reduces insulin resistance in type 2 diabetes mellitus via mediating the lncRNA MALAT1/MicroRNA-382-3p/resistin axis. Mol Ther Nucleic Acids. 2019;18:34–44.
12. Yan B, Tao Z-F, Li X-M, Zhang H, Yao J, Jiang Q. Aberrant expression of long noncoding RNAs in early diabetic retinopathy. Invest Ophthalmol Vis Sci. 2014;55(2):941.
13. Zhang M, Gu H, Xu W, Zhou X. Down-regulation of lncRNA MALAT1 reduces cardiomyocyte apoptosis and improves left ventricular function in diabetic rats. Int J Cardiol. 2016;203:214–6.
14. Hu M, Wang R, Li X, Fan M, Lin J, Zhen J, et al. LncRNA MALAT1 is dysregulated in diabetic nephropathy and involved in high glucose-induced podocyte injury via its interplay with beta-catenin. J Cell Mol Med. 2017;21(11):2732–47.
15. American Diabetes A. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S13–27.
16. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017;128:40–50.
17. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017;389(10085):2239–51.
18. Lin C, Yang L. Long noncoding RNA in cancer: wiring signaling circuitry. Trends Cell Biol. 2018;28(4):287–301.
19. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019;21(5):542–51.
20. Zhang N, Geng T, Wang Z, Zhang R, Cao T, Camporez JP, et al. Elevated hepatic expression of H19 long noncoding RNA contributes to diabetic hyperglycemia. JCI Insight. 2018;3(10):e120304.
21. Ghaedi H, Zare A, Omrani MD, Doustimotlagh AH, Meshkani R, Alipoor S, et al. Genetic variants in long noncoding RNA H19 and MEG3 confer risk of type 2 diabetes in an Iranian population. Gene. 2018;675:265–71.
22. Zhao X, Rong C, Pan F, Xiang L, Wang X, Hu Y. Expression characteristics of long noncoding RNA uc.322 and its effects on pancreatic islet function. J Cell Biochem. 2018;119(11):9239–48.
23. Zhang Y, Qu L, Ni H, Wang Y, Li L, Yang X, et al. Expression and function of lncRNA MALAT1 in gestational diabetes mellitus. Adv Clin Exp Med. 2020;29(8):903–10.
24. Shi S, Yang J, Fan W, Zhou Z, Chen G, Zhang J. Effects of LncRNA MALAT1 on microangiopathy and diabetic kidney disease in diabetic rats by regulating ERK/MAPK signaling pathway. Minerva Med. 2020;111(2):184–6.
25. Liu P, Jia SB, Shi JM, Li WJ, Tang LS, Zhu XH, et al. LncRNA-MALAT1 promotes neovascularization in diabetic retinopathy through regulating miR-125b/VE-cadherin axis. Biosci Rep. 2019;39(5):BSR20181469.
26. Feng T, Shao F, Wu Q, Zhang X, Xu D, Qian K, et al. miR-124 downregulation leads to breast cancer progression via LncRNA-MALAT1 regulation and CDK4/E2F1 signal activation. Oncotarget. 2016;7(13):16205–16.
27. Ding H, Wang F, Shi X, Ma H, Du Y, Hou L, et al. LncRNA MALAT1 induces the dysfunction of beta cells via reducing the histone acetylation of the PDX-1 promoter in type 1 diabetes. Exp Mol Pathol. 2020;114:104432.
28. Gong W, Zhu G, Li J, Yang X. LncRNA MALAT1 promotes the apoptosis and oxidative stress of human lens epithelial cells via p38MAPK pathway in diabetic cataract. Diabetes Res Clin Pract. 2018;144:314–21.
29. Chen J, Ke S, Zhong L, Wu J, Tseng A, Morpurgo B, et al. Long noncoding RNA MALAT1 regulates generation of reactive oxygen species and the insulin responses in male mice. Biochem Pharmacol. 2018;152:94–103.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):77–81
https://doi.org/10.1007/s13410-021-00949-1

ORIGINAL ARTICLE

Association between rs619586 (A/G) polymorphism in the gene encoding lncRNA-MALAT1 with type 2 diabetes susceptibility among the Isfahan population in Iran

Abbas Samadi-Khouzani[1] · Payam Kheirmand Parizi[2,3] · Fatemeh Ghafari[1] · Seyed-Alireza Esmaeili[4,5] · Maryam Peymani[1] · Amir Abbas Momtazi-Borojeni[6,7,8]

Received: 12 May 2020 / Accepted: 5 April 2021 / Published online: 26 April 2021

Abstract

Background and aim Type 2 diabetes mellitus (T2DM) is a global human disease that affects millions of people. Long non-coding RNAs (LncRNAs) are transcripts with more than two-hundred nucleotides that play essential roles in the management of mRNAs. In the present study, we examined whether the rs619586 (A/G) polymorphism in the gene encoding lncRNA-MALAT1 is associated with the susceptibility to T2DM among the Isfahan population, Iran.

Methods To this end, a case-control study was conducted on 200 healthy persons and 200 patients with T2DM. The genomic DNA was extracted from blood samples to strengthen the intended fragments containing rs619586 SNP. Using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), the wild allele (A) and the mutant allele (G) were examined.

Result and conclusion Results indicated that the mutant allele (G) and mutant genotypes (AG/GG) were absent in T2DM patients. This absence suggests that the rs619586 (A/G) polymorphism in the gene encoding lncRNA-MALAT1 might not be associated with the susceptibility to T2DM among the Isfahan population.

Keywords Long non-coding RNA · The metastasis-related lung adenocarcinoma transcript 1 · Polymorphism · Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic multifactorial metabolic disease manifested by insulin resistance and β-cell disappointment, which causes a deleterious increase in the levels of blood glucose [1]. The outbreak of diabetes was evaluated in 2017 when there were 451 million people, aged 18–99, with diabetes worldwide. It has been estimated that the number of cases will be increased to 693 million up to 2045 [2]. Generally, 90–95% of patients with diabetes suffer T2DM [3]. The pervasiveness of T2DM is known to be the highest among developing countries [4]. Both the hereditary and ecological factors are involved in the development of T2DM [5]. Long non-coding RNAs (LncRNAs) are transcripts with more than 200 nucleotides, which do not encode proteins in terms of appearance similar to mRNAs [6]. LncRNAs are involved in a diversity of genetic procedures such as chromosome imprinting, epigenetic regulation, cell-cycle control, and apoptosis [7]. The human β-cell transcriptome examination indicates that lncRNAs progressively control the eccentric expression in T2DM [8]. Metastasis-associated lung adenocarcinoma

✉ Maryam Peymani
m.peymani@iaushk.ac.ir

[1] Department of Biology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[2] Cellular, Molecular and Genetics Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

[3] Medical Genetics Research Center of Genome, Isfahan University of Medical Sciences, Isfahan, Iran

[4] Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[5] Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[6] Department of Medical Biotechnology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran

[7] Iran's National Elites Foundation, Tehran, Iran

[8] Department of Medical Biotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

transcript *1* (MALAT-1) is a non-coding RNA, which is highly conserved among mammals and mainly located in chromosome 11q13 [9]. LncRNA-MALAT1 deregulation is involved in the pathogenesis of diabetes-related microvascular illness, diabetic retinopathy (DR) [10]. Single nucleotide polymorphisms (SNPs) have been found to be associated with the pathogenesis of diabetes [11]. SNPs in lncRNA are accounted for modification of their relating lncRNA operation by direct control of the lncRNA expression [12]. Rs619586 is a MALAT1 gene polymorphism that plays an imperative role in the pathogenesis and incidence of various diseases [13]. Complex genetic and environmental effects create a diverse incidence of genetic SNPs in numerous national populations [14]. It is particularly essential to discover its relationship in the Isfahan population, as the number of cases with T2DM is rapidly increasing [15]. Thus, the present study aimed to determine whether there is an association between rs619856 SNP in the gene encoding lncRNA-MALAT1 with T2DM in the Isfahan population.

Materials and methods

The study design

A case-control study was done to assess the association between rs619586 SNP and T2DM. The included patients were determined regarding the adjustment in blood sugar levels (>126 mg/dl). All patients and controls were Iranians from Isfahan. The questionnaire was used to collect all data about the age, gender, and clinical and supplementary information of study participants. Diabetes is defined by the World Health Organization (WHO) as fasting blood sugar (FBS) above 126 mg/dl (≥7.0 mmol/l) or 2-h value of ≥200 mg/dl (≥11.1 mmol/l) in 75 g oral glucose tolerance test (OGTT) [16, 17]. The patient inclusion criteria were (i) FBS level of ≥126 mg/dl; (ii) 2-h value of ≥200 mg/dl in 75 g OGTT; or (iii) casual plasma glucose level of ≥200 mg/ dl. The control group was randomly selected from healthy subjects who did not have any criteria for diabetes and a family history of diabetes in relatives. Indeed, no family history, FBS level of <110 mg/dl, and 2-h value of <140 mg/dl (<7.8 mmol/l) in OGTT were considered the normal subject inclusion criteria.

DNA extraction

Genomic DNA was extricated from whole-blood samples by a DNA extraction Kit (GENET BIO, Chungnam, Korea) according to the manufacturer's instructions. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel), and the purity checking (A260/A280). Extracted DNA was subjected to polymerase chain reaction (PCR).

PCR analysis

PCR-based analysis was performed by the PCR restriction fragment length polymorphism (RFLP) (PCR-RFLP). The applied primers for the PCR amplification were as follows: Forward, 5`-CCACTTCTCAACCGTCCCT-3`; Reverse, 5`-AGACGGAGAACAACTCGCATC-3` (425 bp particular restriction site to determine diverse alleles of the rs619586 SNP). PCR was done using a programmed thermal cycler (Eppendorf, Hamburg, Germany) under the subsequent circumstances: 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 5 min.

Restriction enzyme analysis

PCR products were subjected to 1 U BspMI (NEB, USA) restriction enzyme (reaction volume 7.5 μL). After 24-h incubation at 37°C, the enzyme cuts the 425-bp PCR product into two fragments of 312 bp and 113 bp in length (Fig. 1). The consequential products were electrophoresed by 2% agarose gel.

Sequencing of products

The acquired PCR product was assessed by sequencing according to Sanger's sequencing method to confirm the LncRNA-MALAT1 encoding gene sequence.

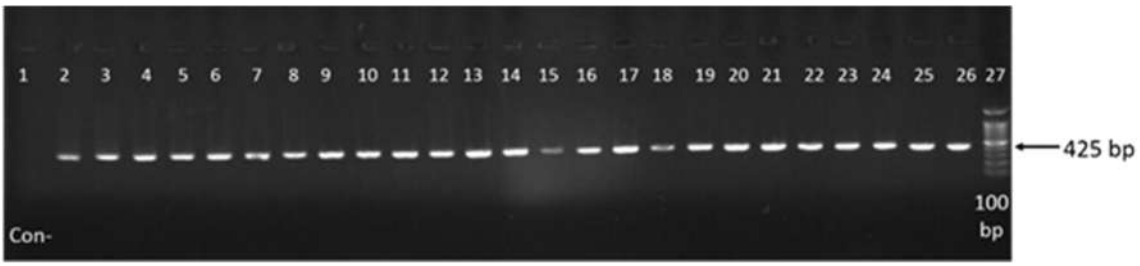


Fig. 1 Electrophoresis of PCR products shows 425-bp fragments

Table 1 Distribution of clinical and biochemical variables in cases and controls

Indexes	Cases	Controls	*p*-value
Fasting blood sugar (mg/dl)	189.80 ± 5.395	83.07 ± 8.90	<.001*
Cholesterol (mg/dl)	211.76 ± 44.65	188.01 ± 38.55	<.001*
Triglyceride (mg/dl)	250 ±20	160 ±37	<.001*
HbA1c (%)	7.3 (6.72-8)	5 (4.9-5.2)	.005#
HDL (mg/dl)	44.44 ±11.20	46.14 ±10.72	.122*
LDL (mg/dl)	102.32 ±41.56	109.25 ±35.24	.073*

*Independent samples *T*-test; mean±std. deviation

#Mann Whitney test; median (percentiles 25, percentiles 75)

Results

The present case-control study involved a total of 400 volunteers. Among them, 200 patients with a history of T2DM were classified as the case group. The remaining 200 members, who did not have T2DM, were classified as the control group. Cases and controls were matched on age. The sample size was calculated using the power calculator for case-control genetic association studies (PGA) [18]. Table 1 signifies the distribution of clinical and biochemical variables in cases and controls. Mean FBS (p <.001), triglyceride (p <.001), total cholesterol (p <.001), and HbA1c (p=.005) were significantly higher in diabetic patients than healthy subjects. There was no significant difference in HDL and LDL levels between the two groups.

The BspMI enzyme was applied to identify the rs619586 genotype. After performing the PCR, its products were examined using a BSPMI restriction enzyme. Fragments with a size of 425 bp represented the genotype (AA), those with sizes of 425, 312, and 113 bp indicated genotype (GA), and those with 312 and 113 bp represented the genotype (GG). In the studied population, no sample could be restricted in the presence of the enzyme. To confirm the results of the RFLP examination, some samples were assessed by sequence analysis, and the results were confirmed (Fig. 2). To verify the enzyme function, a fragment with a specific sequence with the BspMI enzyme cutting site, where there was no polymorphism, was also considered as a positive control; and the enzyme digestion was evaluated on this part (Figs. 2 and 3).

Discussion

Although the etiology of T2DM is defined and resulted from a collaboration of ecological elements with a mix of hereditary variations, its vast majority is still unknown [18]. The investigation of possible key polymorphisms in a few genes of the genome has created a robust methodology to realize complex interaction between the genotype of patients and multifactorial ailments like T2DM [19]. According to studies, there is an association between lncRNA-MALAT1 and microvascular disease and eye disorders relating to diabetes mellitus [10]. Studies have also shown that the MALAT1 expression is increased at the higher concentrations of glucose [20], and there was a relationship between lncRNA-MALAT1 and diabetes-mediated cardiomyopathy [21]. Previous studies also reported that the increased expression of lncRNA-MALAT1 was linked to hyperglycemia-induced inflammation and endothelial dysfunction [22].

In the present study, the gene encoding lncRNA-MALAT1 polymorphism (rs619586 A>G) was analyzed to explore a possible relationship between explicit polymorphic patterns and T2DM susceptibility. In the studied population, there was no sample that could restrict the presence of an enzyme. The results of the experiment were confirmed using the sequencing of some samples.

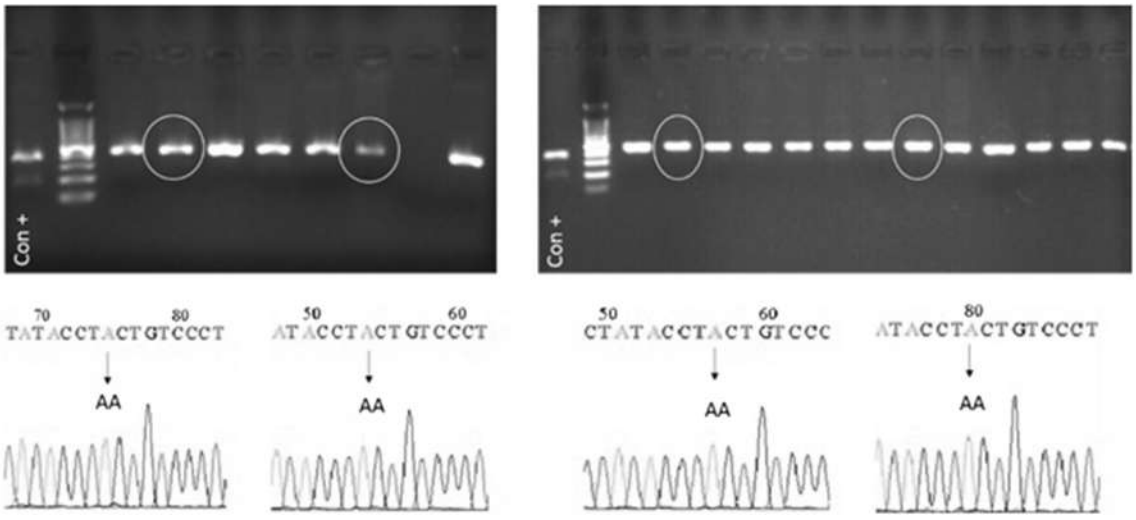


Fig. 2 Result of electrophoresis of some PCR products treated with the BspMI enzyme. To confirm the results, we got help from Sanger's sequencing method that in the all AA genotype was confirmed. Cut in positive control shows the proper enzyme function

Fig. 3 A fragment sequence that has two cutting sites for the BspMI enzyme that cut in the Specified Area Indicates proper enzyme function and confirms enzyme activation

Findings indicated that the wild allele (A) in this polymorphism suggests that there is no detectable mutant allele in the studied population. According to other studies, the minor allele frequency (MAF) of rs619586 is diverse in different populations [23], and it can confirm our findings and represent a specific frequency of the mutant allele in rs916586 in the Isfahan population. To guarantee the enzyme function, a fragment with a particular sequence, which had a BspMI enzyme cutting site where there was no polymorphism, was also considered a positive control; and the enzyme digestion was examined in this part. Indeed, the enzyme function was confirmed because it successfully cuts positive control samples. Of note, there was no evidence to confirm that the polymorphism rs619586 in the lncRNA-MALAT1 encoding gene is a susceptible factor for the development of T2DM. Mutant genotype and allele frequency were missing in T2DM patients and controls in the Isfahan population and BspMI enzyme could not digest gene encoding lncRNA- MALAT1, because G allele required for the enzymatic digestion was absent in the target population. It was found that the rs619586 A/G polymorphism in the MALAT1 encoding gene might not be associated with the vulnerability to T2DM. Nevertheless, this absence of association might be owing to the ethnic variety, ecological risk profiles, body arrangement, the sample size limitations, or different indeterminate factors.

Different genes in various populations are involved in disease susceptibility, and there may be specific types of pathogenic mutations in the whole group. There are racial and geographical differences, what is called genetic diversity [24]. Thus, although different populations are all susceptible to the same disease, different antigenic and environmental factors activate different mutations in the genome, which predisposes the individual. On the other hand, these findings may indicate a different gene distribution in the genetic pool of the population in each geographical area [22, 25, 26]. Therefore, the absence of a mutant allele in the samples under study in the present research can support this principle.

Conclusion

Due to the lack of polymorphism in both control and patient groups, it can be concluded that there is probably no relationship between rs619586 (A/G) polymorphism in the gene encoding lncRNA-MALAT1 with T2DM among the Isfahan population in Iran. Supplementary studies should be conducted on different SNPs (rs3200401 and rs11227209) in the MALAT1 encoding gene to examine their roles and susceptibility to T2DM. We need to test more genomic sites to verify the association between MALAT1 and T2DM. The sample size was relatively not sufficiently large in our study. Since the populations selected in our research were all from Isfahan in Iran, the results need to be validated in larger samples, other regions and ethnic groups.

Acknowledgment We are thankful for supporting this research from Shahrekord, Isfahan University of Medical Sciences, and Mashhad University of Medical Sciences.

Funding The study has been financially supported by Islamic Azad University of Shahrekord, Shahrekord, Iran (Grant number; IR.IAU.SHK.REC.1398.022).

Declarations

The samples were collected from subjects using EDTA-coated tubes, and the informed agreement was taken from the patients and control participants.

The survey was confirmed by the ethical council of the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran (Ethical code number: IR.IAU.SHK.REC.1398.022).

Conflict of interests The authors declare no competing interests.

References

1. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194–222.

2. Cho N, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
3. Association, A.D. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(Supplement 1):S62–9.
4. Haghdoost A, et al., Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. 2009.
5. Nithya K, Angeline T, Isabel W, Asirvatham AJ. SOD1 gene+ 35A/C (exon3/intron3) polymorphism in type 2 diabetes mellitus among south indian population. Gen Res Int. 2016;2016:1–5.
6. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10(3):155–9.
7. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol. 2013;20(3): 300–7.
8. Morán I, Akerman İ, van de Bunt M, Xie R, Benazra M, Nammo T, et al. Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. Cell Metab. 2012;16(4):435–48.
9. Schmidt LH, Spieker T, Koschmieder S, Schäffers S, Humberg J, Jungen D, et al. The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. J Thorac Oncol. 2011;6(12):1984–92.
10. Liu J, et al. Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. Cell Death Dis. 2014;5(10): e1506.
11. Karaderi T, Drong AW, Lindgren CM. Insights into the genetic susceptibility to type 2 diabetes from genome-wide association studies of obesity-related traits. Curr Diabetes Rep. 2015;15(10):83.
12. Li Q, Zhu W, Zhang B, Wu Y, Yan S, Yuan Y, et al. The MALAT1 gene polymorphism and its relationship with the onset of congenital heart disease in Chinese. Biosci Rep. 2018;38(3):BSR20171381.
13. Motawi TM, et al. The expression of long non coding RNA genes is associated with expression with polymorphisms of HULC rs7763881 and MALAT1 rs619586 in hepatocellular carcinoma and HBV Egyptian patients. J Cell Biochem. 2019;120:14645–56.
14. Garte S, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Prevent Biomark. 2001;10(12): 1239–48.
15. Janghorbani M, Amini M. Metabolic syndrome in type 2 diabetes mellitus in Isfahan, Iran: prevalence and risk factors. Metab Syndr Relat Disord. 2007;5(3):243–54.
16. von Eckardstein A, Schulte H, Assmann G. Risk for diabetes mellitus in middle-aged Caucasian male participants of the PROCAM study: implications for the definition of impaired fasting glucose by the American Diabetes Association. J Clin Endocrinol Metab. 2000;85(9):3101–8.
17. Seino Y, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig. 2010;1(1):2–20.
18. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445(7130):881–5.
19. Abuhendi N, Qush A, Naji F, Abunada H, al Buainain R, Shi Z, et al. Genetic polymorphisms associated with type 2 diabetes in the Arab world: A systematic review and meta-analysis. Diabetes Res Clin Pract. 2019;151:198–208.
20. Puthanveetil P, Chen S, Feng B, Gautam A, Chakrabarti S. Long non-coding RNA MALAT 1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J Cell Mol Med. 2015;19(6):1418–25.
21. Zhang M, Gu H, Chen J, Zhou X. Involvement of long noncoding RNA MALAT1 in the pathogenesis of diabetic cardiomyopathy. Int J Cardiol. 2016;202:753–5.
22. Puthanveetil P, et al. Long non-coding RNA MALAT 1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J Cell Mol Med. 2015;19(6):1418–25.
23. Qian XR, Chen L, Liu JT, Zhu BL, Zhao QN, Ding EM, et al. Association between Polymorphisms of MALAT1 and Blood Lead Levels in Lead-exposed Workers. Biomed Environ Sci: BES. 2018;31(7):527–30.
24. Marron MP, et al. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. Hum Mol Genet. 1997;6(8):1275–82.
25. Che D, et al. The lncRNA MALAT1 rs619586 G variant confers decreased susceptibility to recurrent miscarriage. Front Physiol. 2019;10:385.
26. Wang G, et al. Association of polymorphisms in MALAT1 with risk of coronary atherosclerotic heart disease in a Chinese population. Lipids Health Dis. 2018;17(1):75.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):82–90
https://doi.org/10.1007/s13410-021-00951-7

ORIGINAL ARTICLE

Investigation of extracellular matrix genes associated with Alzheimer's disease in the hippocampus of experimental diabetic model rats

Lütfiye Özpak[1] · Ayfer Pazarbasi[2] · Işıl Öcal[3] · M. Bertan Yılmaz[2] · Hülya Binokay[4]

Received: 10 June 2020 / Accepted: 5 April 2021 / Published online: 17 April 2021

Abstract

Backround Both type 2 diabetes (T2D) and Alzheimer's disease affect large number of people all over the world, especially in developed countries, and involve common molecular mechanisms in degenerative processes.

Aim Our study is to investigate the expression levels of the ADAMTS4, TIMP3, RELN, and BCAN genes which encode extracellular matrix molecules and are thought to be related to the pathophysiology of Alzheimer's disease in rats that we used to develop a T2D model by injection of a streptozotocin (STZ) and feeding with high-fat diet (HFD).

Material and methods In total, 40 rats were divided into four groups: HFD + STZ (10), HFD (10), STZ (10), and the control (10). The weight and blood glucose levels of all rats were recorded, and the insulin tolerance test was performed. At the end of the experimental period, the hippocampus of the rats was isolated and a part of this was used for gene expression analysis through real-time PCR, whereas other parts were used for histological analyses.

Results Our results show that plaque-like structures were found in the histological examination of the experimental T2D model. In molecular studies, the expression levels of the ADAMTS4, TIMP3, RELN, and BCAN genes were decreased in the HFD +STZ and STZ groups compared with the control, whereas the same expression levels, except that of inhibitor TIMP3, were found to increase in the HFD group.

Conclusion These genes encoding proteases that regulate neuronal activity have decreased levels in the T2D model. There may be potential in the adoption of new treatment approaches for Alzheimer's and T2D.

Keywords Type 2 diabetes · Alzheimer · Brain extracellular matrix

Introduction

The incidence of type 2 diabetes (T2D) is rapidly increasing worldwide, along with the increasing prevalence of unhealthy diet (e.g., excessive consumption of fast food) and sedentary lifestyle. According to the data from the International Diabetes Federation 2017, the current number of individuals with diabetes, that is, 425 million worldwide, is expected to increase by 48% in 2045, reaching up to 629 million [1, 2]. T2D, which constitutes 90–95% of all diabetes cases and whose

✉ Lütfiye Özpak
lutfiyeozpak@gmail.com; lutfiyeozpak@ksu.edu.tr

Ayfer Pazarbasi
payfer@cu.edu.tr

Işıl Öcal
iocal@cu.edu.tr

M. Bertan Yılmaz
mbyilmaz@cu.edu.tr

Hülya Binokay
hbinokay@cu.edu.tr

[1] Department of Medical Biology, School of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

[2] Department of Medical Biology, School of Medicine, Çukurova University, Adana, Turkey

[3] Department of Biophysics, School of Medicine, Çukurova University, Adana, Turkey

[4] Department of Biostatistics, School of Medicine, Çukurova University, Adana, Turkey

pathophysiology remains unclear, is a hyperglycemic disease that develops due to impaired insulin secretion or due to impaired insulin resistance [3–5]. Epidemiological studies have found that the most important risk factor for T2D is obesity, the incidence of which is dramatically increasing, especially in Western countries [6–8].

Alzheimer's disease is an irreversible brain disease that causes neurodegenerative and neuropsychiatric disorders, and common molecular mechanisms in type 2 diabetes (T2D) degenerative processes [9, 10]. In all types of dementia, including Alzheimer's disease, degenerative processes that cause diabetes increase the risk of dementia two to three times [11].

Extracellular matrix (ECM) molecules, which fill the extracellular space in the brain and have a hydrodynamic structure in charge of neuronal development and plasticity, also play an important role in disease processes [12]. Neuronal activity is regulated by extracellular protease families and inhibitors that play a selective role on ECM proteins [13]. In recent studies, ECM proteins and proteases have been identified as one of the most important regulators of memory, learning, and plasticity [14, 15].

In this study, the expression levels of the ADAMTS4, TIMP3, RELN, and BCAN genes encoding brain ECM molecules were investigated in rats that we used to develop a T2D model by injection of streptozotocin (STZ) and a high-fat diet (HFD).

Materials and methods

Forty male Wistar albino rats (180–200 g body weight, 7 to 10 weeks old) obtained from Çukurova University Health Sciences Experimental Application and Research Center were used in this study. The rats were under conditions of 12:12 h of light–dark cycle, 40–60% humidity, and 21 ± 2 °C temperature with free access to standard rat chow/high-fat rat chow and water ad libitum.

Experimental design

After a week of adaptation, all the animals were randomly divided into four groups of 10 animals each: the normal diet (standard chow-control) group, the HF chow diet (HFD), the STZ + normal diet group (control + STZ), the STZ + HFD chow diet group (HFD + STZ) (Hazman 2014 Adjusted Calories Diet) [15]. Fifty percent of the total calories of the high-fat chow and 4% of the total calories of the standard chow came from fat. The HF chow diet was manually prepared every week to contain 50% tallow, 40% standard chow powder, 5% poultry, and 5% fat soybean and stored at − 20 °C. The rats were given fresh chow daily after defrosting chow. From the start of the experiments, the weight changes of all rats were recorded weekly. At the end of the sixth week, an insulin tolerance test (ITT) was conducted. Fasting blood glucose levels were measured in overnight fasting animals at the end of the eighth week of the study. In the HFD and STZ + normal diet groups, to induce T2D and type 1 diabetes, the rats were given STZ (dissolved in citrate buffer, pH 4.5) via tail vein injection as a single dose of 45 mg/kg BW; 0.1 molar citrate was also given to the control group via tail vein injection. A previous study used this dosage of STZ to induce T2D in rats [16]. The plasma glucose levels were checked with an automated glucose analyzer device (Accu-Check Go, Bayer) 48 h after STZ injection. Animals with a blood glucose level above 300 mg/dl were considered diabetic. The blood glucose changes of all rats were recorded throughout 12 weeks. At the end of 20 weeks, all animals were sacrificed after an overnight fast (16 h) under general anesthesia through intraperitoneal injection of a 65 mg/kg ketamine and 7 mg/kg xylazine mixture.

Insulin tolerance test

To determine the peripheral insulin sensitivity, the rats were fasted for 2–3 h to normalize their plasma glucose levels. The rats were injected intraperitoneally at a dose of 0.5 U/kg body weight insulin (Humalog/Novo Rapid). Thereafter, their blood glucose levels were measured and recorded at 0, 30, 60, and 90 min.

Determination of the serum insulin level

At the end of the study, 3–5 ml blood samples taken from the heart were centrifuged at 3000×*g* at + 4 °C, and the serum sample was separated. Then, serum insulin levels from the blood serum samples were measured with a rat insulin ELISA kit (Cloud Clone).

Histopathological examination

The harvested rat hippocampus tissue was fixed in 10% formaldehyde for routine pathological examination. The hippocampus tissues were dehydrated using ethanol (70%, 90%, 100%) and then cleaned in xylene and embedded in wax. Sagittal sections were cut with a microtome at 4–5-mm thicknesses on to adhesive glasses and stained with hematoxylin and eosin, as well as cresyl violet.

Quantitative real-time PCR

At the end of the experiment, the hippocampus tissue samples were separated for RNA isolation and stored at − 80 °C until use. For total RNA extraction, 10 mg hippocampus tissue of

the rat was used. RNA extraction was made using TRIzol reagent (Life Technologies) according to the manufacturer's instruction, and then, cDNA was synthetized from 1 μg of the total RNA (using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit). A quantitative PCR was performed using the TaqMan gene expression analysis kit (Thermo Fisher) containing a FAM stained probe designed for the expression analysis of the ADAMTS4, TIMP3, RELN, and BCAN genes. Gene expression was quantified in triplicate for each reaction, and GAPDH was used as the housekeeping gene.

Elevated plus maze (EPM) experiments

The learning acquisition was evaluated in the 20th week of the study protocol using elevated plus maze as explained by Pentkovski et al [17]. EPM was conducted in a relatively dark, quiet room, and each animal was placed slowly in the center of the device facing the open arm. The time before an animal enters and time spent in each arm were recorded throughout 5 min.

Statistical analyses

IBM SPSS Statistics version 20.0 was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements were summarized as mean and standard deviation (median and minimum–maximum, as necessary). Bonferroni or Tamhane tests were used in the pairwise comparisons of the groups, depending on whether the variances within the group were homogeneous. The statistical significance level was set as 0.05 in all tests. For the elevated plus maze experiment results, to evaluate the change in the measurements obtained in the time interval, Friedman test was applied. The statistical level of significance for all tests was considered to be 0.05. Serum insulin levels were evaluated as descriptive. Real-time PCR results, fold change, and p values were evaluated at datanalysis.qiagen.com.

Results

Weight and blood glucose level changes in the rats

Body weight levels throughout 20 weeks and the blood glucose levels of the control, HFD, STZ + normal diet, and HFD + STZ rats throughout 16 weeks were measured and recorded. Figure 1 shows the differences in average body weight and blood glucose level data of the rats during a 4-month period.

At the end of 20 weeks, the average body weight of the rats was 314 ± 23.53 in the HFD + STZ group, 398 ± 10.34 in the HFD group, 275 ± 7.36 in the STZ group, and 294 ± 6.18 in the control group (Fig. 1b, c). While the average weights of the experimental groups identified from randomly selected rats during the first week of the study were not statistically significant ($p > 0.05$), a statistically significant difference was found between the groups from the fourth week of the study until the 20th week ($p < 0.05$). As of the second week of the experiment, a significant difference was observed in the average weights of the HFD and HFD + STZ groups fed with a HFD compared with the STZ and control groups fed with a normal diet.

In the eighth week of the study, the blood sugar levels of the rats were determined using blood taken from their tail vein; the rats were fasted overnight after a single dose of STZ injection. At the end of 20 weeks, the average blood glucose level was 387 ± 46.39 in the HFD + STZ group, 137 ± 4.61 in the HFD group, 369 ± 41.89 in the STZ group, and 87 ± 5.07 in the control group (Fig. 1a). While the mean blood glucose levels of the experimental groups were not statistically significant during the first week of the study ($p >0.05$), a statistically significant difference was found between the groups from the eighth week of the study until the 20th week ($p <0.05$). The fasting blood glucose level in the HFD + STZ and STZ groups increased with STZ injection performed at the end of the eighth week. Whereas the blood glucose level of the control group rats is stable throughout the study, that of the HFD group rats that were given an HFD tended to increase (Fig. 1a).

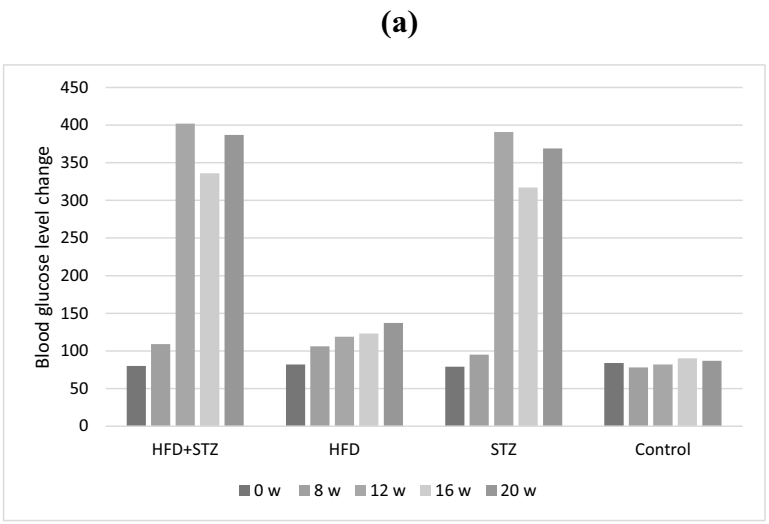
Serum insulin level and insulin tolerance test

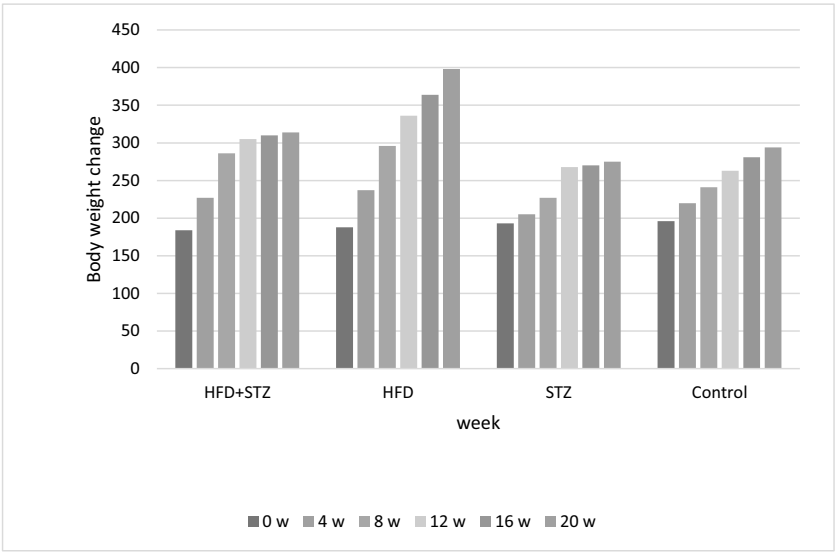
The STZ and control group serum insulin levels were found to be lower than those of the HFD and HFD + STZ groups fed with HFD (Fig. 2).

The ITT was conducted in all groups at the end of the sixth week. Blood glucose values of 100 mg/dl were considered acceptable before insulin application using the reference set by Hazman et al. [18]. The half-life of insulin is 226–314 s in rats. For this reason, it has been reported that, after the first decrease in blood sugar, differences in glucose concentration continue until 30 min, as gluconeogenesis and glycogenolysis are activated [16, 17, 19]. In the HFD and HFD + STZ groups fed with HFD, the drop in blood sugar is less than 50% in the first 30 min. Therefore, it is assumed that insulin resistance occurs in these two groups. The decrease in blood sugar is more than 50% in the control and STZ groups. All groups are statistically significant in terms of all minutes taken ($p < 0.05$) Table 1.

Learning in the plus maze test

In HFD + STZ, STZ, and control groups, there was no significant difference between the time before animals enter from the open arm to the enclosed arm ($p > 0.05$). While, in the





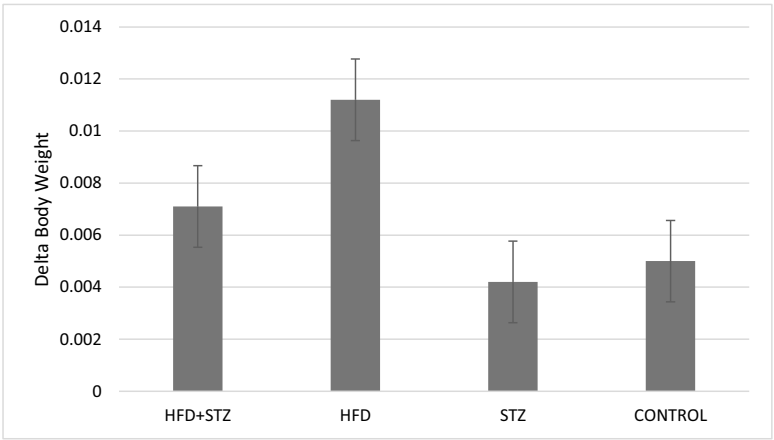


Fig. 1 **a** Changes in blood glucose level of the rats. Data are means ± SD (n = 10). Significant at $p < 0.05$, from the eighth week of the study until the 20th week, compared with control. **b** Changes in body weights of the rats. Significant at $p < 0.05$, from the fourth week of the study until the 20th week, compared with control. **c** Delta body weights. The increase in bodyweight was calculated. HFD, high-fat diet; STZ, streptozotocin (45 mg/kg); w, week

Table 1 Changes of glucose induced by insulin injection

Groups		Time			
		0′	30′ $\overline{X} \pm$ SS	60′ $\overline{X} \pm$ SS	90′ $\overline{X} \pm$ SS
HFD + STZ	Blood glucose levels	100	86±3.23	52±6.28	48±5.29
HFD		100	88±4.52	74±4.73	70±3.52
STZ		100	44±3.36	40±1.49	37±1.56
Control		100	45±2.74	41.5±2.06	37±1.82

Blood glucose values of 100 mg/dl were considered acceptable before insulin application. Data are means ± SD ($n = 10$). Significant at $p < 0.05$, in terms of all minutes taken. $\overline{X}$ average, *SS* standard deviation, *HFD* high-fat diet, *STZ* streptozotocin (45 mg/kg)

HFD group, there was a significant difference between the first day and second day, and the first day and third day the time before animals enter from the open arm to the enclosed arm ($p = 0.004$, $p = 0.040$, respectively).

Histological assessment

The HFD and control group rat hippocampus tissue sections presented a compact arrangement of neurons in the stratum pyramidal in CA1 and CA3. Pyramidal cells had pale nuclei and a deep basophilic cytoplasm. Neurons and neuronal processes, as well as astrocytes in the stratum molecular, were normal.

The HFD + STZ group showed numerous shrunk pyramidal cells with dark nuclei, clumping of neuronal processes and wide capillaries, a shrunk dark granular cell layer, and a preserved molecular layer with many astrocytes. Some plaque-like staining areas were also seen in the stratum molecular layer. These histological findings are similar to those in the STZ group (Fig. 3 and Fig. 4).

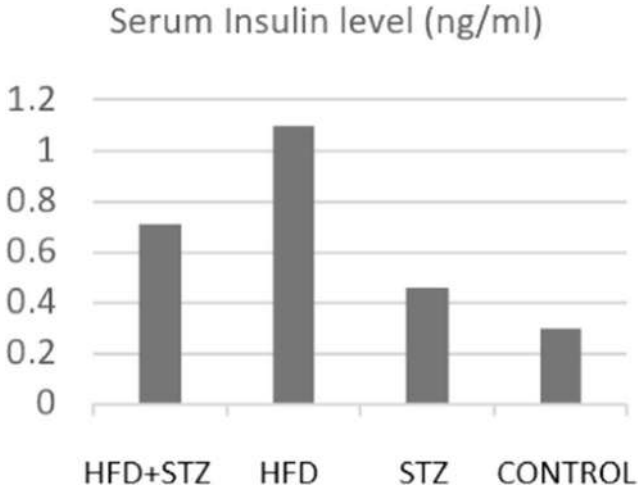


Fig. 2 Serum insulin level (ng/ml) at the end of 20th week. HFD and HFD + STZ groups fed with an HFD were higher versus control and STZ groups fed with normal diet. HFD, high-fat diet; STZ, streptozotocin (45 mg/kg)

Molecular assessment

In our study, the expression levels of the Alzheimer disease–associated ADAMTS4, TIMP3, RELN, and BCAN genes from the brain hippocampus tissue of all rats were examined by the real-time PCR method (Table 2). The findings were analyzed with the $2^{-\Delta\Delta Ct}$ formula.

The expression levels of the ADAMTS4, TIMP3 (inhibitor of ADAMTS4), RELN, and BCAN genes were observed to decrease in the HFD + STZ group, but this was not statistically significant ($p > 0.05$). While the expression of the TIMP3 gene decreased in the HFD group, the expression of the ADAMTS4, RELN, and BCAN genes increased. The increase in the expression of the RELN and BCAN genes was statistically significant ($p < 0.05$). In all genes, a relative decrease in expression levels in the STZ group compared with the control group was observed, but this was not statistically significant ($p > 0.05$).

Discussion

T2D and Alzheimer's disease are common diseases worldwide. Numerous studies have focused on the prevention and treatment of both diseases, and some studies are ongoing. In this line of research, the common molecular mechanisms involved in the degenerative processes in both diseases have been identified [20–23]. Dementia occurs with cerebral insulin resistance accompanying peripheral insulin resistance in T2D. Insulin plays a role in the occurrence of Alzheimer's disease, which affects the metabolism of Aβ, and the tau protein is the most significant link that determines the relation between the two diseases [24].

The lecticas found in the structure of perineuronal networks with reticular formation surrounding the surface of neurons include CNS-specific brevican, neurocan, aggrecan (abundant

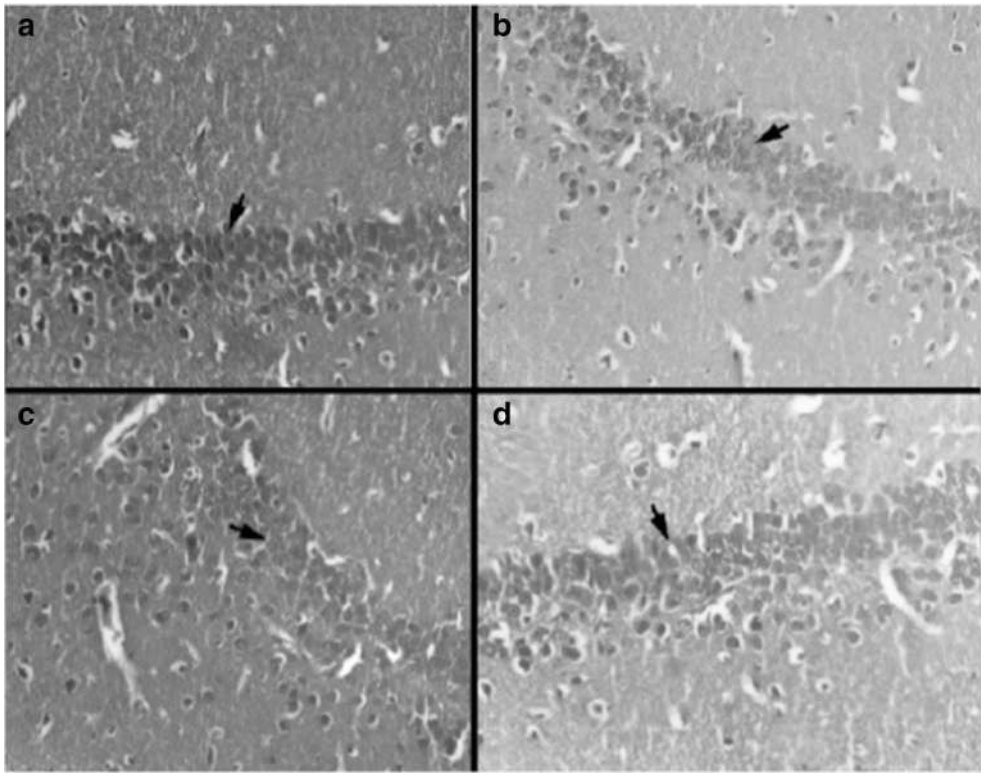


Fig. 3 Hippocampus tissue from HFD and control animal rats. **a** and **c** were stained with hematoxylin and eosin, whereas **b** and **d** were stained with cresyl violet. **a** and **b** were from the HFD group, whereas **c** and **d** were from the control. Normal pyramidal (arrows) cells appear. Magnification: × 200

in cartilage), and versican (located near all tissues containing the basement membrane and blood vessels). During embryonic development, ECM directly affects neuronal and glial migration and differentiation, neurogenesis, and axonal growth, and it guides synaptogenesis [13, 25]. Age-related changes in ECM composition and volume are also one of the most important parameters that play a role in the onset and development of Alzheimer's disease [14]. ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) regulates changes in the amount of matrix proteins in the central nervous systems (CNS), as well as neuronal activity [12]. The interaction of TIMP3 with ECM chondroitin sulfate proteoglycans (such as aggrecan) also enables the inhibition of extracellular ADAMTS enzymes [24]. In one study, the ADAMTS4 mRNA level was found to be high in a rat astrocyte culture given Aβ [26]. The enzymes encoded by the ADAMTS genes contribute to the neurodegenerative process if a disorder occurs while facilitating the normal physiological functions of brain cells [27].

Lemerchant et al. in their study with amyotrophic lateral sclerosis (ALS) model mice when compared to wild type mice, it was determined that ADAMTS4 mRNA level was associated with motor neuron loss and degeneration in the last stage of the disease. When the expression levels of ADAMTS 1, 4, 5, and 9 proteoglycans were evaluated in wild-type mice, in the spinal cord and brain cortex, the expression levels of ADAMTS4 of wild-type mice were found to be eight times higher than those of the others. In the same study, the expression level of the ADAMTS4 inhibitor TIMP3 was found to be increased in the ALS model male mice [28]. In our study, the ADAMTS4 expression decreased, but it was high in the obese group we fed with HFD, which is not compatible with the work done by Lemarchant et al. The TIMP3 expression was lower in all three groups compared with the control group and that in the study of Lemarchant et al. Our finding is not compatible with the work done by Lemarchant et al.

In terms of immunohistochemistry results, in the study of Pehlivan et al., ADAMTS4 and ADAMTS5 expressions were observed to be suppressed in the autopsy samples taken from the brains of patients with Alzheimer's disease compared with a healthy group. According to this study, ECM degradation is insufficient in patients with AD; as a result, there is excessive accumulation of ECM components [29]. The data of Pehlivan et al. in human brain tissue are consistent with those of our study, and ADAMTS4 mRNA expression was found to be decreased compared with that of the control group.

In a study by Gibb et al., a decrease in TIMP3 level in post-traumatic brain injury (TBI) was observed in the hippocampus of the mouse model with TBI. In this study, the findings indicate that TIMP3 has a neuro-cognitive impairment effect in post-TBI, and it has a function in neuronal protection [30]. In our study, the low level of TIMP3 in the hippocampus of rats that we used to develop the T2D and obesity model suggests that there may be a neuro-cognitive disorder in rats based on the study of Gibb et al.

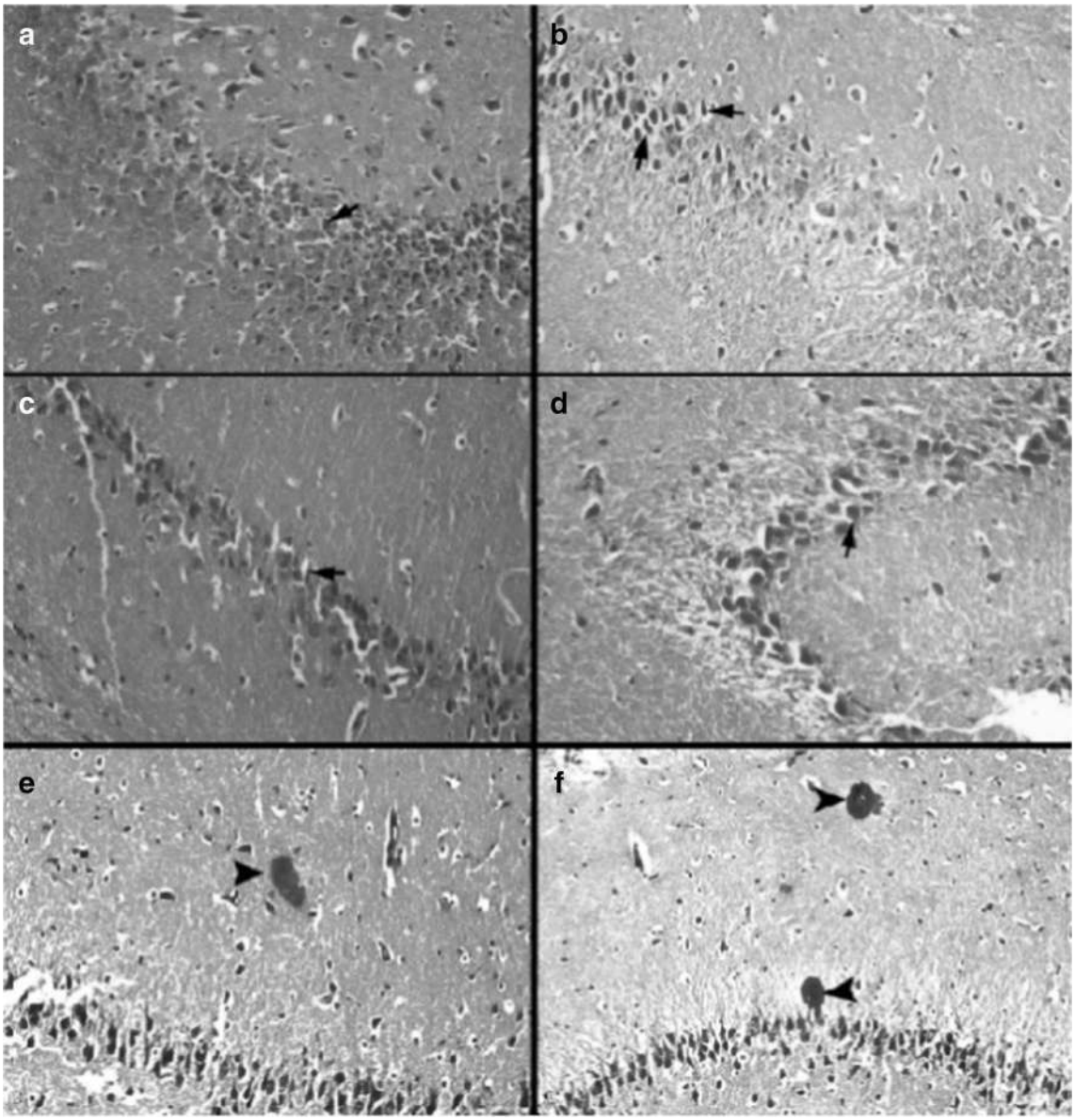


Fig. 4 Hippocampus tissues from HFD + STZ and STZ animal rats. **a** HFD + STZ animal hippocampus; the arrow shows the shrunk pyramidal cell. Hematoxylin and eosin; magnification: × 200. **b** HFD + STZ; cresyl violet staining. Shrunk pyramidal cells are increasing (arrow); magnification: × 200. **c** STZ animal hippocampus; the arrow shows a shrunk pyramidal cell. Hematoxylin and eosin; magnification: × 200. **d** STZ; cresyl violet staining. Shrunk pyramidal cells are increasing (arrow); magnification: × 200. **e** and **f** show plaque-like hematoxylin and cresyl violet positive areas (arrow heads) in the molecular layer

Reelin is an extracellular glycoprotein secreted in the CNS and has important role in neuronal development. Studies have shown that the expression of reelin in the entorhinal cortex and the protein level of reelin in the brain are important in the initial process of Alzheimer's disease. In addition, it has been shown that there is an increase in the mRNA level of RELN in the frontal cortex in the final stages of Alzheimer's disease. These findings indicate that the reelin may contribute to the disease process or may be a potential balancing molecule in such a process [31].

With the presence of ADAMTS4, a protease stimulated with inflammation that cuts the reelin from both the C and N terminals, an acceleration of the aggregation of the reelin in immune defense has been demonstrated in wild-type mice. Endogenous ADAMTS4 and ADAMTS5 inhibitors TIMP1 and TIMP3, which are expressed with reelin, have been shown to block the proteolytic segment of reelin [32].

Some genetic and biochemical studies show reel-mediated signaling changes in Alzheimer's disease. In one of these studies, a decrease in reelin expression was suggested to contribute to the onset and development of Alzheimer's disease by disrupting synaptic function, cytoskeleton stability, and axonal transport [33]. In our study, the mRNA level of reelin was found to be decreased in the T2D model rats and obtained

Table 2 Real-time PCR data (mRNA levels) of ADAMTS4, TIMP3, RELN, and BCAN

	HFD+STZ		HFD		STZ	
Gene	$2^{-\Delta\Delta Ct}$	*p*	$2^{-\Delta\Delta Ct}$	*p*	$2^{-\Delta\Delta Ct}$	*p*
ADAMTS4	0.646	0.07	1.279	0.10	0.963	0.92
TIMP3	0.783	0.51	0.738	0.43	0.857	0.15
RELN	0.724	0.83	1.398	0.0007	0.899	0.43
BCAN	0.722	0.18	1.426	0.02	0.987	0.93

The *p* values were significant for two genes (RELN, $p = 0.007$; BCAN, $p = 0.02$) in the HFD versus control ($p < 0.05$). GAPDH was used as the housekeeping gene. *HFD* high-fat diet, *STZ* streptozotocin (45mg/kg)

results consistent with Saez-Valero et al. suggest that the onset or development of Alzheimer's disease may be caused by T2D.

Brevican, as a member of lectican family of chondroitin sulfate proteoglycans, is involved in brain ECM formation [34]. In the study by Valenzuela et al., the brevican section plays an important role in synaptic plasticity, so it is also important for ECM homeostasis [35]. In another study, brevican was found to be associated with synapse loss in parallel with the disappearance of a brevican-based axonal crust in the structure of amyloid plaques in Alzheimer's disease [36]. Consistent with the result of such a study, in our study the level of expression of the BCAN gene encoding brevican decreased in T2D model rats, and so it is thought that brevican may accelerate the degenerative process in Alzheimer's disease.

Conclusion

Cerebral insulin resistance is thought to be accompanied by peripheral insulin resistance occurring in T2D, as well as by ADAMTS4, TIMP3, RLEN, and BCAN, which encode proteases that regulate neuronal activity by determining the changes in the amount of ECM proteins, which are thought to be associated with Alzheimer's disease in previous studies. The level of gene expression has been investigated. The mRNA level of reelin was found to be decreased in the T2D model rats, so it is thought that the onset or development of Alzheimer's disease may be caused by T2D.

In the literature, there is no clear finding about the increase or decrease of these genes, but such genes are thought to be associated with Alzheimer's disease. More studies are needed to examine the role that these genes play in the T2D and Alzheimer's disease pattern to help prevent these diseases and adopt new approaches to treatment. The negative aspect of our study is that it could not be evaluated in terms of protein. In the next studies we planned, the proteins encoded by ADAMTS4, TIMP3, RELN, and BCAN genes will be evaluated.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Lütfiye Özpak, Ayfer Pazarbasi, Işıl Öcal, M. Bertan Yılmaz, and Hülya Binokay. The first draft of the manuscript was written by Lütfiye Özpak and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by "Cukurova University Research Projects Funding Unit" with project number 2016-5883.

Declarations

Throughout the study, all interventions applied to the animals were carried out in accordance with the approval (reference number: ÇÜTF-DETAUM-09; date: 27.11.2015) of Çukurova University Animal Experiments Local Ethics Committee.

Conflict of interest The authors declare no competing interests.

References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, 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. Ley SH, Meigs JB. Epidemiology and risk factors of type 2 diabetes. Switzerland: Endocrinology; 2018.
3. Introduction. Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(1):S1–2.
4. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. Diabetes Care. 2016;39(11):2065–79.
5. Goldstein BJ, Müller-Wieland D. Type 2 diabetes. 2nd ed. USA: Informa Healthcare; 2008. p. 13–26.
6. Kyrou I, Tsigos C. Obesity in the elderly diabetic patient: ıs weight loss beneficial? No. Diabetes Care. 2009;32(2):403–9.
7. Goyal R, Jialal I. Diabetes mellitus type 2. Treasure Island: StatPearls; 2020.
8. Pulgaron ER, Delamater AM. Obesity and type 2 diabetes in children: epidemiology and treatment. Curr Diab Rep. 2014;14(8):508.
9. Ridge PG, Ebbert MT, Kauwe JS. Genetics of Alzheimer's disease. Biomed Res Int. 2013;25:254954.
10. Ciudin A. Diabetes mellitus and Alzheimer's disease: an unforgettable relation. Endocrinol Nutr. 2016;93(5):191–3.
11. Cole AR, Astell A, Green C, Sutherland C. Molecular connexions between dementia and diabetes. Neurosci Biobehav Rev. 2007;3:1046–63.
12. Miyata S, Kitagawa H. Formation and remodeling of the brain extracellular matrix in neural plasticity: roles of chondroitin sulfate and hyaluronan. Biochim Biophys Acta. 2017;1861(10):2420–34.
13. Gottschall PE, Howell MD. ADAMTS expression and function in central nervous system injury and disorders. Matrix Biol. 2015;44-46:70–6.
14. Morawski M, Filippov M, Tzinia A, Tsilibary E, Vargova L. ECM in brain aging and dementia. Prog Brain Res. 2014;214:207–27.
15. Frischknecht R, Happel MFK. Impact of the extracellular matrix on plasticity in juvenile and adult brains. e-Neuroforum. 2016;7:1–6. https://doi.org/10.1007/s13295-015-0021-z January 13,2021.
16. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des Devel Ther. 2015;9:2515–25.

17. Pentkowski NS, Litvin Y, Blanchard DC, Vasconcellos A, King LB, Blanchard RJ. Effects of acidic-astressin and ovine-CRF microinfusions into the ventral hippocampus on defensive behaviors in rats. Horm Behav. 2009;56(1):35–43.
18. Hazman Ö, Ovalı S. Investigation of the anti-ınflammatory effects of safranal on high-fat diet and multiple low-dose streptozotocin ınduced type 2 diabetes rat model. Inflammation. 2015;38(3):1012–9.
19. Okamoto T, Kanemoto N, Ohbuchi Y, Okano M, Fukui H, Sudo T. Characterization of STZ-ınduced type 2 diabetes in Zucker fatty rats. Exp Anim. 2008;57(4):335–45.
20. Sandhir R, Gupta S. Molecular and biochemical trajectories from diabetes to Alzheimer's disease: a critical appraisal. World J Diabetes. 2015;6(12):1223–42.
21. De la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes evidence reviewed. J Diabetes Sci Technol. 2008;2:1101–13.
22. Mittal K. Katare DP Shared links between type 2 diabetes mellitus and Alzheimer's disease: a review. Diabetol Metab Syndr. 2016;10(2):144–9.
23. Qin L, Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim Biophys Acta. 2017;1863(5):1037–45.
24. Biessels GJ, Kappelle LJ. Increased risk of Alzheimer's disease in type II diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? Biochem Soc Trans. 2005;33(5):1041–4.
25. Lemarchant S, Pruvost M, Montaner J, Emery E, Vivien D, Kanninen K, et al. ADAMTS proteoglycanases in the physiological and pathological central nervous system. J Neuroinflammation. 2013;10:133.
26. Satoh K, Suzuki N, Yokota H. ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs) is transcriptionally induced in beta-amyloid treated rat astrocytes. Neurosci Lett. 2000;289:177–80.
27. Gurses MS, Ural MN, Gulec MA, Akyol O, Akyol S. Pathophysiological function of ADAMTS enzymes on molecular mechanism of Alzheimer's disease. Aging Dis. 2016;7(4):479–90.
28. Lemarchant S, Pomeshchik Y, Kidin I, Kärkkäinen V, Valonen P, Lehtonen S, et al. ADAMTS-4 promotes neurodegeneration in a mouse model of amyotrophic lateral sclerosis. Mol Neurodegener. 2016;11:10.
29. Pehlivan S, Fedakar R, Eren B, Akyol S, Eren F, Turkmen Inanır N, et al. ADAMTS4, 5, 9, and 15 expressions in the autopsied brain of patients with Alzheimers disease: a preliminary immünohistochemistry study. Bull Clin Psychopharmacol. 2016;26(1):7–14.
30. Gibb SL, Zhao Y, Potter D, Hylin MJ, Bruhn R, Baimukanova G, et al. TIMP3 attenuates the loss of neural stem cells, mature neurons and neurocognitive dysfunction in traumatic brain ınjury. Stem Cells. 2015;33(12):3530–44.
31. Yu NN, Tan MS, Yu JT, Xie AM, Tan L. The role of reelin signaling in Alzheimer's disease. Mol Neurobiol. 2016;53(8):5692–700.
32. Krstic D, Rodriguez M, Knuesel I. Regulated proteolytic processing of reelin through interplay of tissue plasminogen activator (tPA), ADAMTS-4, ADAMTS-5, and their modulators. PLoS One. 2012;7(10):47793.
33. Saez-Valero J, Costell M, Sjogren M, Andreasen N, Blennow K. Luque JM Altered levels of cerebrospinal fluid reelin in frontotemporal dementia and Alzheimer's disease. J Neurosci Res. 2003;72(1):132–6.
34. Gary SC, Zerillo CA, Chiang VL, Gaw JU, Gray G, Hockfield S. cDNA cloning, chromosomal localization, and expression analysis of human BEHAB/brevican, a brain specific proteoglycan regulated during cortical development and in glioma. Gene. 2000;256(1-2):139–47.
35. Valenzuela JC, Heise C, Franken G, Singh J, Schweitzer B, Seidenbecher CI, et al. Hyaluronanbased extracellular matrix under conditions of homeostatic plasticity. Philos Trans R Soc Lond Ser B Biol Sci. 2014;369(1654):20130606.
36. Morawski M, Brückner G, Jäger C, Seeger G, Matthews RT, Arendt T. Involvement of perineuronal and perisynaptic extracellular matrix in Alzheimer's disease neuropathology. Brain Pathol. 2012;22(4):547–61.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):91–100
https://doi.org/10.1007/s13410-021-00947-3

ORIGINAL ARTICLE

Risk factors associated with gestational diabetes mellitus: a retrospective case-control study

Xie Yaping[1] · Liu Chunhong[2] · Zhao Huifen[3] · Huang Fengfeng[3] · Huang Huibin[4] · Zhao Meijing[5]

Received: 3 April 2020 / Accepted: 25 March 2021 / Published online: 7 April 2021

Abstract

Objectives The prevalence of gestational diabetes mellitus (GDM) has increased year-after-year globally, especially in low-income and developing countries. This study aims to identify the prevalence of GDM, the risk factors, and the effect on pregnancy outcome based on a retrospective case-control study.

Methods Two hundred ninety-three parturients with GDM who delivered in a general hospital in Fujian province and met the inclusion criteria were selected as the case group from January to June 2018. Two hundred ninety-three parturients without GDM who delivered in the same period served as the control group. Risk factors for GDM were determined by univariate and binary logistic regression analysis. The prevalence of pregnancy outcomes was determined by a chi-square test.

Results The prevalence of GDM was 15.69%. The percentages of 1, 2, and 3 abnormal OGTT values were 55.6%, 30.7%, and 13.7%, respectively. Gravidas with GDM have a higher risk of macrosomia, polyhydramnios, pre-eclampsia, placenta previa, and gestational hypertension than gravidas without GDM ($p < 0.05$). Analysis of the factors influencing the development of GDM was advanced age, married, parents with a history of diabetes, gestational hypertension, and number of abortions.

Conclusions The prevalence of GDM was 15.69% in this geographic region, and > 50% of the patients had one abnormal OGTT value. The risk factors for GDM were advanced age, parents with diabetes, gestational hypertension, and the number of abortions. Pregnancy outcomes of the two groups of patients were different with respect to macrosomia, polyhydramnios, pre-eclampsia, placenta previa, and hypertensive disorders of pregnancy.

Keywords Case-control study · Gestational diabetes mellitus · Pregnancy outcome · Random · Two-child policy

Précis Since China implemented the "Two-Child Policy" in 2016, a large number of women with advanced maternal age achieved second pregnancies, which may lead to an increase in the prevalence of GDM.

Quick points

(1) The prevalence of GDM was 15.69% in this geographic region, and > 50% of the patients had 1 abnormal OGTT value.

(2) The risk factors for GDM were advanced age, parents with diabetes, gestational hypertension, and the number of abortions.

(3) The pregnancy outcomes in the two groups of patients differed with respect to macrosomia, polyhydramnios, pre-eclampsia, placenta previa, and hypertensive disorders of pregnancy.

Xie Yaping is the first author. Liu Chunhong is the co-first author.

✉ Zhao Huifen
624633497@qq.com

Xie Yaping
609227027@qq.com

Liu Chunhong
41443078@qq.com

Huang Fengfeng
625351253@qq.com

Huang Huibin
huibinhuang@aliyun.com

Zhao Meijing
502475834@qq.com

Extended author information available on the last page of the article

Introduction

Gestational diabetes mellitus (GDM) is a decrease in glucose tolerance that occurs or is first detected after pregnancy. The level of blood glucose is lower than that of dominant diabetes [1], which accounts for 80% ~ 90% of gestational hyperglycemia [2]. GDM can cause maternal and infant complications, such as pre-eclampsia, premature rupture of membranes, and premature delivery, and increase the risk of long-term endocrine disorders [3, 4]. Gravidas with GDM have a 70% probability of developing diabetes within 28 years after delivery [5]. The prevalence of GDM is increasing year-after-year globally, especially in low-income and developing countries [6–8]. International studies have shown that the risk factors for the occurrence of GDM are not the same in gravidas of different races and residing in different geographic regions [9–11]. It is also known that the prevalence and risk factors for GDM are not uniform in different geographic regions of China, and the resulting pregnancy outcomes may also be different [8]. China implemented the "Two-Child Policy" in 2016. A large number of women with advanced maternal age achieved second pregnancies. Lifestyle and dietary imbalance may lead to an increase in the prevalence of GDM. Fujian province has one of the highest GDP rankings in China with a population of 40 million; however, pregnant women in Fujian province with GDM still have a poor sense of how to manage their blood glucose and a deep-rooted belief in eating more and not exercising during pregnancy. Understanding the prevalence and risk factors in the region is conducive to the development of targeted interventions to mitigate the adverse consequences of GDM. This study may determine the prevalence, risk factors, and pregnancy outcomes of parturients with GDM in this geographic region, which is of great significance for researchers to design effective intervention measures further, so as to carry out early diet, exercise, and other interventions for parturients with GDM to improve adverse pregnancy outcomes.

Participants

Participants

This study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University in 2019(NO.54). In this study, the convenience sampling method was adopted that maternal inpatients in a general hospital in Fujian province were selected as the study subjects from January to June 2018. The inclusion criteria of the case group were as follows: (1) met the diagnostic criteria for GDM according to the Chinese guidelines for the prevention and treatment of type 2 diabetes [2], (2) single live birth, and (3) no severe pregnancy complications. The exclusion criteria of the case group were as follows: (1) pre-pregnancy diagnosis of diabetes, gestational-dominant diabetes; (2) endocrine diseases, such as hypothyroidism and Cushing's syndrome; (3) heart, liver, kidney, and other chronic diseases, benign tumors, and multiple pregnancies; and (4) incomplete information. Two hundred ninety-three parturients with GDM were included as the case group, and 293 parturients without GDM hospitalized in the same period were randomly selected as the control group. Inclusion criteria (2 and 3) were the same as the case group, and the exclusion criteria (2–4) were the same as the case group.

Sample

The sample size was based on a maximum likelihood estimation. Thus, the sample size should be > 10 times the number of variables to obtain robust regression analysis results [12]. A total of 17 influencing factors were included in this study, so the minimum sample size for each group was 170 ($17 \times 10 = 170$) and the minimum sample size for two groups was 340. A total of 586 patients were included eventually.

A total of 2666 cases were obtained through the electronic medical record system (EMRS), and 293 cases were included in the case group. A total of 1525 patients in the control group met the standard and were coded according to the sequence of admission numbers and input into an Excel worksheet. The RAND function was used to generate random numbers, with a small-to-large order. The first 293 patients were selected as the control group. A total of 586 patients were included in the two groups (Fig. 1).

Materials and Methods

Instruments

Maternal information questionnaires were used to collect data, including general and disease data, as follows: (1) a self-designed basic information questionnaire was used, including age, marital status, educational level, height before delivery, weight, cigarette smoking, alcohol consumption, family history of diabetes, family history of hypertension, menstrual cycle characteristics, parity, live births and abortions, cesarean section history, and blood pressure (general data); (2) a relevant research group was established to review the literature and group members brainstormed to form the final 21

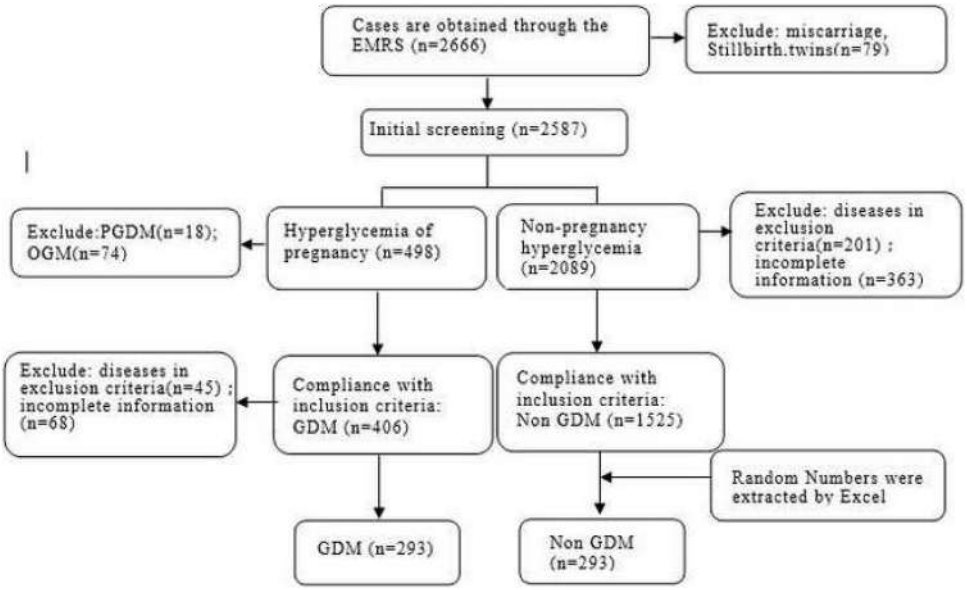


Fig. 1 Flow chart of case screening

indicators, including ① neonatal weight, body length, gestational age, preterm birth history, fetal macrosomia, low birth weight, neonatal hypoglycemia, fetal distress, and admission to the NICU; ② oligohydramnios, polyhydramnios, anemia, pre-eclampsia, placental abruption, premature rupture of membranes, placenta previa, hypertensive disorders of pregnancy (HDP), perineal lateral incision, and delivery mode; and ③ the results of the first glucose tolerance test (OGTT) and outliers it contains, and the glycosylated hemoglobin (HbA1c) values examined in the hospital before delivery of the case group (disease data).

Diagnosis of GDM and blood testing

Diabetes can be classified into a single gene diabetes syndrome, post-transplantation diabetes mellitus, cystic fibrosis-related diabetes, prediabetes and type 2 diabetes, type 1 diabetes, and GDM [13]. Gestational hyperglycemia can be divided into pre-pregnancy diabetes mellitus (PGDM), overt gestational diabetes mellitus (OGM), and GDM. GDM accounts for 80% ~ 90% of gravidas with gestational hyperglycemia [2]. In this study, pregnant women with previously undiagnosed diabetes were diagnosed with gestational diabetes using a 75-g glucose tolerance test (OGTT) at 24–28 weeks of gestation. OGTT was performed the morning after a $\geq$ 8-h overnight fast. GDM was diagnosed when any of the following plasma glucose parameters were reached or exceeded: fasting plasma glucose (FPG) $\geq$ 5.1 mmol/L but < 7.0 mmol/L, 1-h plasma glucose (1-h PG) $\geq$ 10.0 mmol/L, and 2-h plasma glucose (2-h PG) $\geq$ 8.5 mmol/L but < 11.1 mmol/L [1]. After venous blood was obtained from the patient, HbA1c was measured in a laboratory with U.S. Hemoglobin A1c Standardization Program (NGSP) certification and diabetes control and complication test (DCCT) analysis standardized methods. The NGSP uses ion-exchange high-performance liquid chromatography (HPLC) as a reference method and is currently the designated comparison method for HbA1c determination [14].

Data collection

The researcher and two members of the research team created the medical questions regarding the subjects' past status, retrospectively. They consulted the EMRS in the medical record room to collect the patients' general and disease data. Before collecting the information, the team members were trained and assessed, including the theoretical knowledge related to GDM, the content of the questionnaire, and the completion specifications. When collecting data, two members worked together, one to consult the electronic medical record and the other to fill in the data form. The collected data were entered into Excel by two members and checked to ensure the accuracy of the data.

Statistical analysis

SPSS 23.0 software was used for statistical analysis. Counting data are shown as frequencies (percentages), and a chi-square test was used for inter-group comparisons. For measurement data conforming to a normal distribution, $\bar{\chi} \pm s$ was used to represent the mean value and standard deviation, and *t*-tests were used for comparison between the two independent samples. Binary logistic regression analysis was used for multivariate analysis. The statistical significance was defined as a $p < 0.05$.

Results

Prevalence of GDM

Overall prevalence of GDM

The study initially included 2587 patients, including 498 with hyperglycemia in pregnancy and 406 with GDM. GDM accounted for 81.53% of gravidas with hyperglycemia, and the prevalence of GDM was 15.69%.

OGTT of GDM

According to the OGTT results of the case group, 163 patients (55.6%) had one abnormal value, 90 patients (30.7%) had two, and 40 patients (13.7%) had three. Patients with one abnormal value included 56 patients (34.4%) with abnormal FPG, 67 patients (41.1%) with abnormal 1-h plasma glucose, and 40 patients (24.5%) with abnormal 2-h plasma glucose. Fifty-two patients (57.8%) had abnormal FPG and 2-h plasma glucose; 17 patients (18.9%) had abnormal FPG and 1-h plasma glucose, and 21 patients (23.3%) had abnormal 1-h and 2-h plasma glucose. Patients with three abnormal values included 40 patients with an abnormal FPG, 1-h plasma glucose, and 2-h plasma glucose.

Comparison of general information between the two groups

The mean maternal age of the case group was 31.95 ± 5.01 years, while the mean maternal age of the control group was 29.97 ± 4.30 years (Table 1).

Risk factor analysis of GDM

Single-factor analysis

Univariate analysis was performed with GDM as the dependent variable and patient general data as the independent variable. The results showed that age, marital status, paternal diabetes history, maternal diabetes history, parity, the number of pre-pregnancy abortions, and blood pressure were statistically significant($p < 0.05$; Table 1).

Multi-factor analysis

Multivariate logistic regression analysis was carried out, including age, marriage, paternal diabetes history, maternal diabetes history, hypertension, the number of pregnancies before the pregnancy, and the number of abortions before the pregnancy. Advanced age, married, paternal diabetes history, maternal diabetes history, HDP, and number of abortions were independent risk factors for GDM. The results are shown in Table 2, according to the size of the standard regression coefficient in order as follows: history of $\geq$3 miscarriages, maternal diabetes history, paternal diabetes history, married, a history of two abortions, HDP, a history of one abortion, and age.

Comparison of disease data between the two groups (Table 3)

Discussion

Prevalence of GDM

Prevalence

The prevalence of GDM in this study was 15.69%. A meta-analysis involving the prevalence of GDM in China showed that the prevalence in different geographic regions and hospitals of China varied greatly, ranging from 11.45–23.19% [8]. The prevalence of GDM in the current study was less than the 23.19% prevalence in Beijing [8], which may be related to the sample size, different attributes of research institutions, and patients' medical habits. There are many general hospitals in Beijing, and high-risk pregnant patients often choose general hospitals for medical treatment. The results of this study are quite consistent with the results of the study on the prevalence of GDM in Fuzhou [8] (14.42%), which may be due to the fact that this region is close to the above area, and the dietary habits, living standards, and lifestyles are similar.

Abnormal blood glucose distribution

Studies have shown that maternal blood glucose levels are associated with the accompanying diabetes status and the prevalence of perinatal complications [15]. In this study, the percentage of abnormal OGTT values in 293 GDM patients was similar to the results of Ding et al. [16]. Saldana et al. [17] reported that as the abnormal OGTT values increased, the risk of complications among gravidas with GDM increased. Moreover, the prevalence of fetal macrosomia with three abnormal OGTT values was the highest [17], which may be due to the fact that two or three outliers may destroy glucose balance and insulin sensitivity more than one outlier, and the higher the number of outliers, the higher the prevalence of adverse pregnancy outcomes [18]. Therefore, abnormal OGTT values have a predictive value for pregnancy outcomes. It has been shown that OGTT fasting hyperglycemia is significantly correlated with macrosomia (OR = 1.84, 95% CI: 1.39 ~ 2.42, $p < 0.001$), and the correlation is stronger with the increase in FPG [18]. Therefore, stratified management for pregnant women with GDM is recommended, especially for

Table 1 General information and univariate analysis results of the two groups (n (%))

Item	Control group (n = 293)	Case group (n = 293)	χ^2	p
Age				
<35	258 (88.05)	224 (76.45)	13.514[1)]	<0.001
≥35	35 (11.95)	69 (23.55)		
Educational level				
Junior high school and below	85 (29.01)	98 (33.44)	4.159[1)]	0.245
Senior high school	40 (13.65)	43 (14.68)		
Junior college	60 (20.48)	67 (22.87)		
Bachelor or above	108 (36.86)	85 (29.01)		
Marital status				
Unmarried	14 (4.78)	4 (1.37)	5.941[2)]	0.031
Married	279 (95.22)	289 (98.63)		
Pre-pregnancy BMI				
<18.5	6 (2.1)	6 (2.1)	6.795	0.079
18.8~23.9	71 (24.2)	53 (18.1)		
24.0~27.9	126 (43.0)	116 (39.5)		
≥28.0	90 (30.7)	118 (40.3)		
Paternal history of diabetes				
None	289 (98.6)	275 (93.9)	7.981[2)]	0.005
Yes	4 (1.4)	18 (6.1)		
Maternal history of diabetes				
None	290 (98.98)	279 (95.22)	6.058[2)]	0.014
Yes	3 (1.02)	14 (4.78)		
Paternal history of hypertension				
None	289 (98.63)	287 (97.95)	0.102[2)]	0.750
Yes	4 (1.37)	6 (2.05)		
Maternal history of hypertension				
None	291 (99.32)	288 (98.29)	0.578[2)]	0.447
Yes	2 (0.68)	5 (1.71)		
Menstrual cycle (day)				
21~35	270 (92.15)	259 (88.40)	2.352[1)]	0.125
≥35	23 (7.85)	34 (11.60)		
The number of pregnancies before pregnancy				
0	94 (32.08)	75 (25.60)	18.683[1)]	<0.001
1	122 (41.64)	92 (31.40)		
2	35 (11.95)	64 (21.84)		
≥3	42 (14.33)	62 (21.16)		
The number of live births before pregnancy				
0	118 (40.28)	107 (36.52)	0.910[1)]	0.634
1	157 (53.58)	168 (57.34)		
2	18 (6.14)	18 (6.14)		
The number of abortion before pregnancy				
0	201 (68.60)	148 (50.51)	21.267[1)]	<0.001
1	56 (19.11)	83 (28.33)		
2	27 (9.22)	40 (13.65)		
≥3	9 (3.07)	22 (7.51)		
Cesarean				
None	198 (67.58)	201 (68.60)	0.071[1)]	0.790
Yes	95 (32.42)	92 (31.40)		

Table 1 (continued)

Item	Control group (n = 293)	Case group (n = 293)	χ^2	p
BP (mmHg)				
<90/60	15 (5.12)	8 (2.73)	16.831[1)]	<0.001
90~140/60~90	260 (88.74)	237 (80.89)		
≥140/90	18 (6.14)	48 (16.38)		

Note: 1) chi-square; 2) continuous correction of chi-square

pregnant women with three abnormal OGTT values and a high FPG.

Factors influencing GDM

The results of this study showed that advanced age, married, history of diabetes in parents, HDP, and number of miscarriages were independent risk factors for GDM.

Advanced age

The results of this study showed that the risk of GDM was 2.117 times higher in mothers ≥35 years of age than mothers <35 (OR = 2.117, $p < 0.05$). This result is consistent with previous studies [6, 19] for the following reasons: ① an increase in age, glucose metabolic differences, lipid metabolic differences, and hormone level changes in females; ② an increase in age, and decline in insulin secretion; and ③ insulin antagonists increased in elderly pregnant women [19]. China implemented the "Universal Two-child Policy" in 2016. The proportion of elderly pregnant women has increased [20]. Therefore, the prevention of GDM in elderly pregnant women is one of the key issues Chinese medical workers are facing, suggesting that we need to focus on exploring the intervention model for pregnant women with advanced maternal age to prevent GDM.

Marriage

In this study, the risk of GDM in married women was 4.393 times higher than unmarried women (OR = 4.393, $p < 0.05$). The effect of marital status on GDM has not been reported. This finding may reflect the younger age of unmarried parturients in this study than married parturients. There was a significant difference in the married-to-unmarried ratio in this study, which may be related to sample size bias. Therefore, it is not prudent to assume that being married is a risk factor for GDM. In the future, the sample size should be further increased and stratified analysis should be adopted to exclude the influence of age and analyze the influence of marriage on GDM.

Table 2 Results of multivariate analysis

Variable	Grouping	B	SE	Wals	p	OR	95% CI of OR	
							Lower limits	Upper limits
Constant		−1.961	0.614	10.204	0.001	0.141		
Age (years)	≥35	0.750	0.253	8.779	0.003	2.117	1.289	3.477
Marriage	Marriage	1.480	0.597	6.137	0.013	4.393	1.362	14.169
Paternal history of diabetes	Yes	1.609	0.601	7.161	0.007	4.999	1.538	16.248
Maternal history of diabetes	Yes	1.627	0.674	5.823	0.016	5.088	1.357	19.071
HDP	Yes	1.28	0.309	17.218	<0.001	3.598	1.965	6.588
The number of pregnancies before pregnancy	0	-	-	5.431	0.143	1		
	1	−0.259	0.232	1.242	0.265	0.772	0.490	1.217
	2	−0.08	0.419	0.036	0.849	0.923	0.406	2.099
	≥3	−1.033	0.577	3.209	0.073	0.356	0.115	1.102
The number of abortion before pregnancy	0	-	-	9.729	0.021	1		
	1	0.841	0.333	6.391	0.011	2.318	1.208	4.448
	2	1.471	0.562	6.849	0.009	4.356	1.447	13.112
	≥3	1.853	0.683	7.355	0.007	6.382	1.672	24.362

Table 3 Comparison of pregnancy outcome between the two groups (*n* (%))

Item	Control group (*n* = 293)	Case group (*n* = 293)	χ^2/T	*p*
Neonatal gender				
Male	170 (58.02)	162 (55.29)	0.445[2)]	0.505
Female	123 (41.98)	131 (44.71)		
Neonatal weight (g)	3085.05 ± 506.94	3103.99 ± 560.84	0.429[1)]	0.668
Body length (cm)	49.45 ± 2.34	49.39 ± 2.81	−0.287[1)]	0.774
Gestational age (day)	268.66 ± 14.30	267.68 ± 13.99	−0.835[1)]	0.404
Premature birth				
None	258 (88.05)	250 (85.32)	0.946[2)]	0.331
Yes	35 (11.95)	43 (14.68)		
Macromacrosia				
None	289 (98.63)	281 (95.90)	4.112[3)]	0.043
Yes	4 (1.37)	12 (4.10)		
Low birth weight				
None	261 (89.08)	260 (88.74)	0.017[2)]	0.895
Yes	32 (10.92)	33 (11.26)		
Fetal distress				
None	264 (90.10)	258 (88.05)	0.631[2)]	0.427
Yes	29 (9.90)	35 (11.95)		
Neonatal hypoglycemia				
None	289 (98.63)	286 (97.61)	0.834[3)]	0.543
Yes	4 (1.37)	7 (2.39)		
Admission to the NICU				
None	250 (85.32)	259 (88.40)	1.211[2)]	0.271
Yes	43 (14.68)	34 (11.60)		
Polyhydramnios				
None	285 (97.27)	273 (93.17)	5.401[2)]	0.020
Yes	8 (2.73)	20 (6.83)		
Oligoamnios				
None	267 (91.13)	272 (92.83)	0.578[2)]	0.447
Yes	26 (8.87)	21 (7.17)		
Anemia				
None	192 (65.53)	197 (67.24)	0.191[2)]	0.662
Yes	101 (34.47)	96 (32.76)		
Preeclampsia				
None	286 (97.61)	275 (93.86)	5.056[2)]	0.025
Yes	7 (2.39)	18 (6.14)		
Placental abruption				
None	290 (98.98)	288 (98.29)	0.127[3)]	0.722
Yes	3 (1.02)	5 (1.71)		
Premature rupture of membranes				
None	236 (80.55)	243 (82.94)	0.560[2)]	0.454
Yes	57 (19.45)	50 (17.06)		
Placenta previa				
None	271 (92.49)	282 (96.25)	3.885[2)]	0.049
Yes	22 (7.51)	11 (3.75)		
Cesarean				
None	135 (46.08)	116 (39.59)	2.516[2)]	0.113
Yes	158 (53.92)	177 (60.41)		

Table 3 (continued)

Item	Control group (n = 293)	Case group (n = 293)	χ^2/T	p
Perineal lateral incision				
None	264 (90.10)	274 (93.52)	2.269[2)]	0.132
Yes	29 (9.90)	19 (6.48)		
HDP				
None	285 (97.27)	262 (89.42)	14.531[2)]	<0.001
Yes	8 (2.73)	31 (10.58)		

Note: 1) t value; 2) chi-square; 3) continuous correction of chi-square

History of diabetes in parents

The results of this study showed that patients whose fathers had a history of diabetes had a 4.999 times higher risk of developing GDM than those whose fathers did not have diabetes (OR = 4.999, $p < 0.05$). Patients whose mothers had a history of diabetes had a 5.088 times greater risk of developing GDM than those whose mothers did not have diabetes(OR = 5.088, $p < 0.05$), indicating that a history of diabetes in either parent is an independent risk factor for GDM. This finding was consistent with the view that a family history of diabetes is a risk factor for GDM [21] and may be related to the genetic susceptibility of pregnant women with a family history of diabetes, which may induce GDM after pregnancy. The study found that if both parents were diabetic, the prevalence of GDM in the offspring increased [22]. Other studies have shown that a maternal diabetes history has a greater impact on the prevalence of GDM than paternal diabetes, which may be associated with abnormal glucose metabolism in some mothers during pregnancy, leading to fetal dysplasia in utero and further development of GDM [23]. For women of childbearing age who have a history of diabetes in their parents, they should pay more attention to their own blood glucose levels, then prevent, detect, and treat hyperglycemia early.

Hypertension during pregnancy

The study results showed that gravidas with HDP had a 3.598 times higher risk of developing GDM than gravidas without HDP (OR = 3.598, $p < 0.05$). In agreement with the findings of Abdalrahman [24], the prevalence of GDM in pregnant women with HDP was 2.6 times than gravidas without HDP (OR = 2.6, 95% CI: 1.1~6.2, $p = 0.03$) for the following reasons: ① insulin resistance, long-term high glucose levels, promotion of fat transformation, feedback imbalance of fat-insulin secretion axis, and change in fat metabolism in pregnant women with GDM; and ② extensive vascular lesions caused by GDM, then vascular endothelial thickening, narrowed lumen, increased vascular resistance, and elevated blood pressure [25]. Therefore, it is necessary to pay attention to blood pressure and blood lipids, as well as blood glucose, in the population with GDM.

Number of miscarriages

The results of this study showed that the risk of GDM was 2.318 times higher in women who had one previous miscarriage than women who did not have a history of miscarriages (OR = 2.318, $p < 0.05$). Women who had two miscarriages had 4.356 times increased risk of GDM (OR = 4.356, $p<0.05$). Women who had three or more miscarriages had 6.382 times increased risk of developing GDM (OR = 6.382, $p < 0.05$). The higher the number of miscarriages, the greater the risk of GDM, which may be related to an increase in the number of pregnancies, the greater the possibility of weight retention-type obesity in pregnant women [26], which may be related to the education level of the pregnant women. It is thought [27] that women who have had multiple abortions are more likely to have poor living habits, a lower social status, and lower educational level and are more likely to have incomplete pre-pregnancy and prenatal health care. Therefore, this group has a greater risk of developing GDM. Attention should be paid to the prevention of GDM for women with a previous history of abortion, especially those who have had three or more abortions.

Effects of GDM on pregnancy outcomes

The results of this study showed that the prevalence of macrosomia, polyhydramnios, pre-eclampsia, placenta previa, and HDP in the case group was statistically different from the control group($p < 0.05$). This conclusion is consistent with the research conducted by Kosus et al. [28] and Kamana et al. [29]. GDM can cause short- and long-term maternal, fetal, and newborn complications [1]. Due to the high glucose status of GDM, the secretion of insulin will be stimulated by β cells, then protein synthesis increases, and the risk of macrosomia also increases [3, 4]. The prevalence of macrosomia in the two groups of this study was lower than the above studies, which

may be related to the differences in population, research institutions, and dietary habits and lifestyle in different countries. In addition, the recent effects of GDM on maternal and infants include polyhydramnios, pre-eclampsia, HDP, placenta previa, ketoacidosis, premature rupture of membranes, and placental abruption. Gravidas with GDM can reduce the risk of adverse pregnancy outcomes, at least in part, through dietary control, enhanced exercise, weight control, and other interventions [1].

Limitations

Data collection was limited by conditions and time. For example, information on pre-pregnancy BMI, changes in BMI during pregnancy, dietary intake, exercise during pregnancy, and long-term prognosis after childbirth could not be accurately obtained. This study only collected data from one hospital, the sample size was small, and there was a low positive rate for some indicators, which may have affected the stability of the research results based on the limited time and researchers.

Conclusions

In conclusion, the prevalence of GDM in this geographic region was relatively high, and the maternal and infant pregnancy outcomes of the two groups of patients were different with respect to macrosomia, polyhydramnios, pre-eclampsia, placenta previa, and HDP. Advanced age, history of diabetes in the parents, HDP, and number of abortions were independent risk factors for GDM. In view of the above risk factors, the community can strengthen education for high-risk populations, determine effective prevention strategies, construct and improve the management model of early intervention for GDM patients, achieve early detection, treatment, and intervention, strictly control blood glucose levels, and improve maternal and infant outcomes.

Availability of data and material Not applicable.

Code availability Not applicable.

Author contribution Inspiration and thinking, guidance: Zhao Huifen

Research design: Zhao Huifen, Xie Yaping, Liu Chunhong, Huang Fengfeng, Zhao Meijing, Huang Huibin

Data collection and statistic analysis: Zhao Huifen, Xie Yaping, Liu Chunhong, Huang Fengfeng, Zhao Meijing, Huang Huibin

Writing manuscript: Zhao Huifen, Xie Yaping, Liu Chunhong

Modify paper and afford guidance: Zhao Huifen, Xie Yaping

Funding This research was funded by Quanzhou science and technology plan project in China.

Declarations

Ethics approval This study was approved by the No.54 file, 2019 of ethics committee of the Second Affiliated Hospital of Fujian Medical University, China.

Consent to participate This study obtained informed consent from the subjects.

Consent for publication The article is the authors' original work. The article has not received prior publication and is not under consideration for publication elsewhere. All authors have seen and approved the manuscript being submitted.

Conflict of interest All authors report no conflict of interest.

References

1. American Diabetes Association. Management of diabetes in pregnancy: standards of medical care in diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S137–43.
2. Yang HX, Song G. Advances in the diagnosis and treatment of gestational diabetes. Chin J Front Med (Electron Ed). 2010;2(3): 29–31.
3. Cheng J. Effect of individualized medical nutrition therapy on blood glucose control efficiency and pregnancy outcome of gestational diabetes mellitus. Shandong: Qingdao University; 2013.
4. Valkama A, Koivusalo S, Lindstrom J, et al. The effect of dietary counselling on food intakes in pregnant women at risk for gestational diabetes: a secondary analysis of a randomised controlled trial radial. Eur J Clin Nutr. 2016;70(8):912–7.
5. Alonso A, Del Rey CG, Navarro A, et al. Effects of gestational diabetes mellitus on proteins implicated in insulin signaling in human placenta. GynecolEndocrinol. 2006;22(9):526–35.
6. Nava GEN, Salcedo GA, Hernández ECE, et al. Prevalence, risk factors and perinatal outcomes of gestational diabetes in Mexican adolescents when applying diagnostic criteria from three different international guidelines. Int J Diabetes Dev Ctries. 2020. https://doi.org/10.1007/s13410-020-00876-7.
7. Bao W, Tobias DK, Hu FB, et al. Pre-pregnancy potato consumption and risk of gestational diabetes mellitus: prospective cohort study. BMJ. 2016;352:h6898.
8. Narenqimuge, Li DM, Mi LX, et al. Meta-analysis of prevalence of gestational diabetes in China. Chin J Evid Based Med. 2018;18(3): 280–5.

9. Mohan MA, Chandrakumar A. Evaluation of prevalence and risk factors of gestational diabetes in a tertiary care hospital in Kerala[J]. Diabetes Metab Syndr. 2016;10(2):68–71.
10. Ganapathy A, Holla R, Darshan BB, Kumar N, Kulkarni V, Unnikrishnan B, et al. Determinants of gestational diabetes mellitus: a hospital-based case–control study in coastal South India. Int J Diabetes Dev Ctries. 2020;41:108–13. https://doi.org/10.1007/s13410-020-00844-1.
11. Han Y, Tong M, Jin L, Yu J, Meng W, Ren A, et al. Maternal age at pregnancy and risk for gestational diabetes mellitus among Chinese women with singleton pregnancies. Int J Diabetes Dev Ctries. 2020;41:114–20. https://doi.org/10.1007/s13410-020-00859-8.
12. Peduzzi P, Concato J, Kemper E, et al. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49(12):1373–9.
13. Nathan DM. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes response to Kilpatrick, Bloomgarden, and Zimmet. Diabetes Care. 2009;32(12):e160.
14. Dong H, Chen WX, Zhang CB, et al. The guideline for glycosylated hemoglobin laboratory. Chin J Diabetes. 2013;21(08):673–8.
15. Jain R, Davey S, Davey A, Raghav SK, Singh JV. Can the management of blood sugar levels in gestational diabetes mellitus cases be an indicator of maternal and fetal outcomes? The results of a prospective cohort study from India. J Fam Community Med. 2016;23(2):94–9.
16. Ding TT, Xiang J, Luo BR, Hu J. Relationship between the iadpsg-criteria-defined abnormal glucose values and adverse pregnancy outcomes among women having gestational diabetes mellitus: a retrospective cohort study. Medicine (Baltimore). 2018;97(43):e12920.
17. Saldana TM, Siega-Riz AM, Adair LS, Savitz DA, Thorp JM. The association between impaired glucose tolerance and birth weight among black and white women in central North Carolina. Diabetes Care. 2003;26(3):656–61.
18. Feng H, Zhu WW, Yang HX, Wei YM, Wang C, Su RN, et al. Relationship between oral glucose tolerance test characteristics and adverse pregnancy outcomes among women with gestational diabetes mellitus. Chin Med J. 2017;130(9):1012–8.
19. Yang HL, Yang Z. Effects of advanced gestation on maternal and fetal outcomes. Chin J Emerg Obstetr. 2016;5(03):129–35.
20. Chen SF, Zhang C, Chen Y, et al. Analysis of factors influencing obstetric safety and countermeasures of reproductive trendon"Two Child Policy". J Shanghai Jiaotong Univ (Med Ed). 2016;36(5).
21. Zhang C, Rawal S, Chong YS. Risk factors for gestational diabetes: is prevention possible? Diabetologia. 2016;59(7):1385–90.
22. Eades CE, Cameron DM, Evans JMM. Prevalence of gestational diabetes mellitus in Europe: a meta-analysis. Diab Res Clin Pract. 2017;129:173–81.
23. Harder T, Plagemann A. A role for gestational diabetes in the excess maternal transmission of type 2 diabetes? Diabetes Care. 2000;23(3):431–2.
24. AbdalrahmanAlmarzouki A. Maternal and neonatal outcome of controlled gestational diabetes mellitus versus high risk group without gestational diabetes mellitus: a comparative study. Med Glas (Zenica). 2013;10(1):70–4.
25. Bardenheier BH, Elixhauser A, Imperatore G, Devlin HM, Kuklina EV, Geiss LS, et al. Variation in prevalence of gestational diabetes mellitus among hospital discharges for obstetric delivery across 23 states in the United States. Diabetes Care. 2013;36(5):1209–14.
26. Tian YM. Preliminary analysis on prevalence of gestational diabetes mellitus and related risk factors. Shanxi: Shanxi Medical University; 2019.
27. Wang JF. Risk factors of gestational diabetes mellitus and its effect on pregnancy outcome. Chin J Med. 2017;14(24):135–8.
28. Kosus N, Kosus A, Duran M, et al. Effect of number of abnormal oral glucose tolerance test (ogtt) values on birthweight in women with gestational diabetes. Indian J Med Res. 2013;137(1):95–101.
29. Kamana KC, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. Ann Nutr Metab. 2015;66(2):14–20.

Affiliations

Xie Yaping[1] · Liu Chunhong[2] · Zhao Huifen[3] · Huang Fengfeng[3] · Huang Huibin[4] · Zhao Meijing[5]

[1] The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

[2] Outpatient Department, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

[3] Clinical Nursing Teaching and Research Department, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

[4] Endocrinology Department, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

[5] Gynaecology and Obstetrics Department, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):101–107
https://doi.org/10.1007/s13410-021-00953-5

ORIGINAL ARTICLE

Risk factors for diabetes mellitus in patients with gastroesophageal reflux disease

Chiu-Hua Chang[1] · Tai-Hsiang Chen[2] · Lan-Lung (Luke) Chiang[2] · Chen-Chung Ma[3]

Received: 14 November 2020 / Accepted: 5 April 2021 / Published online: 16 April 2021

Abstract

Aim Diabetes mellitus (DM) and gastroesophageal reflux disease (GERD) may have a bidirectional association in clinical practice, and both diseases have caused considerable damage to the burden of health care and even hindered the economic development of the society. We sought to discover the risk factors for DM in patients with GERD.

Methods The research data was collected from the database of the health management center at a medical center in Southern Taiwan as a retrospective cross-sectional research from January 1, 2016, to December 31, 2018. We used logistic regression to analyze the related factors for DM in patients with GERD.

Results Of the 5578 patients with GERD, 739 had DM. The statistically significant risk factors for DM in GERD were gender, age, body mass index, waistline, betel quid chewing habit, lack of exercise habit, and family history of DM.

Conclusion We conclude that DM in patients with GERD should have regular physical examinations that include a thorough patient history, appropriate laboratory tests, and possible endoscopy. We also suggest that patients with either DM or GERD should be evaluated and treated immediately if they have symptoms, including regular physical examinations and lifestyle interventions, to improve health-related quality of life.

Keywords Diabetes mellitus · Gastroesophageal reflux disease · Physical examination · Lifestyle habits · Family histories

Introduction

Gastroesophageal reflux disease (GERD) is one of the most common upper gastrointestinal diseases in developed countries; as much as 40% of the adult population exhibits reflux symptoms [1]. Diabetes mellitus (DM), especially type 2, has also increased dramatically worldwide, accounting for 95% of DM cases. It is estimated that there will be as many as 439 million diabetic adults in the world by 2030 [2]. In DM, autonomic neuropathy and physiological changes caused by metabolic disorders, and the variations in neural control of the lower esophageal sphincter, can cause the aggressive stomach and duodenum contents to invade the esophageal mucosa. Therefore, certain DM symptoms are more common in the gastrointestinal tract and can affect the digestive tract from the mouth to the rectum [3]. Gastrointestinal symptoms adversely affect quality of life and have major medical consequences. Both diseases have increasingly higher medical and socioeconomic significance.

Previous studies indicated a potential correlation between GERD and DM [4–9]. Studies found the prevalence of GERD in patients with DM was significantly higher than in the general population, and the prevalence of GERD symptoms in patients with neuropathy was higher than in patients without this complication [10]. Diabetes mellitus may be related to unhealthy lifestyle factors such as smoking, further increasing the likelihood of developing GERD [11]. A study of 2836 veterans found that diabetics had esophagus and heart adenocarcinoma more frequently than non-diabetics, yet the increased incidence of esophageal adenocarcinoma in DM patients was not associated with obesity. Esophageal

Chiu-Hua Chang and Tai-Hsiang Chen contributed equally and are both joint first authors to this work.

✉ Chen-Chung Ma
up000238@isu.edu.tw

[1] Nursing Department, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, Republic of China

[2] College of Management, Yuan Ze University, Taoyuan, Taiwan, Republic of China

[3] Department of Healthcare Administration, I-Shou University, No. 8, Yida Rd., Jiaosu Village Yanchao District, Kaohsiung 82445, Taiwan, Republic of China

adenocarcinoma was found to be more common in GERD patients with high values of glycosylated hemoglobin [12]. The results of previous studies found that GERD and DM had some correlation. We summarized the common risk factors from past studies and found that age, dietary habits, family history, gender, obesity, and smoking habit increase the risk of both GERD and DM [13–22]. While GERD and DM should be viewed as a bidirectional association in clinical practice, most previous studies have explored the risk of GERD in DM patients. However, different environments may create different lifestyle habits and family medical histories. We aimed to determine the independent risk factors for DM in patients with GERD. Our model incorporated demographic characteristics, lifestyle habits, and family medical histories. We analyzed patient information from a medical center database in Southern Taiwan.

Methods

Data and sampling design

The study sample was derived from the database of the health management center at a medical center in Southern Taiwan from January 1, 2016, to December 31, 2018, collecting a total of 5601 research samples. Each patient would receive a health questionnaire by mail prior to the physical examination. They were required to complete the questionnaire and submit it for review before their physical examination. The main purpose of the questionnaire is to provide doctors with a quick confirmation of the health status of the patients, so the answers answered by the patients are not the finals. The finals are to proofread the questionnaire from the medical records to increase the accuracy. Subsequently, the patient medical record number linked each questionnaire with blood test and gastroscopy reports. We extracted and confirmed the data related to GERD and DM. To ensure privacy, patient case information could not be directly or indirectly identified. We reviewed the data for each case to ensure the integrity of the data. After deleting samples with missing data, 5578 patient cases were used in the study; the cases were required to have a GERD diagnosis after a physical examination with gastroscopy.

The clinical diagnosis of GERD mainly refers to the Los Angeles classification, the most commonly used to suggest the endoscopic appearance of reflux esophagitis and grade its severity, from mild (grade A) to severe (grade D) [23]. The classification was developed by the International Working Group for the Classification of Oesophagitis, supported by the World Organization of Gastroenterology, and published in its final form in 1999. Grade A esophagitis is defined as a Z1 mucosal fracture not exceeding 5 mm and not extending between the tops of the two mucosal folds. Grade D esophagitis is defined as a rupture of the Z1 mucosa which accounts for 75% or more of the esophageal circumference [23]. All patients with varying degrees of severity are classified as having GERD. This study was approved by the institutional review board of the Kaohsiung Veterans General Hospital on September 9th, 2020 (approval No. KSVGH20-CT10-01). The review board waived the need for consent.

Research variables and definitions

The dependent variable of this study was whether the participating patients with GERD also were diagnosed with DM. All of the patient samples had GERD diagnoses with DM assessment via a physical examination and blood test. According to the American Diabetes Association Standards of Medical Care in Diabetes-2020, the DM diagnostic criteria were defined as glycosylated hemoglobin (A1c) greater than or equal to 6.5, or fasting plasma glucose (FPG) greater than or equal to 126 [24]. We performed these two blood tests on the same sample and specified DM if they exceeded the criteria, and thus defined patients clinically diagnosed with DM and those who were not. These two categories were the dependent variables in the logistic regression model.

The independent variables in the logistic regression model included personal characteristics, lifestyle habits, and family medical histories. Personal characteristics included gender (female, male), age (≤ 30, 31–50, 51–70, > 70), body mass index (BMI) (< 18.5 kg/m^2, 18.5–23.9 kg/m^2, ≥ 24 kg/m^2), and waistline measurement (male < 90 cm and female < 80 cm; male ≥ 90 cm and female ≥ 80 cm) [25]. Waistline variable was defined as ≥ 90 cm (males) and ≥ 80 cm (females) using obesity criteria [26]. Lifestyle habits included dietary habits (non-vegetarian, vegetarian), alcohol consumption (no, yes), smoking (no, yes), betel quid chewing habit (no, yes), and exercise habit (no, yes). Family histories included DM (no, yes), hyperlipidemia (no, yes), cardiac disease (no, yes), and myocardial infarction (no, yes).

Statistical analysis

Descriptive analysis was performed to calculate the distribution and percentage of the different factors. Then, logistic regression was used to model the dependent variables ("with DM" and "without DM") to explore the related factors for DM in GERD patients. We used IBM SPSS (V22) for data analysis to calculate 95% confidence interval and descriptive and logistic regression statistics. The test was statistically significant at $p < 0.05$.

Results

The results showed that in the 5578 patients with GERD, 739 had DM; descriptive data are summarized in Table 1. In terms

Table 1 The distribution and percentage of different factors with or without DM (*N*=5578)

Categories	Variables	Without DM		With DM	
		N	Percent (%)	*N*	Percent (%)
Personal characteristics	Gender				
	Female	2650	54.76	591	79.97
	Male	2189	45.24	148	20.03
	Age				
	≤ 30	189	3.91	3	0.41
	31–50	2189	45.24	148	20.03
	51–70	2279	47.10	518	70.09
	>70	182	3.76	70	9.47
	BMI (kg/m^2)				
	<18.5	197	4.07	7	0.95
	18.5–23.9	2245	46.39	192	25.98
	≥24	2397	49.54	540	73.07
	Waistline (cm)				
	Non-obesity	2708	55.96	238	32.21
	Obesity	2131	44.04	501	67.79
Lifestyle habits	Dietary				
	Non-vegetarian	4707	97.27	714	96.62
	Vegetarian	132	2.73	25	3.38
	Alcohol consumption				
	No	1906	39.39	283	38.29
	Yes	2933	60.61	456	61.71
	Smoking				
	No	3025	62.51	427	57.78
	Yes	1814	37.49	312	42.22
	Betel quid chewing				
	No	4612	95.31	685	92.69
	Yes	227	4.69	54	7.31
	Exercise				
	No	1147	23.70	221	29.91
	Yes	3692	76.30	518	70.09
Family histories	DM				
	No	3638	75.18	395	53.45
	Yes	1201	24.82	344	46.55
	Hyperlipidemia				
	No	4622	95.52	696	94.18
	Yes	217	4.48	43	5.82
	Cardiac disease				
	No	4405	91.03	672	90.93
	Yes	434	8.97	67	9.07
	Myocardial infarction				
	No	4556	94.15	695	94.05
	Yes	283	5.85	44	5.95

BMI, body mass index; *DM*, diabetes mellitus

of gender, females were more than males, with 54.76% and 79.97% of the patients without and with DM, respectively. Most of the patients without and with DM were 31 to 70 years old, and even 70.09% of patients aged 51 to 70 years had DM. The majority of patients without DM and DM had a BMI between 18.5 and 23.9 and ≥24, and 540 (73.07%) patients

with DM had a BMI ≥24. Males with waistlines greater than or equal to 90 cm or females with waistlines greater than or equal to 80 cm were considered obese. There were 238 obese participants (32.21%) and 501 non-obese participants (67.79%) with DM. Those without DM were nearly evenly divided. Almost all patients with dietary habits were non-vegetarian. Approximately 60% of both patients without DM and those with DM had alcohol consumption and smoking habits. Almost all patients did not have the habit of betel quid chewing. Approximately 70% of both patients without DM and those with DM had an exercise habit. In terms of family histories, 24.82% of patients without DM had family histories of DM, while 46.55% of those with DM had family histories. Only about 5% of patients without and with DM had family histories of hyperlipidemia and myocardial infarction. Moreover, only about 9% of patients without and with DM had family histories of cardiac disease.

The variables were analyzed via logistic regression. After controlling for other variables, we analyzed the relationship between the variables and DM. Table 2 shows statistically significant differences in gender, age, BMI, waistline, betel quid chewing habit, exercise habit, and family history of DM. These factors were predictors of DM in patients with GERD. Males had significantly higher odds of having DM than did females (odds ratio [OR], 1.566; 95% confidence interval [CI], 1.247–1.968). Compared with patients under 30 years old, older patients with DM had higher odds. Patients aged 51–70 had a significantly higher OR of 13.215 (95% CI, 4.162–41.955), and those over 70 had a significantly higher OR of 23.990 (95% CI, 7.311–78.719). Patients with DM with BMI ≥24 had the highest odds compared with those with BMI < 18.5 (OR, 2.346; 95% CI, 1.053–5.229). Compared to patients whose waistlines were considered non-obese, obese patients with DM had significantly higher odds (OR, 1.829; 95% CI, 1.488–2.247). Patients with betel quid chewing habits were significantly associated with having DM (OR, 1.416; 95% CI, 1.002–2.001), while patients with exercise habits were less likely to develop DM (OR, 0.799; 95% CI, 0.665–0.961). Compared to patients with no family histories of DM, a significant positive influence was observed for patients with family histories of DM (OR, 3.153; 95% CI, 2.646–3.758).

Table 2 Logistic regression results of different risk factors for DM in patients with GERD (*N*=5578)

Variables	OR	95% CI		*p*
		Lower	Upper	
Gender				
Female	(Reference)			
Male	1.566	1.247	1.968	<0.001
Age				
≤ 30	(Reference)			
31–50	3.784	1.183	12.105	0.025
51–70	13.215	4.162	41.955	<0.001
>70	23.99	7.311	78.719	<0.001
BMI (kg/m^2)				
<18.5	(Reference)			
18.5–23.9	1.365	0.62	3.004	0.44
≥24	2.346	1.053	5.229	0.037
Waistline (cm)				
Non-obesity	(Reference)			
Obesity	1.829	1.488	2.247	<0.001
Dietary				
Non-vegetarian	(Reference)			
Vegetarian	1.195	0.75	1.903	0.454
Alcohol consumption				
No	(Reference)			
Yes	0.916	0.761	1.103	0.354
Smoking				
No	(Reference)			
Yes	0.953	0.787	1.154	0.622
Betel quid chewing				
No	(Reference)			
Yes	1.416	1.002	2.001	0.049
Exercise				
No	(Reference)			
Yes	0.799	0.665	0.961	0.017
Family history of DM				
No	(Reference)			
Yes	3.153	2.646	3.758	<0.001
Family history of Hyperlipidemia				
No	(Reference)			
Yes	0.933	0.644	1.351	0.713
Family history of cardiac disease				
No	(Reference)			
Yes	0.812	0.605	1.089	0.165
Family history of myocardial infarction				
No	(Reference)			
Yes	0.849	0.596	1.209	0.364
Constant	0.004			<0.001

OR, odds ratio; *CI*, confidence interval; *BMI*, body mass index; *DM*, diabetes mellitus

The statistical test was statistically significant at $p < 0.05$

Discussion

This study explored the independent risk factors for DM in patients with GERD; the results indicated that both clinical conditions are interrelated and that patients with type 2 DM were the majority of DM patients. We reviewed the risk factors from previous studies and found that age, dietary habits, family history, gender, obesity, and smoking habit increased the risk of both GERD and DM. In this study, the statistically significant risk

factors for DM in GERD were gender, age, body mass index, waistline, betel quid chewing habit, lack of exercise habit, and family history of DM. First of all, our study found that gender was a risk factor. Males had a higher risk of DM than did females, consistent with previous studies showing that gender is an important modifier and a fundamental biological factor with a key role in regulating dynamic balance in health [27]. Gender has with risk factors for cardiometabolic susceptibility, clinical presentation, and the need for DM management. For various diabetes-related comorbidities, particularly cardiovascular and kidney disease, severity of injury also varies by gender. Psychosocial factors influence the development and progression of DM and were addressed in a gender-dimorphic way [28]. As in previous studies, we found that increased age, BMI, and waistline were important predictors of DM in GERD patients [7, 11, 27]. Age is a well-known risk factor; the body's immune and metabolic systems deteriorate with age. Similar to previous studies, we found that the incidence of hiatal hernia, a GERD risk factor, was extremely high in the elderly. The average size of hiatal hernia increases with patient age due to tissue aging effects [29]. A previous study showed that hiatal hernia size was related to the severity of esophagitis [30]. Our study also demonstrated that BMI was related to the incidence and severity of DM in patients with GERD, and that elevated BMI could be an important causative factor. When a high BMI leads to an imbalance in body fat, various physiological and metabolic functions may deteriorate. Treatment should include proton pump inhibitor treatment and other specific measures, as well as DM treatment, especially blood glucose control and weight loss [27, 31]. Han and Lean proposed using waistline as a measure of obesity to predict health risks. Because of the multiple clinical and personal benefits of obesity management, there is a rationale for providing evidence-based obesity management for all patients with large waistline to reduce the risk of metabolic syndrome, DM, and cardiovascular disease [32]. Along with BMI, waistline provides information about health risks, and with training and standardization, it is conceptually easy to measure. In the aforementioned study, obesity was thought to be caused by a variety of factors, including an increased gastroesophageal sphincter gradient, the incidence of hiatal hernia, and intraperitoneal pressure. Some DM patients, especially those with type 2 DM, are obese [7]. Therefore, the present study included both indicators to measure obesity criteria.

Lifestyle habits are clear risk factors for DM in GERD patients, since poor habits accumulate long-term, causing blood sugar fluctuations and potentially obesity. Behavioral changes and healthy lifestyle habits can help prevent or slow DM and are crucial to treating DM. However, many people have insufficient knowledge of practices for healthier lifestyles. In a study of 207 people with type 2 DM in India, up to 83.3% had a poor knowledge of lifestyle changes as a non-drug treatment [33]. The present study explored the effects of lifestyle habits on DM in patients with GERD and found that statistically significant variables were betel quid chewing habit and exercise habit, consistent with previous studies. Betel quid is one of the most commonly used psychoactive substances in the world, with an estimated 600 million betel quid users worldwide [34]. Some studies have found that the habit was associated with chronic kidney disease and an increased risk of cardiovascular disease [35, 36], while others found it frequently increased blood pressure and risk of arterial stiffness [37, 38]. Previous studies confirmed the connection between betel quid chewing and DM. The incidence of newly diagnosed, age-specific DM increased with age, peaking in the 60–69 age range. In betel quid chewers over 70, incidence of DM decreased, partly because chewers may die early due to diseases. Furthermore, most chewers are typically diagnosed with DM at an earlier age, peaking at 60–69 years [39]. The relationship of betel quid usage with biological mechanisms is worthy of further discussion.

Another important factor is that a regular exercise habit improves metabolism. Habitual high-intensity exercise alters motor function of the esophagus and ventricles, but may exacerbate symptoms of GERD in the upper digestive tract [40]. We sought to explore the independent risk factors for DM in patients with GERD. Although the above-mentioned study found that high-intensity exercise may aggravate gastrointestinal symptoms, GERD patients should maintain a moderate exercise habit to reduce DM risk. Regular exercise is essential to DM management, especially during aging and as adiposity increases. A previous study showed that people engaged in physical activity had a much lower risk of developing DM than the sedentary. While weight loss may be difficult to attain, regular exercise can reduce the risk of DM by increasing the muscle disposal of insulin-mediated glucose [41]. The 495 participants in a study by the American Diabetes Prevention Program who met their physical activity goals had a 44% reduction in DM [42].

These findings are directly in line with our results that increased exercise can reduce DM risk in GERD patients. Changing poor lifestyle habits is important for physical health, especially for those with a family history of related diseases. The results of our study show that GERD patients with a family history of DM are more likely to have DM. Family history is a common and unalterable risk factor for most chronic non-communicable diseases, as a collective reflection of genetic predisposition, common environment, and behavior. Information about family history can be a unique, useful tool for public health and preventive medicine. The aforementioned study demonstrated that subjects with a family history of DM had significantly higher BMIs, waistlines, and hip circumferences than did subjects without a family history of DM [43]. Another recent study in India found that healthy individuals with a family history of DM had higher anthropometric values [44]. A family history of DM is associated with the prevalence of obesity, hypertension, and metabolic

syndrome. It was concluded that a positive family history of DM was significantly associated with metabolic syndrome [45]. A previous study also confirmed the link between family history of DM and hypertension and the aggregation of these diseases in the offspring of affected patients [46].

The advantage of family history as a risk assessment tool is that it truly reflects common genetic and lifestyle habit factors. Although family history is an immutable risk factor in its own right, it contributes to increased risk awareness, risk stratification, targeted interventions, and positive effects on healthy behaviors. There is ample evidence in randomized controlled trials and observational studies that adopting simple, healthy lifestyle changes can prevent type 2 DM. Accurate risk perception may have a positive impact on behavioral change and contribute to DM prevention [47]. The use of family history as a public health tool to prevent DM has broad prospects.

Limitations

There are several limitations of this study to consider. First, we analyzed a secondary database as a cross-sectional study and could not further clarify the causal relationship between the independent and dependent variables. Therefore, we suggest that future researchers employ longitudinal studies to explore long-term trends. Second, the subjects of this study are patients of the health management center at a medical center in Southern Taiwan. Due to the limitations inherent in regional data, the results of this study may not be applied to those of the national population. Furthermore, the sample was obtained from the health management center, so it may not be representative of outpatients. The data collection scope should be expanded to the nationwide in order to provide more representative information.

Conclusions

The present study investigated the clinical correlation between DM and GERD, and identified associated risk factors. We provide insights into the prevalence of DM in patients with GERD and offer clinical reference for managed caregivers. A physical examination should begin with a complete family history, appropriate laboratory examinations, and possible endoscopy. In general, we suggest that patients with either DM or GERD should be evaluated and treated immediately if they have symptoms. The predictive factors of this study can help patients seek early attention, including regular physical examinations and lifestyle interventions, to reduce the incidence of DM.

Acknowledgment We thank the Kaohsiung Veterans General Hospital in Southern Taiwan for providing the database of records from the health management center, and the director and other doctors in the health management center for their support during the planning of the study and the data collection process.

Declarations

Ethical approval This study was approved by the institutional review board of the Kaohsiung Veterans General Hospital (approval No. KSVGH20-CT10-01).

Conflict of interest The authors declare no competing interests.

References

1. Moayyedi P, Talley NJ. Gastro-oesophageal reflux disease. Lancet. 2006;367(9528):2086–100.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(1):4–14.
3. Gatopoulou A, Papanas N, Maltezos E. Diabetic gastrointestinal autonomic neuropathy: current status and new achievements for everyday clinical practice. Eur J Intern Med. 2012;23(6):499–505.
4. Fujiwara M, Miwa T, Kawai T, Odawara M. Gastroesophageal reflux disease in patients with diabetes: preliminary study. J Gastroen Hepatol. 2015;30:31–5.
5. Gokturk S, Akyuz F, Arici S, et al. Gastroesophageal reflux in asymptomatic patients with diabetes: an impedance study diabetes, obesity and gastroesophageal reflux. Exp Clin Endocr Diab. 2020;128(01):52–8.
6. Ikeda Y, Furukawa S, Sakai T, Niiya T, Miyaoka H, Miyake T, et al. Age and prevalence of esophageal reflux disease in japanese patients with type 2 diabetes mellitus: the dogo study. Dig Dis Sci. 2016;61(12):3530–6.
7. Sun XM, Tan JC, Zhu Y, Lin L. Association between diabetes mellitus and gastroesophageal reflux disease: a meta-analysis. World J Gastroenterol. 2015;21(10):3085–92.
8. Takeshita E, Furukawa S, Sakai T, Niiya T, Miyaoka H, Miyake T, et al. Eating behaviours and prevalence of gastroesophageal reflux disease in japanese adult patients with type 2 diabetes mellitus: the dogo study. Can J Diabetes. 2018;42(3):308–12.
9. Yarandi SS, Srinivasan S. Diabetic gastrointestinal motility disorders and the role of enteric nervous system: current status and future directions. Neurogastroenterol Motil. 2014;26(5):611–24.
10. Wang X, Pitchumoni CS, Chandrarana K, Shah N. Increased prevalence of symptoms of gastroesophageal reflux diseases in type 2 diabetics with neuropathy. World J Gastroenterol. 2008;14(5):709–12.
11. Huang W, Ren H, Ben Q, Cai Q, Zhu W, Li Z. Risk of esophageal cancer in diabetes mellitus: a meta-analysis of observational studies. Cancer Causes Control. 2012;23(2):263–72.
12. Dixon JL, Copeland LA, Zeber JE, MacCarthy AA, Reznik SI, Smythe WR, et al. Association between diabetes and esophageal cancer, independent of obesity, in the United States Veterans Affairs population. Dis Esophagus. 2016;29(7):747–51.
13. Chan JC, Malik V, Jia W, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA. 2009;301(20):2129–40.
14. Eckel N, Mühlenbruch K, Meidtner K, Boeing H, Stefan N, Schulze MB. Characterization of metabolically unhealthy normal-weight individuals: risk factors and their associations with type 2 diabetes. Metabolism. 2015;64(8):862–71.
15. Edelstein ZR, Bronner MP, Rosen SN, Vaughan TL. Risk factors for Barrett's esophagus among patients with gastroesophageal

reflux disease: a community clinic-based case-control study. Am J Gastroenterol. 2009;104(4):834–42.
16. Eusebi LH, Ratnakumaran R, Yuan Y, Solaymani-Dodaran M, Bazzoli F, Ford AC. Global prevalence of, and risk factors for, gastro-oesophageal reflux symptoms: a meta-analysis. Gut. 2018;67(3):430–40.
17. Jaiswal M, Divers J, Dabelea D, Isom S, Bell RA, Martin CL, et al. Prevalence of and risk factors for diabetic peripheral neuropathy in youth with type 1 and type 2 diabetes: search for diabetes in youth study. Diabetes Care. 2017;40(9):1226–32.
18. Kariri AM, Darraj MA, Wassly A, Arishi HA, Lughbi M, Kariri A, et al. Prevalence and risk factors of gastroesophageal reflux disease in southwestern saudi arabia. Cureus. 2020;12(1):e6626.
19. Ness-Jensen E, Hveem K, El-Serag H, Lagergren J. Lifestyle intervention in gastroesophageal reflux disease. Clin Gastroenterol H. 2016;14(2):175–82.
20. Pohl H, Wrobel K, Bojarski C, Voderholzer W, Sonnenberg A, Rösch T, et al. Risk factors in the development of esophageal adenocarcinoma. Am J Gastroenterol. 2013;108(2):200–7.
21. Richter JE, Rubenstein JH. Presentation and epidemiology of gastroesophageal reflux disease. Gastroenterology. 2018;154(2):267–76.
22. Talmud PJ, Hingorani AD, Cooper JA, Marmot MG, Brunner EJ, Kumari M, et al. Utility of genetic and non-genetic risk factors in prediction of type 2 diabetes: Whitehall II prospective cohort study. BMJ. 2010;340:b4838.
23. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut. 1999;45(2):172–80.
24. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S14–31.
25. Pan WH, Yeh WT, Chen HJ, Chuang SY, Chang HY, Chen L, et al. The U-shaped relationship between BMI and all-cause mortality contrasts with a progressive increase in medical expenditure: a prospective cohort study. Asia Pac J Clin Nutr. 2012;21(4):577–87.
26. Li W, Wang D, Wang X, Gong Y, Cao S, Yin X, et al. The association of metabolic syndrome components and diabetes mellitus: evidence from China National Stroke Screening and Prevention Project. BMC Public Health. 2019;19(1):192.
27. Natalini J, Palit A, Sankineni A, Friedenberg FK. Diabetes mellitus is an independent risk for gastroesophageal reflux disease among urban African Americans. Dis Esophagus. 2015;28(5):405–11.
28. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocr Rev. 2016;37(3):278–316.
29. Pilotto A, Franceschi M, Leandro G, Scarcelli C, D'Ambrosio LP, Seripa D, et al. Clinical features of reflux esophagitis in older people: a study of 840 consecutive patients. J Am Geriatr Soc. 2006;54(10):1537–42.
30. Kermansaravi M, Kabir A, Mousavimaleki A, Pazouki A. Association between hiatal hernia and gastroesophageal reflux symptoms after one-anastomosis/mini gastric bypass. Surg Obes Relat Dis. 2020;16(7):863–7.
31. Kase H, Hattori Y, Sato N, Banba N, Kasai K. Symptoms of gastroesophageal reflux in diabetes patients. Diabetes Res Clin Pract. 2008;79(2):e6–7.
32. Han TS, Lean ME. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. JRSM Cardiovasc Dis. 2016;5:2048004016633371.
33. Malathy R, Narmadha M, Ramesh S, Alvin JM, Dinesh BN. Effect of a diabetes counseling programme on knowledge, attitude and practice among diabetic patients in Erode district of South India. J Young Pharm. 2011;3(1):65–72.
34. Das A, Orlan E, Duncan K, Thomas H, Ndumele A, Ilbawi A, et al. Areca nut and betel quid control interventions: halting the epidemic. Subst Use Misuse. 2020;*55*(9):1552–9.
35. Do DV, Wang X, Vedula SS, et al. Blood pressure control for diabetic retinopathy. Cochrane Database Syst Rev. 2015;1:CD006127.
36. Chou CY, Cheng SY, Liu JH, Cheng WC, Kang IM, Tseng YH, et al. Association between betel-nut chewing and chronic kidney disease in men. Public Health Nutr. 2009;12(5):723–7.
37. Lin SH, Liao YS, Huang SH, Liao WH. Relationship between betel quid chewing and risks of cardiovascular disease in older adults: a cross-sectional study in Taiwan. Drug Alcohol Depend. 2014;141:132–7.
38. Wei YT, Chou YT, Yang YC, Chou CY, Lu FH, Chang CJ, et al. Betel nut chewing associated with increased risk of arterial stiffness. Drug Alcohol Depend. 2017;180:1–6.
39. Tseng CH. Betel nut chewing and incidence of newly diagnosed type 2 diabetes mellitus in Taiwan. BMC Res Notes. 2010;3:228.
40. Jozkow P, Wasko-Czopnik D, Medras M, Paradowski L. Gastroesophageal reflux disease and physical activity. Sports Med. 2006;36(5):385–91.
41. Horowitz JF. Exercise-induced alterations in muscle lipid metabolism improve insulin sensitivity. Exerc Sport Sci Rev. 2007;35(4):192–6.
42. Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, et al. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care. 2006;29(9):2102–7.
43. Papazafiropoulou A, Sotiropoulos A, Skliros E, Kardara M, Kokolaki A, Apostolou O, et al. Familial history of diabetes and clinical characteristics in Greek subjects with type 2 diabetes. BMC Endocr Disord. 2009;9:12.
44. Padaki S, Vijayakrishna K, Dambal A, et al. Anthropometry and physical fitness in individuals with family history of type-2 diabetes mellitus: a comparative study. Indian J Endocrinol Metab. 2011;15(4):327–30.
45. Das M, Pal S, Ghosh A. Family history of type 2 diabetes and prevalence of metabolic syndrome in adult Asian Indians. J Cardiovasc Dis Res. 2012;3(2):104–8.
46. Wada K, Tamakoshi K, Yatsuya H, Otsuka R, Murata C, Zhang H, et al. Association between parental histories of hypertension, diabetes and dyslipidemia and the clustering of these disorders in offspring. Prev Med. 2006;42(5):358–63.
47. Shreck E, Gonzalez JS, Cohen HW, Walker EA. Risk perception and self-management in urban, diverse adults with type 2 diabetes: the improving diabetes outcomes study. Int J Behav Med. 2014;21(1):88–98.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):108–115
https://doi.org/10.1007/s13410-021-00958-0

ORIGINAL ARTICLE

Diabetic nephropathy with and without retinopathy: comparison between urine and serum vascular endothelial growth factor

Hanisah Abdul Hamid[1] · Wei Yen Kong[2] · Norwahidah Abdul Karim[3] · Meng Hsien Yong[1] · Norfilza Mohd Mokhtar[4] · Norshamsiah Md Din[1]

Received: 6 May 2020 / Accepted: 13 May 2021 / Published online: 2 June 2021

Abstract

Aim To evaluate vascular endothelial growth factor (VEGF) levels in serum and urine among diabetic nephropathy (DN) patients with and without diabetic retinopathy (DR).

Methods This is a cross-sectional study involving stage 3 and 4 DN patients. A fundus examination was done to determine the DR status. VEGF levels were measured using VEGF-A ELISA kits. Serum creatinine, glycated hemoglobin (HbA1c), and urine protein creatinine index (UPCI) were analyzed per laboratory protocol. Clinical data collected includes height, weight, visual acuity, fundus photo, and macula thickness measured by the optical coherence tomography.

Results Seventy-nine patients were divided into group 1: DN with no DR ($n = 45.57\%$) and group 2: DN with DR ($n = 34.43\%$). The two groups were equally distributed in age, gender, ethnicity, duration of diabetes, and smoking status. Mean serum VEGF (244.9 pg/mL), urine VEGF (230.4 pg/mL), and HbA1c (7.6%) were significantly higher in group 2. Mean serum to urine VEGF ratios were 0.72 in group 1 and 1.06 in group 2. Poorer renal function was seen in group 2, although the UPCI was not significantly different. There was no significant correlation between serum and urine VEGF levels and between serum VEGF and other parameters.

Conclusion The occurrence of DR in patients with DN is associated with higher mean serum than urine VEGF levels, as compared to lower serum to urine ratio of VEGF levels in DN patients without DR.

Keywords Diabetic nephropathy · Diabetic retinopathy · Vascular endothelial growth factor · Serum · Urine

Introduction

Diabetes mellitus (DM) is one of the major health problems and is increasing worldwide. The global diabetes prevalence in 2019 was estimated to be 9.3% (463 million), and the prevalence is estimated to rise by 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. In Malaysia, the National Health and Morbidity Survey 2015 showed an increase in the prevalence of DM from 15.2% in 2011 to 17.5% in 2015 [2]. With the improvement in the patient's survival rate, diabetic retinopathy (DR) and nephropathy have emerged as a major microvascular complication. Diabetic nephropathy (DN) was

✉ Norshamsiah Md Din
shamsiahdr@hotmail.com

Hanisah Abdul Hamid
hanisahabdulhamid1805@gmail.com

Wei Yen Kong
wykong1979@yahoo.com

Norwahidah Abdul Karim
wahida2609@gmail.com

Meng Hsien Yong
yongmenghsien@ppukm.ukm.edu.my

Norfilza Mohd Mokhtar
norfilza@ppukm.ukm.edu.my

[1] Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000, Cheras, Kuala Lumpur, Malaysia

[2] Department of Internal Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[3] Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[4] Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

found to be the commonest complication (91%), followed by neuropathy (54.4%), retinopathy (39.3%), and other macrovascular complications (17.5%) [3]. According to the Diabetic Nephropathy Clinical Practice Guideline (CPG) Malaysia 2004, approximately 70% of type 2 diabetic patients with nephropathy will have retinopathy as well. DN has been known to be the risk factor not only for DR but also for vision-threatening DR [4].

Various molecular mechanisms contributing to these microvascular complications have been implicated [5], but vascular endothelial growth factor (VEGF) is one of the main factors involved [6, 7]. VEGF is a potent cytokine that plays an important role in inducing angiogenesis and increased vascular permeability [8]. The VEGF family consists of VEGF-A (generally called VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) [9]. VEGF-A is the most potent mediator found in DR [10] and DN [11].

VEGF in the eye is produced by various cells including retinal pigment epithelial cells, glial cells, retinal capillary pericytes, endothelial cells, Muller cells, and ganglion cells [12]. Elevated VEGF levels are seen more in patients with DR than those without [13] and are seen in various bodily fluids including serum, vitreous, and aqueous humor [14, 15]. Diabetes affects the retina in a multitude of mechanism, one of it being increased vascular permeability in and around the macula leading to variable changes in macula thickness. While there are reports of macular thickness to be thicker by at least 40 mm in eyes with DR compared to non-diabetic controls [16], others reported thinner pericentral macular thickness [17] or no difference between the two groups of patients [18]. These changes in macula thickness may contribute to deranged visual functions in diabetics.

VEGF and VEGF receptor (VEGFR) expressions are critical in maintaining normal glomerular podocytes and renal tubular function. Glomerular podocytes are the key regulators of macromolecule permeability and the major sites of VEGF synthesis. VEGF expressed in glomerular podocytes activates VEGFR2 on glomerular capillary endothelial cells, thus maintaining and regulating the endothelial fenestrations and permeability in a similar manner to its role in the ocular choriocapillaris [19].

In experimental, non-diabetic renal studies, stimulating angiogenesis with VEGF led to improved renal function, [20] reenforcing the importance of VEGF for normal podocyte function and glomerular filtration. However, in diabetic animal models, the opposite appears to be true: VEGF antibodies (which lowers available VEGF) improved renal function through reduced glomerular hyperfiltration and hypertrophy [19, 20] and reduced proteinuria [20]. These findings suggest that VEGF may be a primary pathologic biomarker similar to its role in ocular diabetic microvascular pathology [19].

High levels of systemic VEGF in patients with type 2 diabetes are induced by various mechanisms including hyperglycemic state, hypoxia, advanced glycation end products (AGEs), angiotensin II, protein kinase C activation, and various growth factors and cytokines [21]. Serum VEGF levels were found to be higher in diabetic patients with DR than those with DN, when compared to normal individuals [22], and higher in diabetic patients with microvascular complications compared to those without [23].

We aim to see whether there is a difference in serum and urine VEGF levels among patients with DN with or without DR and see if there is an association between these VEGF levels and other parameters such as glycated hemoglobin (HbA1c) level; renal function parameters such as serum creatinine, glomerular filtration rate (GFR), and urine protein creatinine index (UPCI); and visual function parameters such as visual acuity and macula thickness.

Subjects, materials, and methods

Patients and methods

This is a cross-sectional study conducted from March 2017 till March 2018 involving 79 patients with chronic kidney disease (CKD) of stages 3 and 4 with underlying type 2 DM. Consecutive eligible patients were recruited from the nephrology and ophthalmology clinic, UKM Medical Centre. Ethical approval was obtained from the Universiti Kebangsaan Malaysia Research and Ethics Committee (Ethical approval code: FF-2016-337). This study adhered to the tenets of the Declaration of Helsinki and the Malaysian Guidelines for Good Clinical Practice (GCP). A signed written informed consent was obtained from all patients prior to enrolment.

The staging of DN was done based on the glomerular filtration rate (GFR). The inclusion criteria were type 2 DM with established DN either in stage 3 (defined as GFR = 30–59 μmol/L) or 4 (GFR = 15–29 μmol/L) [24]. The exclusion criteria were patients who have received intravitreal anti-VEGF injections, CKD other than from diabetes (e.g., obstructive uropathy), pregnant/lactating women, underlying malignancy, and underlying autoimmune diseases.

Only one eye per patient was included and the eye with worse vision was chosen. Visual acuity was assessed using the Snellen chart and converted to a LogMAR value for ease of analysis. Fundus photography following the ETDRS seven standard photograph was taken using a fundus camera (Topcon Medical System Inc.). The photographs were evaluated and staged by an ophthalmologist using the Malaysian DR screening Clinical Practice Guideline (2011) and categorized into (1) no DR, (2) mild non-proliferative diabetic retinopathy (NPDR—the presence of microaneurysms only), (3) moderate NPDR (presence of more than just microaneurysms but less than severe NPDR), (4) severe NPDR (presence of any of the following: (i) more than 20 intraretinal hemorrhages

in each of 4 quadrants, (ii) definite venous beading in 2 or more quadrants, and (iii) prominent intraretinal microvascular abnormalities in 1 or more quadrants), (5) proliferative DR (PDR—the presence of retinal neovascularization or vitreous/preretinal hemorrhage), and (6) advanced diabetic eye disease (ADED—the presence of any of the following: (i) fibrovascular tissue proliferation, (ii) tractional retinal detachment, (iii) rhegmatogenous retinal detachment, and (iv) dragging/distortion of the retina) [4]. All eyes were grouped into group 1 (DN with no DR) and group 2 (DN with the presence of DR changes either mild, moderate, severe, PDR, or ADED).

The macula thickness was measured using the Heidelberg Spectralis OCT (Heidelberg Spectralis Inc., Germany) by a trained technician. For the purpose of this study, central subfield thickness was taken for analysis [25, 26].

Laboratory investigations

Glycated hemoglobin (HbA1c) was measured with the ion-exchange high-performance liquid chromatography (HPLC) method, with normal value ranges between 4.4 and 6.4%. Serum creatinine was measured using the compensation Jaffe method with reference values of 50.4–98.1 μmol/L. Urine protein creatinine index was measured using an automated turbidimetric benzalkonium chloride method, with normal values of < 0.02 g/mmol creatinine. The estimated glomerular filtration rate was measured using the Modification of Diet in Renal Disease (MDRD) study equation.

VEGF specimen collection and processing

Blood samples of at least 3 mL were collected in a plain tube, and urine samples of at least 1.5 mL were obtained from the patients. Blood samples were allowed to clot in room temperature for 30 min [27] and subsequently centrifuged for approximately 10 min with 3000 rpm to obtain the serum and stored in a – 80 °C freezer. Urine samples were also stored in a – 80 °C freezer. When enough samples have been collected and ready for processing, the urine and serum samples were taken out from the freezer and thawed in ice. The samples were then centrifuged for 20 min with 3000 rpm to remove the supernatant. These processes were done by single trained and credentialed staff that were blinded from the status of the patient.

VEGF level measurement

VEGF levels in the urine and serum were measured using a commercially available double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) VEGF-A kits (Sunred Biological Technology Co Ltd Shanghai). This kit has a low intra- and inter-assay error range (intra-assay error: < 10% and inter-assay error: 8.8 < 12%). The sensitivity of the assay is 2.677 pg/mL as stated by the manufacturer. Sample processing was done as per the assay protocol. The optical density (OD) level of the standard and sample was measured under 450 nm light wavelength using a Perkin Elmer Multimode plate reader. The standard concentration curve was obtained, and the concentration of VEGF level was measured in picogram/milliliter (pg/mL).

Statistical analysis

Data were analyzed using SPSS version 25.0 (SPSS Inc. Chicago, IL, USA). Categorical data were tested using the chi-squared test or Fisher's exact test for datasets less than 5 and continuous data were analyzed using Mann-Whitney U/Kruskal-Wallis for non-parametric data and unpaired t-test for parametric data. Parametric and non-parametric datasets were expressed as mean with standard deviation (SD) and median with interquartile range (IQR) respectively. The Spearman rank test was used to find a correlation, and multiple regression analysis was used to find associations between serum and urine VEGF level with HbA1c, GFR, and UPCI. p values of < 0.05 were considered to be statistically significant.

Results

A total of 79 eligible patients were included and grouped into group 1 (n = 45, 57%) and group 2 (n = 34, 43%). The demographic data of the study population were summarized in Table 1. The two groups were equally distributed in terms of age, gender, ethnicity, duration of diabetes, and smoking status. However, more patients in group 2 had more severe DN (stage 4) compared to group 1, which was also reflected by the significantly higher serum creatinine and lower GFR in group 2. The diabetic control is also poorer in group 2 as evidenced by the significantly higher HbA1c by almost 0.8% difference. With both groups being equally overweight, there was no significant difference in BMI between the two groups. Although there was no significant difference in CSFT of the macula between the two groups, there was a significant difference in the best-corrected visual acuity (BCVA).

Both mean serum and urine VEGF were significantly higher in group 2 than in group 1. The mean serum VEGF was higher by almost twofold in group 2 while mean urine VEGF is approximately 1.3 times higher in group 2. Additionally, while mean serum VEGF was lower than mean urine VEGF in group 1, the reverse was seen in group 2. This results in a serum to urine ratio of VEGF levels to be 0.72 in group 1 and 1.06 in group 2. However, further analysis did not find any correlation between serum VEGF with urine VEGF levels, HbA1c, and vision in LogMAR (Fig. 1a–c).

Table 1 Demographic, clinical, and laboratory data between group 1 and group 2

	Group 1 DN no DR (n = 45)	Group 2 DN with DR (n = 34)	p value (< 0.05)
Age (mean ± SD), years	70 ± 9	67 ± 7	0.078[a]
Gender, n (%)			
Male	30 (62.5%)	18 (37.5%)	0.216[b]
Female	15 (48.4)	16 (51.6%)	
Race, n (%)			
Malay	26 (55.3%)	21 (44.7%)	
Chinese	15 (55.6%)	12 (44.4%)	0.666[c]
Indian	4 (80%)	1 (20%)	
Duration of DM, n (%)			
≤ 5 years	6 (75%)	2 (25%)	
5–10 years	5 (55.6%)	4 (44.4%)	0.499[c]
11–15 years	9 (64.3%)	5 (35.7%)	
16–20 years	11 (64.7%)	6 (35.3%)	
> 20 years	14 (45.2%)	17 (54.8%)	
Smoking status, n (%)			
Non-smoker	30 (56.6%)	23 (43.4%)	
Ex-smoker	14 (66.7%)	7 (33.3%)	0.204[c]
Active smoker	1 (20%)	4 (80%)	
Severity of DN			
Stage 3 DN	30 (69.8%)	13 (30.2%)	0.012[b]
Stage 4 DN	15 (41.7%)	21 (58.3%)	
Urine protein creatinine index (UPCI)	0.09 (0.04–0.20)	0.08 (0.07–0.50)	0.087[d]
Serum creatinine, median (IQR), (μmol)	170 (147–200)	191 (159–254)	0.038[d]
GFR median (IQR), mL/min/1.73 m^2	32 (25–38)	27 (21–34)	0.034[d]
HbA1c (%)	6.8 (6.0–7.5)	7.6 (7.3–9.4)	0.000[d]
BMI median (IQR), kg/m^2	27.78 (24.68–33.57)	28.62 (24.34–31.01)	0.589[d]
Serum VEGF-A (pg/mL)	123.63 (59.82–245.36)	244.91 (90.64–312.91)	0.046[d]
Urine VEGF-A (pg/mL)	171.91 (56.91–223.45) Ratio S:U = 0.72	230.37 (168.27–285.09) Ratio S:U = 1.06	0.025[d]
Central subfield macula thickness (μm)	267 (251–284)	266 (237–300)	0.706[d]
LogMAR	0.2 (0.2–0.3)	0.3 (0.2–0.5)	0.029[d]

[a] t-test; [b] chi-squared test; [c] Fisher's exact test; [d] Mann-Whitney U test

A comparison of serum and urine VEGF levels did not find any significant difference in different grades of DR severity (Table 2). This could be due to the small number of eyes in each grading.

We found no correlation between serum/urine VEGF with visual function parameters (visual acuity and macula thickness) and renal function parameters (serum creatinine, GFR, and UPCI) (Table 3). Multiple regression analyses also failed to find any significant associations between the VEGF levels and other parameters.

Discussion

Serum VEGF levels have been found to be significantly elevated in diabetic patients compared to healthy controls, even without diabetic retinopathy [22, 28]. We wanted to investigate whether there is any difference in VEGF levels in the serum and urine in diabetic-related nephropathy and retinopathy, the two most common microangiopathies in diabetes. Establishing this may help further understand the cascade of events as far as VEGF is concerned.

We found that in patients with DN without DR, both serum and urine VEGF levels were elevated, but not correlated, and that the urine VEGF is higher than the serum levels, indicating that the kidney disease process is dominant at this stage.

However, in patients with diabetic nephropathy and retinopathy, we found that serum and urine VEGF levels were both elevated, and the serum VEGF elevation is more compared to elevation in urine VEGF levels. The mean serum VEGF level was almost doubled that of the mean urine VEGF levels. This is almost similar to the findings by Guo et al. where they found the highest VEGF levels in retinopathy patients followed by nephropathy and lastly diabetic hypertension [22]. This may indicate that retinopathy occurs when serum VEGF levels reached a certain level. A higher serum to urine VEGF ratio is probably an indicator of the state of microangiopathy in DM.

Apart from higher serum VEGF level, the urine VEGF level was also found to be higher in group 2. Serum VEGF appears to increase alongside elevated urine VEGF levels in

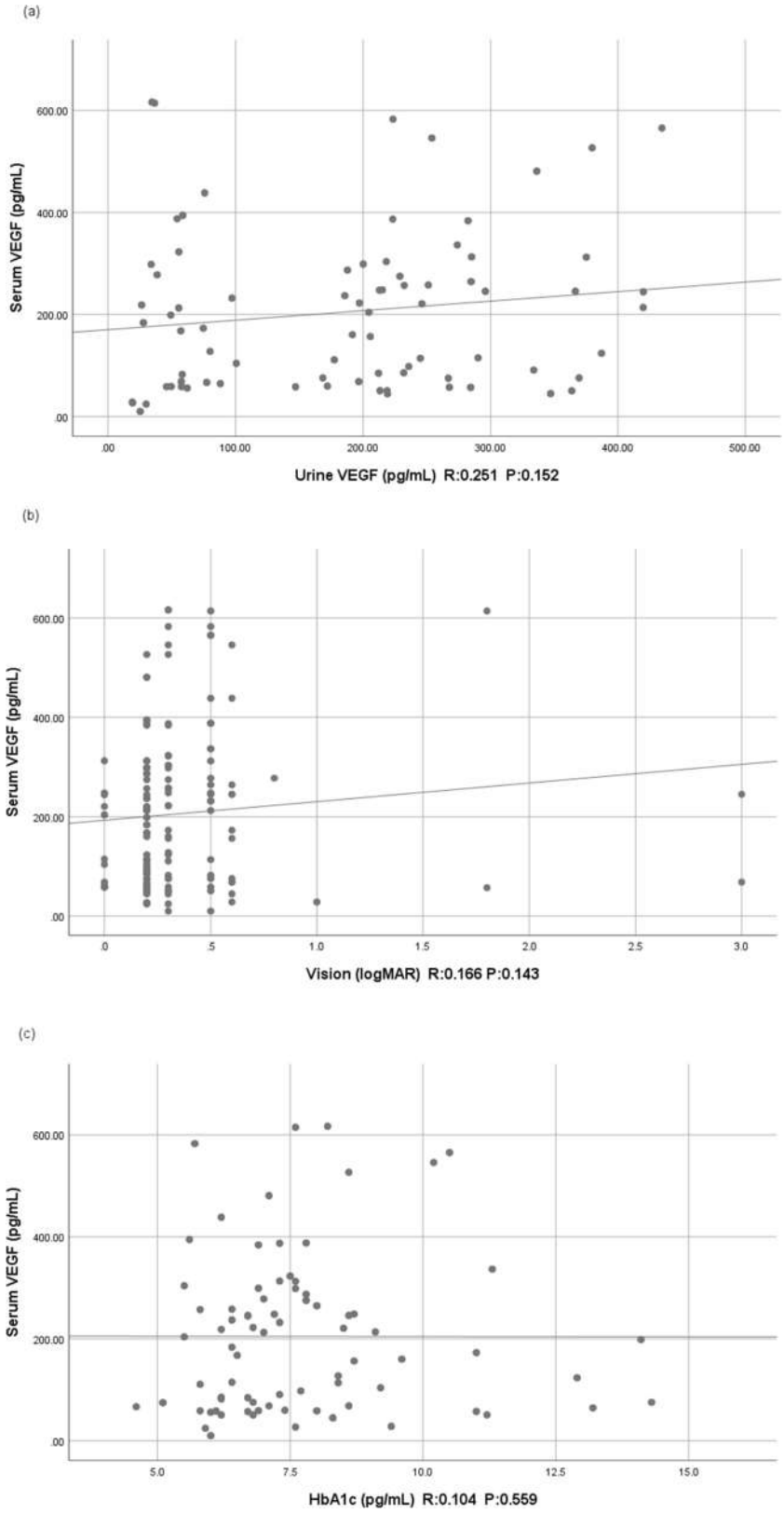


Fig. 1 Correlation between serum VEGF and urine VEGF level (**a**), vision in LogMAR (**b**), and HbA1c (**c**)

patients with both DN and DR. In the absence of any literature describing higher circulating serum VEGF being excreted in the kidney and the fact that we do not find a significant correlation between serum and urine VEGF, we postulate that the increase in serum VEGF is due to the disease process in the target organs, while

Table 2 Serum and urine VEGF level according to the stage of diabetic retinopathy

	No DR (n = 45) Median (IQR)	Mild NPDR (n = 19) Median (IQR)	Moderate NPDR (n = 11) Median (IQR)	PDR (n = 4) Median (IQR)	p value (< 0.05)
Serum VEGF-A (pg/mL)	123.63 (59.82–245.36)	222.36 (104.03–298.27)	287 (75.50–387.91)	63.17 (39.58–256.91)	0.059[a]
Urine VEGF-A (pg/mL)	171.91 (56.91–223.45)	235.82 (191.55–333.82)	228.82 (168.27–284.73)	144.41 (47.32–291.32)	0.102[a]

[a] Kruskal-Wallis, median (IQR)

the increase in urine VEGF could be due to a combination of the disease process in the kidneys and also the excretion of circulating serum VEGF through the poorly functioning kidneys.

We found significantly poorer renal function, indicated by higher serum creatinine and GFR, and poorer visual acuity in group 2 patients. With higher serum VEGF levels, the state of microangiopathy is poorer in these two target organs. Rodriguez-Poncelas et al. reported the association between diabetic chronic kidney disease and diabetic retinopathy [29]. Grunwald et al. also found that the presence of retinopathy was associated with lower GFR [30].

The higher serum to urine VEGF ratio is in line with higher HbA1c in group 2 (Table 1), in agreement with Amen et al. where they also found higher HbA1c in DR patients [31]. Higher levels of HbA1c reflect poorer glycemic control with higher blood glucose concentration. The formation of advanced glycation end products (AGEs) increases VEGF gene transcription and mRNA production, thereby upregulating VEGF production [32]. Chronic hyperglycemia also triggers a chain of reactions contributing to the accumulation and expression of VEGF and its receptors VEGFR1 and VEGFR2 [11, 33]. Mociran et al. demonstrated that higher HbA1c and diabetic nephropathy both are risk factors for diabetic retinopathy [34]. Our study agrees with this finding and has shown that more severe nephropathy had higher urine and serum VEGF when they develop retinopathy. UKPDS study state that, by maintaining the HbA1c level to ≤ 6.5%, the disease progression will be minimized and there is less risk of developing retinopathy [35].

Table 3 Correlation of serum and urine VEGF level with other parameters

Variables	Serum VEGF		Urine VEGF	
	R value	p value	R value	p value
Urine VEGF	0.251	0.152[a]		
Macula thickness	0.024	0.767[a]	− 0.047	0.562[a]
LogMAR	0.166	0.143[a]	0.021	0.855[a]
HbA1c	− 0.104	0.559[a]	− 0.193	0.274[a]
Serum creatinine	− 0.022	0.849[a]	− 0.145	0.201[a]
GFR	0.021	0.851[a]	− 0.176	0.122[a]
UPCI	− 0.044	0.804[a]	− 0.152	0.390[a]
BMI	0.263	0.133[a]	0.004	0.980[a]

[a] Spearman correlation test

We did not find any correlation between HbA1c and VEGF levels in our patients, contradicting the results of Zehetner et al. who found HbA1c levels correlating with increased plasma levels of VEGF [36]. We also did not find any correlation between VEGF levels with parameters for both renal and visual functions. This suggests that the functional outcome of nephropathy and retinopathy is not directly related to the levels of VEGF in the serum or urine levels but perhaps related to the local changes seen in the kidney and eye. Although hyperglycemic state activates multiple pathways leading to expression and increase of various growth factors including VEGF, there are inter and intra-individual variations of biochemical and physiological response to hyperglycemic state among diabetic patients [37]. This causes variation in the development of complications despite significant or strong risk factors, possibly contributed by a difference in genetic susceptibility [37]. Further biochemical and genetic study is recommended to look into these factors.

VEGF in the eye increases retinal capillary permeability by causing increased phosphorylation of proteins involved with tight junctions causing leakage and subsequent macula edema [38, 39]. It also promotes angiogenesis in the retinal capillaries leading to vascular proliferative changes of DR [15]. Both mechanisms threaten the vision by causing macula edema or proliferative diabetic retinopathy. Despite seeing no difference in macula thickness, we found significantly worse visual acuity in group 2 (Table 1). This may suggest other sources of visual dysfunction such as cataracts or dry eyes contributing to this poorer visual acuity [40].

Cross-sectional studies only give a "snapshot" of patients' condition; all the measurement is made at one point of time. Therefore, a causal relationship cannot be established. However, this cross-sectional study is useful in identifying associations that can then be further studied using a cohort of patients [41]. A prospective study involving both DN and DR patients alike may further explain the sequence of VEGF elevation to elicit the role and potential treatment option for these two microangiopathies.

Other limitations of our study include other confounding factors not assessed, such as hypertension, ischemic heart disease,

and dyslipidemia which were known to increase the risk of developing DR [42] and contribute to higher levels of VEGF [43]. We also did not investigate the influence of treatment modalities on VEGF levels such as ACE inhibitors and insulin which has been shown to reduce circulating VEGF levels [44, 45].

Although it has been reported that VEGF levels in serum tend to be higher than the actual levels when measured in the plasma due to the release of platelet-derived VEGF during platelet aggregation (clotting) [46], we chose to measure serum levels because some readings from plasma VEGF levels were lower than the sensitivity of the ELISA kits as recommended by the manufacturer and therefore will not give any meaningful analysis [47]. Lastly, due to financial constrain in this study, we were only able to obtain 79 study samples which is relatively small. This may have led to non-significant results, especially in correlation and regression analysis.

In conclusion, patients with type 2 diabetes with nephropathy and retinopathy had a poorer renal function, poorer diabetic control, and poorer BCVA, compared to patients with nephropathy but without retinopathy. They also have higher serum VEGF by about twofold, and 1.3 times higher urine VEFG, with a higher serum to urine VEGF ratio than those with nephropathy but without retinopathy. However, the serum and urine VEGF levels were not correlated with each other nor were they correlated with visual or renal function parameters. These changes may suggest elevated urine VEGF levels are predominant in type 2 diabetic patients with kidney disease without retinopathy before microangiopathy worsened to involve both the kidneys and eyes with poorer diabetic control alongside elevated serum and urine VEGF levels.

Acknowledgment The authors thank Dr. Hazlita Mohd Isa for the study ideation and Prof. Suzana Makpol together with her lab technicians for providing the lab facilities and support in running the specimens.

Author contributions Conceptualization: Norshamsiah Md Din; data curation: Hanisah Abdul Hamid; investigation: Norwahidah Abdul Karim; methodology: Wei Yen Kong; project administration: Hanisah Abdul Hamid; resource: Norwahidah Abdul Karim, Wei Yen Kong; supervision: Norfilza Mohd Mokhtar, Norwahidah Abdul Karim, Wei Yen Kong; writing-original draft: Hanisah Abdul Hamid; writing-review and editing: Meng Hsien Yong, Norshamsiah Md Din.

Funding This work was supported by Universiti Kebangsaan Malaysia (grant numbers: FF-2016-337).

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Ethical approval was obtained from the Universiti Kebangsaan Malaysia Research and Ethics Committee (Ethical approval code: FF-2016-337). This study adhered to the tenets of the Declaration of Helsinki and the Malaysian Guidelines for Good Clinical Practice (GCP).

Consent to participate A signed written informed consent was obtained from all patients prior to enrolment.

Consent for publication Upon signing off informed consent prior to enrolment, the patient is aware that the data gathered will be used for publication purposes.

Conflict of interest The authors declare no competing interests.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, 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. https://doi.org/10.1016/j.diabres.2019.107843.
2. National Health & Morbidity Survey. Malaysia: Ministry of Health; 2015. http://www.moh.gov.my/moh/resources/nhmsreport2015vol2.pdf. Accessed 28 May 2017.
3. Abougalambou SSI, Mohamed M, Sulaiman SAS, Abougalambou AS, Hassali MA. Current clinical status and complications among type 2 diabetic patients in Universiti Sains Malaysia hospital. Int J Diabetes Mellit. 2010;2(3):184–8.
4. Screening of Diabetic Retinopathy. Clinical Practice Guidelines. Malaysia; 2011. www.acadmed.org.my/view_file.cfm?fileid=656 Accessed 20 May 2017.
5. Vithian K, Hurel S. Microvascular complications: pathophysiology and management. Clin Med Lond Engl. 2010;10(5):505–9.
6. Benjamin LE. Glucose, VEGF-A, and diabetic complications. Am J Pathol. 2001;158(4):1181–4.
7. Wirostko B, Wong TY, Simó R. Vascular endothelial growth factor and diabetic complications. Prog Retin Eye Res. 2008;27(6):608–21.
8. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci. 1995;108(Pt 6):2369–79.
9. Takahashi S. Vascular endothelial growth factor (VEGF), VEGF receptors and their inhibitors for antiangiogenic tumor therapy. Biol Pharm Bull. 2011;34(12):1785–8.
10. Amadio M, Govoni S, Pascale A. Targeting VEGF in eye neovascularization: What's new? A comprehensive review on current therapies and oligonucleotide-based interventions under development. Pharmacol Res. 2016;103:253–69.
11. Tufro A, Veron D. VEGF and podocytes in diabetic nephropathy. Semin Nephrol. 2012;32(4):385–93.
12. Duh E, Aiello LP. Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. Diabetes. 1999;48(10):1899–906.
13. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, Hartnett ME. Vascular endothelial growth factor in eye disease. Prog Retin Eye Res. 2008;27(4):331–71.
14. Rasol HAA, Azab A. Vitreous, aqueous, and serum levels of vascular endothelial growth factor and angiopoietin-2 in patients with proliferative diabetic retinopathy and diabetic macular edema. Middle East Afr J Ophthalmol. 2007;14(1):3.
15. Funatsu H, Yamashita H, Nakanishi Y, Hori S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of patients

with proliferative diabetic retinopathy. Br J Ophthalmol. 2002;86(3):311–5.
16. Lattanzio R, Brancato R, Pierro L, Bandello F, Iaccher B, Fiore T, et al. Macular thickness measured by optical coherence tomography (OCT) in diabetic patients. Eur J Ophthalmol. 2002;12(6):482–7.
17. Biallosterski C, van Velthoven MEJ, Michels RPJ, Schlingemann RO, DeVries JH, Verbraak FD. Decreased optical coherence tomography-measured pericentral retinal thickness in patients with diabetes mellitus type 1 with minimal diabetic retinopathy. Br J Ophthalmol. 2007;91(9):1135–8.
18. Kashani AH, Zimmer-Galler IE, Shah SM, Dustin L, Do DV, Eliott D, et al. Retinal thickness analysis by race, gender, and age using Stratus OCT. Am J Ophthalmol. 2010;149(3):496–502.e1.
19. Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int. 2004;65(6):2003–17.
20. Nakagawa T, Sato W, Sautin YY, Glushakova O, Croker B, Atkinson MA, et al. Uncoupling of vascular endothelial growth factor with nitric oxide as a mechanism for diabetic vasculopathy. J Am Soc Nephrol. 2006;17(3):736–45.
21. Khamaisi M, Schrijvers BF, De Vriese AS, Raz I, Flyvbjerg A. The emerging role of VEGF in diabetic kidney disease. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2003;18(8):1427–30.
22. Guo L, Jiang F, Tang Y-T, Si M-Y, Jiao X-Y. The association of serum vascular endothelial growth factor and ferritin in diabetic microvascular disease. Diabetes Technol Ther. 2014;16(4):224–34.
23. Mahdy RA, Nada WM, Hadhoud KM, El-Tarhony SA. The role of vascular endothelial growth factor in the progression of diabetic vascular complications. Eye Lond Engl. 2010;24(10):1576–84.
24. Diabetic Nephropathy. Clinical Practice Guidelines. Malaysia; 2004. www.acadmed.org.my/view_file.cfm?fileid=271. Accessed 20 May 2017.
25. Chalam KV, Bressler SB, Edwards AR, Berger BB, Bressler NM, Glassman AR, et al. retinal thickness in people with diabetes and minimal or no diabetic retinopathy: Heidelberg Spectralis Optical Coherence Tomography. Invest Ophthalmol Vis Sci. 2012;53(13):8154–61.
26. Ozturk BT, Bozkurt B, Kerimoglu H, Okka M, Kamis U, Gunduz K. Effect of serum cytokines and VEGF levels on diabetic retinopathy and macular thickness. Mol Vis. 2009;15:1906–14.
27. Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, et al. Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. J Proteome Res. 2009;8(1):113–7.
28. Mahdy RA, Nada WM. Evaluation of the role of vascular endothelial growth factor in diabetic retinopathy. Ophthalmic Res. 2011;45(2):87–91.
29. Rodríguez-Poncelas A, Mundet-Tudurí X, Miravet-Jiménez S, Casellas A, la Puente JFB-D, Franch-Nadal J, et al. Chronic kidney disease and diabetic retinopathy in patients with type 2 diabetes. PLoS One. 2016;11(2):e0149448.
30. Grunwald JE, Alexander J, Ying G-S, Maguire M, Daniel E, Whittock-Martin R, et al. Retinopathy and chronic kidney disease in the chronic renal insufficiency cohort study (CRIC). Arch Ophthalmol. 2012;130(9):1136–44.
31. Mokhtar A, Attia FA, El-Seid SS, El MAA. Vascular endothelial growth factor serum level in patients with diabetic retinopathy. Asian Acad Manag J. 2013;33.
32. Treins C, Giorgetti-Peraldi S, Murdaca J, Van Obberghen E. Regulation of vascular endothelial growth factor expression by advanced glycation end products. J Biol Chem. 2001;276(47):43836–41.
33. Ruszkowska-Ciastek B, Sokup A, Socha MW, Ruprecht Z, Hałas L, Góralczyk B, et al. A preliminary evaluation of VEGF-A, VEGFR1 and VEGFR2 in patients with well-controlled type 2 diabetes mellitus. J Zhejiang Univ Sci B. 2014;15(6):575–81.
34. Mihaela M, Cristian D, Nicolae H. Risk factors and severity of diabetic retinopathy in Maramureş. Appl Med Inform. 2009;24.
35. Stratton IM, Kohner EM, Aldington SJ, Turner RC, Holman RR, Manley SE, et al. UKPDS 50: risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis. Diabetologia. 2001;44(2):156–63.
36. Zehetner C, Kirchmair R, Kralinger M, Kieselbach G. Correlation of vascular endothelial growth factor plasma levels and glycemic control in patients with diabetic retinopathy. Acta Ophthalmol. 2013;91(6):e470–3.
37. Klein R. Diabetic retinopathy and nephropathy. In: Cortes P, Mogensen CE, editors. Contemporary diabetes: the diabetic kidney. Totowa: Humana Press Inc; 2007. p. 473–98.
38. Antonetti DA, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. J Biol Chem. 1999;274(33):23463–7.
39. Romero-Aroca P, Baget-Bernaldiz M, Pareja-Rios A, Lopez-Galvez M, Navarro-Gil R, Verges R. Diabetic macular edema pathophysiology: vasogenic versus inflammatory. J Diabetes Res. 2016;2016:2156273.
40. Skarbez K, Priestley Y, Hoepf M, Koevary SB. Comprehensive review of the effects of diabetes on ocular health. Expert Rev Ophthalmol. 2010;5(4):557–77.
41. Mann CJ. Observational research methods. Research design II: cohort, cross sectional, and case-control studies. Emerg Med J. 2003;20(1):54–60.
42. Management of type 2 diabetes mellitus. Clinical Practice Guidelines. Malaysia; 2015. https://www.moh.gov.my/moh/resources/Penerbitan/CPG/Endocrine/3a.pdf. Accessed 13 April 2017.
43. Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. Endocrinol Metab Clin N Am. 2006;35(3):491–510.
44. Yoshiji H, Kuriyama S, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, et al. The angiotensin-I-converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: possible role of the vascular endothelial growth factor. Clin Cancer Res Off J Am Assoc Cancer Res. 2001;7(4):1073–8.
45. Dandona P, Aljada A, Mohanty P, Ghanim H, Bandyopadhyay A, Chaudhuri A. Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. Diabetes Care. 2003;26(12):3310–4.
46. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem. 2001;47(4):617–23.
47. McIlhenny C, George WD, Doughty JC. A comparison of serum and plasma levels of vascular endothelial growth factor during the menstrual cycle in healthy female volunteers. Br J Cancer. 2002;86(11):1786–9.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):116–125
https://doi.org/10.1007/s13410-021-00962-4

ORIGINAL ARTICLE

Obesity and dyslipidemia among Bhil tribal population: A cross-sectional study from India

Divya Mishra[1,2] · Imnameren Longkumer[1] · Kallur Nava Saraswathy[1] · Rupalika[1,3] · Naorem Kiranmala Devi[1]

Received: 8 October 2020 / Accepted: 22 May 2021 / Published online: 23 June 2021

Abstract

Background Rising obesity epidemic and dyslipidemia majorly contribute to cardiovascular disease burden. Emerging evidence has reported an increasing trend of metabolic disorders among rural tribal populations in developing countries. Hence, the present study attempts to estimate the prevalence of obesity and dyslipidemia and explore their association among Bhil tribal population from India.

Materials and methods This was a cross-sectional study consisting of 277 adults recruited through household survey. Data on socio-demographic and lifestyle variables were collected and obesity was determined using anthropometric measurements. Biochemical assay was done using fasting blood samples. Logistic regression was employed to identify obesity variables associated with dyslipidemia.

Results Almost 40% of the population had generalized obesity (high BMI) while abdominal obesity was found to be relatively higher in terms of WHR (76.9%) followed by WHtR (59.2%) and WC (44.8%). Dyslipidemia in form of low HDL-C was found to be highest (52.3%) followed by high TG (40.8%), TC (7.9%), and LDL-C (6.5%). Sex-wise distribution of obesity and dyslipidemia variables revealed women were significantly more obese and dyslipidemic (low HDL-C) as compared to men. Logistic regression analysis after controlling for confounders revealed a significant association of abdominal obesity with all abnormal lipids except for high TG.

Conclusion The observed high prevalence of obesity and dyslipidemia, particularly low HDL-C and hypertriglyceridemia, warrants a public health concern. This study emphasizes the need for population-specific disease documentation to highlight underlying mechanisms of risk factors and to set forth a targeted approach to disease treatment and management.

Keywords Tribal population · Obesity · Dyslipidemia · Cardiovascular risk factors · Malnutrition

Introduction

Cardiovascular diseases (CVDs) have emerged as the leading cause of mortality with an estimated 17.8 million deaths worldwide, of which 80% occur in low- and middle-income countries (LMICs) [1]. India, being a LMIC, is currently undergoing drastic economic development, accompanied by epidemiological and nutritional transition [2, 3]. Zou et al. [4] reiterated the burden of CVDs among Indians with almost 62% mortality within the age group of 35 to 60 years. Further, this proportion was highest in less developed states, which is a major concern owing to the challenges posed by lack of proper healthcare systems in these relatively poorer regions [5]. Most of the risk factors attributed to CVDs in India include poor dietary intake with increasing incidence of hypertension, obesity, and abnormal lipid profile [1, 2, 5]. However, regional and socioeconomic variations have been observed with respect to these risk factors [2, 5, 6].

The fast-paced increase in CVDs has been majorly contributed by the rising obesity epidemic and dyslipidemia [7–9]. In India, Luhan et al. [8] predicted a doubling in overweight and obesity prevalence by 2040, which is observed to be increasing faster than the global average. Moreover, lipid

✉ Naorem Kiranmala Devi
kmaladevi@gmail.com

[1] Department of Anthropology, University of Delhi, -110007, Delhi, India

[2] Present address: Aurora Health Innovations LLP, -560038, Bangalore, Karnataka, India

[3] Present address: Department of Pulmonary, Critical Care & Sleep Medicine, All India Institute of Medical Sciences, -110029, New Delhi, India

abnormalities, known to promote atherosclerosis, are associated with an increased risk of adverse cardiovascular events including coronary heart disease, myocardial infarction, and stroke [10, 11]. Thus, documenting these risk factors will aid in policy intervention and treatment to reduce premature deaths caused by CVDs.

In India, with enormous diversity at every level, huge variations in the prevalence of overweight/obesity and dyslipidemia have been observed, especially of rural-urban variation [12, 13]. Another aspect accounting for diversity in India is the tribal populations, which account for more than 8.6% of the total population [14]. Traditionally, chronic diseases have been labelled to the affluent societies, attributable to changing lifestyles in terms of dietary habits and sedentary lifestyle [15, 16]. However, rising number of evidence has shown an increasing trend in lifestyle disorders such as obesity, dyslipidemia, hypertension, hyperglycemia, and metabolic syndrome among tribal populations in different parts of India [17–22]. Nevertheless, owing to differential ethnic backgrounds, cultural practices, dietary habits, geographical locations, flora, and fauna, tribal populations are expected to have community-specific risk factors. Thus, it is imperative that lifestyle determinants accounting for major CVD risk should be captured in specific tribal community so as to develop disease prevention strategies and implementation of management programmes.

Bhil is a tribal population inhabiting the Indian states of Gujarat, Maharashtra, Karnataka, Rajasthan, Chhattisgarh, and Madhya Pradesh and they account for the largest tribal population groups in India [14]. Because of rapid globalization and epidemiological transition, Bhil community is expected to be burdened with lifestyle disorders. Understanding the variability in different population groups within the country could provide useful information for targeted intervention for those who need most. In view of this, the present study aimed to estimate the prevalence of obesity and dyslipidemia and explore their association among Bhil tribal population from India.

Method

Area and people

The present study was a cross-sectional study conducted among Bhil tribal population from Rajasthan and rural Delhi (tracing their ancestry from Rajasthan). They are the largest tribal community in Rajasthan and share both linguistic and ethnic affiliation with South Indian tribal population groups [23]. Agriculture is their primary economy, although some are involved in daily-wage labor work.

Participants and data collection

Participants for the present study were recruited as part of a major government funded project with strict inclusion criteria of only Bhil tribe from Rajasthan or tracing their ancestry from Rajasthan and residing in Delhi at the time of sample collection. Both males and females between the age 25 and 70 years were recruited. As genetic screening was part of the project, individuals unrelated up to first cousin were recruited through household surveys. Since the major aim of the project was to estimate cardiovascular adversities in the selected population, sample size was calculated based on pooled estimate for mean prevalence of hypertension (16.1%) among adult tribal populations in India. With confidence interval of 96% and permissible error of 4%, the sample size was calculated to be 357. However, 80 individuals were excluded from the present study due to their refusal during blood collection and/or on medication for chronic diseases (CAD, blood pressure, diabetes). Finally, a total of 277 individuals were included for the present study. The study was approved by the Ethics Committee, Department of Anthropology, University of Delhi. Pre-informed written consent, transcribed in local language, was obtained from each individual prior to recruitment and data collection.

Detailed data on socio-demographic (name, age, sex, migration history, household composition, education, occupation, income, etc.) and lifestyle (smoking status, alcohol consumption, physical activity, and dietary intake) variables were collected using pretested interview schedules. Anthropometric variables, i.e., height vertex, body weight, waist circumference (WC), and hip circumference, were measured following standard protocol. Three measurements were taken and the mean of the replicates was used for analyses.

Definition of overweight and obesity

Overweight was defined as body mass index (BMI) $\geq$ 23.0 kg/m^2 but < 25.0 kg/m^2 while generalized obesity was defined as BMI $\geq$ 25.0 kg/m^2 and underweight as BMI < 18.5 kg/m^2 based on World Health Organization Asia Pacific Guidelines [24].

Abdominal obesity was defined as WC $\geq$ 90 cm for men and $\geq$ 80 cm for women [25]; waist–hip ratio (WHR) $\geq$ 0.90 for men and $\geq$ 0.80 for women and calculated as WC (in cm) divided by hip circumference (in cm) [25]; waist–height ratio (WHtR) $\geq$ 0.5 and calculated as WC (in cm) divided by height vertex (in cm) [26].

Blood collection and biochemical analysis

Overnight fasting intravenous blood (5 ml) was collected into vacutainers with and without ethylenediaminetetraacetic acid (EDTA) (2.5 ml each) from each of the recruited participants

by a trained personnel. Plasma and serum were separated from vacutainers with EDTA and without EDTA, respectively. Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were assayed with spectrophotometer (Dialab instrument) using commercial kits (Randox, USA). Low-density lipoprotein cholesterol (LDL-C) was estimated using Friedewald equation [27].

Classification

Dyslipidemia was classified according to National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) guidelines as [28]:

Hypercholesterolemia–serum cholesterol levels ≥ 200 mg/dl (≥ 5.2 mmol/l).

Hypertriglyceridemia–serum triglyceride levels ≥ 150 mg/dl (≥ 1.7 mmol/l).

Low HDL-C–serum HDL-C < 40 mg/dl (< 1.04 mmol/l) for men and < 50 mg/dl (< 1.3 mmol/l) for women.

High LDL-C–LDL-C levels ≥ 130 mg/dl (≥ 3.4 mmol/l) calculated using the Friedewald equation [27].

Statistical analysis

Statistical analysis was performed using SPSS v.20 (SPSS Inc., Chicago, IL, USA). Student t-test was used to perform test for difference in mean values. Prevalence was reported as number with percent. Chi-square test was used to perform test for difference in categorical variables. Logistic regression test was performed to find the association of obesity variables with dyslipidemia. A p-value of ≤ 0.05 was considered statistically significant.

Results

General characteristics of the study population (Table 1)

Mean age of the studied population was 42.61 (SD ± 11.48) years and females outnumbered males, i.e., 67.5% females as compared to 32.5% of males. Illiteracy was quite high (62.1%) and thus is reflected in the type of occupation they pursue, i.e., manual laborers predominantly agriculturists, which comprised the highest proportion (46.6%) in comparison to other occupation these tribal people practiced. Moreover, 91.0% of the population had per capita annual family income < 45,000 INR indicating that the Bhils belonged to a lower socioeconomic strata. In terms of lifestyle characteristics, approximately one-fourth of Bhils were each smokers (29.3%) as well as alcohol consumers (21.7%).

Prevalence of cardiometabolic risk factors among Bhil tribal population (Table 2)

Individuals with generalized obesity (overweight + obese BMI) were found to be around 39.8%. On the contrary, prevalence of abdominal obesity was much higher with 44.8% of the studied population having high WC; three-fourth of the population had high WHR (76.9%) and more than half of the population had high WHtR (59.2%). Dyslipidemia in the present population varied notably with lowest prevalence consisting of high LDL-C and high TC (6.5% and 7.9%, respectively), while the highest prevalence of abnormal lipid variable was found to be low HDL-C (52.3%). Moreover,

Table 1 General socio-demographic and lifestyle characteristics of the Bhil tribal population

General characteristics		Bhil population
Age (years)	Mean ± SD	42.61 ± 11.48
Sex, n (%)	Male	90 (32.5)
	Female	187 (67.5)
Education, n (%)	Non-literate	172 (62.1)
	Literate	105 (37.9)
Occupation, n (%)	Household work	81 (29.2)
	Manual labor	129 (46.6)
	Employed	52 (18.8)
	Dependent	15 (5.4)
Per capita annual income (INR), n (%)	< 45,000	252 (91.0)
	≥ 45,000	25 (9.0)
Smoking status, n (%)	No	198 (71.5)
	Yes	79 (28.5)
Alcohol consumption, n (%)	No	218 (78.7)
	Yes	59 (21.3)

Abbreviations: *n*, number of individuals; *SD*, standard deviation; *INR*, Indian National Rupees

Table 2 Total and sex-wise prevalence of cardiometabolic risk factors (obesity and abnormal lipids) among the study population

Cardiometabolic risk factors	Bhil population			
	Total, n (%)	Male, n (%)	Female, n (%)	χ^2 *p*-value
Overweight/obese BMI	110 (39.8)	30 (42.3)	80 (51.9)	0.17
High WC	124 (44.8)	25 (27.8)	99 (52.9)	< 0.001*
High WHR	213 (76.9)	59 (65.6)	154 (82.4)	0.002*
High WHtR	164 (59.2)	46 (51.1)	118 (63.1)	0.05*
High TC	22 (7.9)	13 (14.4)	9 (4.8)	0.005*
High TG	113 (40.8)	42 (46.7)	71 (38.0)	0.16
Low HDL-C	145 (52.3)	32 (35.6)	113 (60.4)	< 0.001*
High LDL-C	18 (6.5)	8 (8.9)	10 (5.3)	0.26

Abbreviations: *BMI*, body mass index; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *n*, number of individuals; *TC*, total cholesterol; *TG*, triglyceride; *WC*, waist circumference; *WHR*, waist–hip ratio; *WHtR*, waist to height ratio

Units: kg/m^2, kilogram per square meter; *cm*, centimeter; *mg/dl*, milligram per deciliter

*Significance at *p*-value ≤ 0.05

TG comprised 40.8% of dyslipidemia in the present population.

When the sex-wise prevalence of cardiometabolic risk factors was considered (Table 2), females were found to be significantly more obese in terms of abdominal obesity as compared to that of males (female vs male: WC = 52.9% vs 27.8%; WHR = 82.4% vs 65.6%, and WHtR = 63.1% vs 51.1%). However, there was no sex-wise difference in the prevalence of generalized obesity. Low HDL-C, which was found to be the most prevalent form of dyslipidemia in the present population, was significantly higher among females (60.4%) as compared to males (35.6%). Intriguingly, males had higher prevalence of high TC (14.4%) as compared to females (4.8%).

Relationship of obesity indices with lipid variables (Table 3)

When the distribution of obesity indices in lipid variables was considered in overall population, abdominal obesity in terms of high WC and high WHtR was significantly higher in abnormal TC. On the other hand, all three abdominal obesity indices were significantly higher in low HDL-C group, except for LDL-C which had only high WC differentially distributed between the normal and abnormal groups. Further, generalized obesity with respect to overweight/obese BMI was found to be significantly higher in only low HDL-C and high LDL-C groups. None of the obesity indices were found to present a relationship with high TG.

Table 3 Relationship of obesity indices with lipid variables among the study population

Variables		TC			TG			HDL-C			LDL-C		
		High, n (%)	Normal, n (%)	χ^2 *p*-value	High, n (%)	Normal, n (%)	χ^2 *p*-value	Low, n (%)	Normal, n (%)	χ^2 *p*-value	High, n (%)	Normal, n (%)	χ^2 *p*-value
BMI	Normal	9 (40.9)	106 (52.2)	0.31	48 (52.2)	67 (50.4)	0.79	54 (44.3)	61 (59.2)	0.02*	5 (27.8)	110 (53.1)	0.03*
	Overweight/obese	13 (59.1)	97 (47.8)		44 (47.8)	66 (49.6)		68 (55.7)	42 (40.8)		13 (72.2)	97 (46.9)	
WC	Normal	7 (31.8)	146 (57.3)	0.02*	62 (54.9)	91 (55.5)	0.91	67 (46.2)	86 (65.2)	0.002*	6 (33.3)	147 (56.8)	0.05*
	High	15 (68.2)	109 (42.7)		51 (45.1)	73 (44.5)		78 (53.8)	46 (34.8)		12 (66.7)	112 (43.2)	
WHR	Normal	3 (13.6)	61 (23.9)	0.27	22 (19.5)	42 (25.6)	0.23	24 (16.6)	40 (30.3)	0.007*	3 (16.7)	61 (23.6)	0.50
	High	19 (86.4)	194 (76.1)		91 (80.5)	122 (74.4)		121 (83.4)	92 (69.7)		15 (83.3)	198 (76.4)	
WHtR	Normal	4 (18.2)	109 (42.7)	0.02*	45 (39.8)	68 (41.5)	0.78	51 (35.2)	62 (47.0)	0.04*	4 (22.2)	109 (42.1)	0.09
	High	18 (81.8)	146 (57.3)		68 (60.2)	96 (58.5)		94 (64.8)	70 (53.0)		14 (77.8)	150 (57.9)	

Abbreviations: *TC*, total cholesterol; *TG*, triglyceride; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *BMI*, body mass index; *WC*, waist circumference; *WHR*, waist–hip ratio; *WHtR*, waist to height ratio; *n*, number of individuals

*Significance at *p*-value ≤ 0.05

Association of obesity indices with abnormal lipid variables (Table 4)

Logistic regression analysis, after adjusting for all the confounding factors (Additional file Table 1), revealed that high WC and high WHtR were significantly associated with increased risk for high TC [high WC 3.95 OR (95% CI = 1.42–10.96); high WHtR 3.86 OR (95% CI = 1.26–12.640)]. Moreover, high WC posed 1.70- and 2.85-fold significant increased risk for low HDL-C and high LDL-C, respectively. On the other hand, overweight/obese BMI was found to pose significant increased risk for only high LDL-C [overweight/obese BMI 3.10 OR (95% CI = 1.05–9.15)].

Discussion

In Indian scenario, there is wide heterogeneity in the prevalence of cardiovascular risk factors throughout the country. Thus, identifying community-specific risk factors and their underlying mechanisms can help in targeted disease intervention and prevention. In light of this, the present study details the prevalence of cardiometabolic risk factors, viz. obesity and dyslipidemia, among a single tribal population of India. Alarmingly high prevalence of obesity was observed in conjunction with more than half of the study population with low HDL-C.

In a rapidly developing economies like India, the notion that overweight and obesity are usually present among higher socioeconomic position (SEP) has become more of a fallacy, as growing body of evidence shows a rise in overweight/obesity among lower SEP, particularly among rural populations [29, 30]. Comparative analysis of the National Family Health Surveys (NFHS) 2, 3, and 4 from 1998 to 2016, assessing the prevalence of overweight and obesity, showed an increasing trend, irrespective of SEP [29]. Although these national representative studies exhibit an increasing burden of overweight/obesity in rural populations, unfortunately, there is no such data on tribal populations in India, except for one conducted by the National Nutrition Monitoring Bureau which reported an overweight prevalence of 2–3% among Indian tribes [31]. The present findings, on the other hand, revealed a high prevalence of generalized obesity (overweight + obesity) around 39.8% and even more higher abdominal obesity in terms of WC, WHR, and WHtR as 44.8%, 76.9%, and 59.2%, respectively, among Bhil tribal population. Kshatriya and Acharya [21] reported a cumulative 12.8% prevalence of overweight/obesity among 9 major tribal populations of India. Several other studies on tribal populations reported similar high prevalence of obesity [19, 20, 22]. Further, the present study observed gender differences in the prevalence of obesity whereby women were significantly more obese as compared to men. It has been postulated that women are at a higher risk of developing obesity, particularly of abdominal obesity, during post-menopausal period because of hormonal changes [32]. Our previous study reported the age at menopause among Bhil tribal women as 44.74 years [33]. Interestingly, age-wise prevalence of obesity among women in the present study showed increasing trend of abdominal obesity after the mean menopausal age (Additional file Figure 1). Thus, this aspect could be attributed to the high prevalence of abdominal obesity among women in this population.

Alternately, an underlying mechanism of unhealthy dietary intake may also be responsible for the overall high prevalence of obesity in the present population. Rising trend of obesity in LMIC has been attributed to increasing globalized food markets with consumption of various low quality processed and

Table 4 Logistic regression showing association of obesity with lipid variables among the study population

Obesity variables	High TC			High TG			Low HDL-C			High LDL-C		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
BMI ≥ 23 kg/m^2	1.69	0.66–4.33	0.27	0.88	0.50–1.54	0.65	1.62	0.93–2.81	0.08	3.10	1.05–9.15	0.04*
High WC	3.95	1.42–10.96	0.008*	1.02	0.61–1.69	0.94	1.70	1.01–2.85	0.04*	2.85	1.02–7.96	0.04*
High WHR	2.44	0.66–9.12	0.18	1.49	0.81–2.74	0.19	1.63	0.89–3.00	0.11	1.70	0.47–6.17	0.41
High WHtR	3.86	1.26–12.64	0.02*	0.97	0.58–1.63	0.91	1.32	0.79–2.21	0.28	2.68	0.85–8.47	0.09

Abbreviations: *TC*, total cholesterol; *TG*, triglyceride; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *OR*, odds ratio; *CI*, confidence interval; *kg/m^2*, kilogram per square meter

Variables adjusted for TC: sex, per capita annual income, alcohol consumption

Variables adjusted for TG: age, education

Variables adjusted for HDL-C: age, sex, alcohol consumption

Variables adjusted for LDL-C: alcohol consumption

*Significance at *p*-value ≤ 0.05

Table 5 Prevalence of abdominal and general obesity in India

S. no.	Region in India/population	Sample size	Abdominal obesity† prevalence (%)	General obesity‡ prevalence (%)	Authors
1.	Chennai, Tamil Nadu	2262	70.2	66.8	Kaur et al. [47]
2.	North — Delhi, Haryana; South — Tamil Nadu, Kerala; East — Assam; West — Maharashtra	U = 15,239 Peri-U = 15,760 R = 13,524	44.3 29.5 20.9	34.7 21.4 11.7	WHO-ICMR Indian NCD risk factor Surveillance [48]
3.	Chennai, Tamil Nadu	2350	46.6	45.9	Deepa et al. [49]
4.	Delhi	459	68.9	50.1	Bharadwaj et al. [50]
5.	Tinsukia, Assam (Mishing tribe)	332	11.4	15.7	Misra et al. [51]
6.	Himachal Pradesh	T = 4000 UT = 4000	43.3 59	13.3 29.6	Kapoor et al. [18]
7.	Assam (Rengma-Naga population)	826	-	10.8	Rengma et al. [19]
8.	Tamil Nadu	U = 1047 R = 2521	37.4 22.1	35.7 20.0	ICMR-INDIAB study [12]
	Maharashtra	U = 1215 R = 2594	26.7 15.0	26.1 12.2	
	Jharkhand	U = 921 R = 2286	37.2 8.7	30.4 4.3	
	Chandigarh	U = 880 R = 2336	46.6 32.1	40.3 27.9	
9.	Uttarakhand (Rang Bhotia tribe)	288	34	56.6	Kandpal et al. [20]
10.	West Bengal, Odisha, & Gujarat (9 tribal populations)	2156	21.0*	12.8**	Kshatriya & Acharya [21]
11.	Kerala (Kani tribe)	298	22.1	10.8	Sajeev & Soman [52]
12.	Manipur (Meitei population)	1142	31.3	33.0	Chhungi et al. [22]
	Manipur (Mizo population)	422	63.7	49.1	
	Manipur (Liangmai population)	352	65.3	38.4	

Abbreviations: *R*, rural; *U*, urban; *T*, tribal population; *UT*, urban tribal population

†Abdominal obesity = WC ≥ 90 cm for men and ≥ 80 cm for women

‡General obesity = BMI ≥ 25.0 kg/m^2

*Overweight/obese waist to hip ratio

**Overweight/obese BMI ≥ 23 kg/m^2

packaged foods, increase in the proportion of dietary energy derived from oils, fats, and caloric sweeteners, coupled with decreased consumption of fruits [21, 34, 35]. Indeed, related findings have been reported in our previous study among Bhil tribal population with daily consumption of vegetable oil and trans-fat, increased intake of artificially sweetened beverages, and very low consumption of fruits [36]. This rapid change in dietary "food-basket," which their genome is not adapted to, may trigger the accumulation of adipose tissue. In light of this, and given the extent of obesity-related cardiovascular complications and mortality [7], addressing the obesity epidemic among Indian tribal populations becomes increasingly important for future mitigation of obesity-induced non-communicable diseases (NCDs).

By and large, the pattern of dyslipidemia in India tends to have a lower prevalence of hypercholesterolemia with elevated low HDL-C and triglyceride, more so among tribal populations [37, 38]. The present findings revealed more than half of the study population have low HDL-C. Other studies have reported higher prevalence of low HDL-C among Indian tribes [38, 39], although an exception was observed among Mizo tribal population from North-East India with greater prevalence of hypercholesterolemia as compared to low HDL-C [22]. It has been observed that dietary micronutrient deficiency and undernutrition is associated with lower level of HDL-C [40]. Moreover, economically marginalized tribal populations in India exhibit a high prevalence of micronutrient deficiency and undernutrition [41]. Perhaps Bhils, being an economically impoverished population, dietary micronutrient deficiency and undernutrition may have resulted in decreased HDL-C level, as reflected in the high prevalence of low HDL-C. However, this postulate should be corroborated by further

Table 6 Prevalence of dyslipidemia (overall lipids and lipoprotein wise) in India

S. no.	Region in India/population	Sample size	Overall dyslipidemia	High TC (%)	High TG (%)	Low HDL-C (%)	High LDL-C (%)	Authors
1.	Mumbai, Maharashtra	18.05	-	30.9	29.9	49	67.6	Sawant et al. [53]
2.	Delhi	459	-	26.6	42.7	37.0	51.6	Bhardwaj et al. [50]
3.	Wardha, Maharashtra	529	40.6	25.9	13.9	28.1	17.4	Joshi et al. [54]
4.	Kangra, Himachal Pradesh	R = 450 T = 450	- -	4.9 0.7	60.2 55.2	57.0 48.2	3.8 0.5	Bhardwaj et al. [39]
5.	Multisite study — North, West, East, South, and Central	6123	-	25.0	36.9	42.5	15.8	Guptha et al. [55]
6.	Multisite study — North, South, East, Central	6198	-	14.7	30.2	37.1	-	Gupta et al. [56]
7.	ICMR-INDIAB study	2042	79	13.9	29.5	72.3	11.8	ICMR-INDIAB study [13]
	Tamil Nadu	657	76.9	18.3	30.6	68.9	15.8	
	Maharashtra	473	77	13.7	22.8	69.6	13.3	
	Jharkhand	410	80	4.9	24.1	76.8	3.4	
	Chandigarh	502	82.9	15.5	38.6	75.5	12	
8.	Multisite study	67,398	-	30.4	30.5	59.7	31.6	Gupta et al. [57]
9.	Tamil Nadu	U = 165 R = 160	- -	30.9 25.1	47.9 40.0	69.1 63.1	33.4 23.1	Raj et al. [58]
10.	Manipur (Meitei population)	1142	47.9	22.3	27.8	17.3	8.9	Chhungi et al. [22]
	Manipur (Mizo population)	422	75.1	58.1	49.7	28.9	51.4	
	Manipur (Liangmai population)	352	76.7	35.2	39.0	51.7	27.9	

Abbreviations: *TC*, total cholesterol; *TG*, triglyceride; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *R*, rural; *U*, urban; *T*, tribal population

High TC ≥ 200 mg/dl

High TG ≥ 150 mg/dl

Low HDL-C < 40 mg/dl for men and < 50 mg/dl for women

High LDL-C ≥ 130 mg/d

studies. Notably, 40% of population exhibited hypertriglyceridemia in the present study. The occurrence of hypertriglyceridemia is particularly common in South Asians including Indians due to consumption of high carbohydrate diet [42]. Our previous study also reported high frequency of food items rich in carbohydrate, mainly wheat and rice in the same population [36]. In view of this, the prevalence of high TG in the present population could be attributed to consumption of carbohydrate-rich diet.

It is known that excessive accumulation of body fat promotes obesity resulting in abnormal levels of circulating lipids [43]. Previous study has noted increasing incidence of obesity among dyslipidemic individuals [44], which has also been observed in the present study. Moreover, our study revealed that central obesity posed significantly increased risk for high TC and low HDL-C while both central and general obesity were found to pose significant increased risk for high LDL-C. Similar results were demonstrated in other study indicating obesity as a major risk factor for abnormal lipid levels [45]. Vekic et al. [46] stated that the characteristic feature of obesity-induced dyslipidemia includes high concentration of TG accompanied by reduced HDL-C and mildly increased LDL-C. However, the present study could not find association of obesity with hypertriglyceridemia indicating an independent mechanism or pathways affecting triglyceride level. Nonetheless, obesity seems to be a major contributing factor to abnormal lipid levels among Bhils. Thus, the need to scale up awareness creation on controlling obesity should be a prime focus and promotion of healthy lifestyles to reduce obesity is pertinent.

The present study adds to the accumulating evidence of obesity epidemic and dyslipidemia in India. A pooled analysis of different studies in India (Table 5) revealed general and abdominal obesity ranging from 10.8 to 66.8% and 11.4 to 70.2%, respectively, with urban populations exhibiting higher burden. Similar analysis on lipoproteins (Table 6) showed varying percentage of dyslipidemia prevalence with low HDL-C manifesting the highest form of lipid abnormality in almost all the studies (range between 17.3 and 76.8%). Moreover, the prevalence of high TC, TG, and LDL-C in the pooled analysis ranged from 0.7 to 58.1%, 13.9 to 60.2%, and 0.5 to 67.6%, respectively. Findings from the present study are in concordance with these studies in India highlighting the accumulating evidence of chronic diseases

emerging as leading health concern even among rural tribal populations. Therefore, it is high time to dissociate from the traditional thought that tribal populations are free from lifestyle disorders and NCDs. Prioritizing tribal health management focussing on reducing obesity and dyslipidemia will be crucial to minimizing future disease complications. Bartlett et al. [59] demonstrated that low HDL-C presented a 30 to 60% risk of CVD when accompanied by high TG and LDL-C. Considering the high prevalence of low HDL-C and hypertriglyceridemia in the present tribal population, with obesity and diet as major contributing factors, weight management and healthy dietary modifications should be of paramount importance.

Conclusion

The present study revealed an emerging epidemic of obesity and dyslipidemia, particularly low HDL and hypertriglyceridemia among a single tribal population. A cross-talk between obesity and imbalanced dietary intake is postulated in the rising dyslipidemia among Bhil tribal population. While lifestyle disorders may ramify into severe complications in all populations, but the underlying cause of such may be different in different populations, particularly in a diverse nation like India, owing to the variation in ethnicity, culture, and lifestyle. Thus, results of present study call attention to refrain from "one umbrella" policy approach in addressing obesity and dyslipidemia in India. Instead, identifying population-specific risk factors for targeted approach of intervention strategies, better suited for the population-in-focus, will help reduce the incidence of these chronic metabolic diseases.

Abbreviations CVD, cardiovascular diseases; LMICs, low-middle-income countries; CAD, coronary artery disease; WC, waist circumference; BMI, body mass index; WHR, waist–hip ratio; WHtR, waist–height ratio; EDTA, ethylenediaminetetraacetic acid; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NCEP-ATP III, National Cholesterol Education Program-Adult Treatment Panel III; SPSS, Statistical Package for Social Sciences; SEP, socioeconomic position; NFHS, National Family Health Survey; NCDs, non-communicable diseases

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-00962-4.

Acknowledgments The authors acknowledge University of Delhi and Department of Biotechnology, Government of India for providing financial assistance in the form of DU-DST PURSE grant and all the study participants for their cooperation during the entire study period. The authors would also like to acknowledge Prof. P. C. Joshi, principal investigator, and all the co-investigators for their valuable suggestions and support.

Availability of data and material Data on the present study will be available through request from the corresponding author.

Code availability Not applicable.

Author contribution KNS conceptualized the manuscript. DM, NKD, and R were involved in fieldwork and data collection. DM and IL jointly conducted the data analysis. All authors contributed to the interpretation of the findings. IL wrote the first draft. KNS, DM, IL, and NKD critically revised the manuscript for intellectual content. The corresponding author (NKD) had full data of the study and accounted for the accuracy and integrity of the work. All authors approved the final version of the paper for publication.

Funding The present study was funded by University of Delhi and Department of Science and Technology, Government of India vide DU-DST PURSE grant [RC/2014/7114].

Declarations

Ethics approval The study was approved by the Ethical Committee, Department of Anthropology, University of Delhi.

Consent to participate Informed written consent was obtained from all participants prior to recruitment.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. J Am Coll Cardiol. 2019;74:2529–32. https://doi.org/10.1016/j.jacc.2019.10.009.
2. Dandona L, Dandona R, Kumar GA, Shukla DK, Paul VK, Balakrishnan K, et al. Nations within a nation: variations in epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. Lancet. 2017;390(10111):2437–60. https://doi.org/10.1016/S0140-6736(17)32804-0.
3. Tak M, Shankar B, Kadiyala S. Dietary transition in India: temporal and regional trends, 1993 to 2012. Food Nutr Bull. 2019;40(2):254–70. https://doi.org/10.1177/0379572119833856.
4. Zou Z, Cini K, Dong B, Ma Y, Ma J, Burgner DP, et al. Time trends in cardiovascular disease mortality across the BRICS: an age-period-cohort analysis of key nations with emerging economies using the global burden of disease study 2017. Circulation. 2020;141(10):790–9. https://doi.org/10.1161/CIRCULATIONAHA.119.042864.
5. Prabhakaran D, Jeemon P, Sharma M, Roth GA, Johnson C, Harikrishnan S, et al. The changing patterns of cardiovascular diseases and their risk factors in the states of India: the Global Burden of Disease Study 1990–2016. Lancet Glob Health. 2018;6(12):e1339–51. https://doi.org/10.1016/S2214-109X(18)30407-8.
6. Geldsetzer P, Manne-Goehler J, Theilmann M, Davies JI, Awasthi A, Danaei G, et al. Geographic and sociodemographic variation of cardiovascular disease risk in India: a cross-sectional study of 797,540 adults. PLoS Med. 2018;15(6):e1002581. https://doi.org/10.1371/journal.pmed.1002581.
7. Cercato C, Fonseca FA. Cardiovascular risk and obesity. Diabetol Metab Syndr. 2019;11(1):74. https://doi.org/10.1186/s13098-019-0468-0.
8. Luhar S, Timæus IM, Jones R, Cunningham S, Patel SA, Kinra S, et al. Forecasting the prevalence of overweight and obesity in India

to 2040. PLoS One. 2020;15(2):e0229438. https://doi.org/10.1371/journal.pone.0229438.
9. Lee JS, Chang PY, Zhang Y, Kizer JR, Best LG, Howard BV. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the strong heart study. Diabetes Care. 2017;40(4):529–37. https://doi.org/10.2337/dc16-1958.
10. Pol T, Held C, Westerbergh J, Lindbäck J, Alexander JH, Alings M, et al. Dyslipidemia and risk of cardiovascular events in patients with atrial fibrillation treated with oral anticoagulation therapy: insights from the ARISTOTLE (Apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation) trial. J Am Heart Assoc. 2018;7(3):e007444. https://doi.org/10.1161/JAHA.117.007444.
11. Ciffone NA, Copple T. Managing dyslipidemia for CVD prevention: a review of recent clinical practice guidelines. The Nurse Practitioner. 2019;44(1):8–16. https://doi.org/10.1097/01.npr.0000550246.96902.de.
12. Pradeepa R, Anjana RM, Joshi SR, Bhansali A, Deepa M, Joshi PP, et al. Prevalence of generalized & abdominal obesity in urban & rural India—the ICMR-INDIAB study (Phase-I)[ICMR-INDIAB-3]. Indian J Med Res. 2015;142(2):139. https://doi.org/10.4103/0971-5916.164234.
13. Joshi SR, Anjana RM, Deepa M, Pradeepa R, Bhansali A, Dhandania VK, et al. Prevalence of dyslipidemia in urban and rural India: the ICMR–INDIAB study. PLoS One. 2014;9(5):e96808. https://doi.org/10.1371/journal.pone.0096808.
14. Ministry of Tribal Affairs (Statistics Division), Government of India. Statistical profile of scheduled tribes in India 2013. https://tribal.nic.in/ST/StatisticalProfileofSTs2013.pdf. [Accessed 17 June 2020].
15. Sobal J, Stunkard AJ. Socioeconomic status and obesity: a review of the literature. Psychol Bull. 1989;105(2):260–75. https://doi.org/10.1037/0033-2909.105.2.260.
16. Popkin BM, Paeratakul S, Zhai F, Ge K. A review of dietary and environmental correlates of obesity with emphasis on developing countries. Obes Res. 1995;3:145s–53s. https://doi.org/10.1002/j.1550-8528.1995.tb00457.x.
17. Meshram II, Laxmaiah A. Prevalence of hypertension and its correlates among adult tribal population (≥ 20 years) of Maharashtra State, India. International Journal of Health Sciences and Research. 2014;4(1):130–9 https://www.ijhsr.org/IJHSR_Vol.4_Issue.1_Jan2014/23.pdf.
18. Kapoor D, Bhardwaj AK, Kumar D, Raina SK. Prevalence of diabetes mellitus and its risk factors among permanently settled tribal individuals in tribal and urban areas in northern state of sub-Himalayan region of India. J Chronic Dis. 2014;2014:1–9. https://doi.org/10.1155/2014/380597.
19. Rengma MS, Sen J, Mondal N. Socio-economic, demographic and lifestyle determinants of overweight and obesity among adults of Northeast India. Ethiop J Health Sci. 2015;25(3):199–208. https://doi.org/10.4314/ejhs.v25i3.2.
20. Kandpal V, Sachdeva MP, Saraswathy KN. An assessment study of CVD related risk factors in a tribal population of India. BMC Public Health. 2016;16(1):434. https://doi.org/10.1186/s12889-016-3106-x.
21. Kshatriya GK, Acharya SK. Triple burden of obesity, undernutrition, and cardiovascular disease risk among Indian tribes. PLoS One. 2016;11(1):e0147934. https://doi.org/10.1371/journal.pone.0147934.
22. Chhungi V, Ningombam SS, Yadav S, Singh HS, Devi NK, Chandel S, et al. Prevalence of cardiovascular risk factors among tribal and non-tribal populations with East Asian Ancestry from North East India. Am J Hum Biol. 2019;31(5):e23263. https://doi.org/10.1002/ajhb.23263.
23. Chaubey G, Kadian A, Bala S, Rao VR. Genetic affinity of the Bhil, Kol and Gond mentioned in epic Ramayana. PLoS One. 2015;10(6):e0127655. https://doi.org/10.1371/journal.pone.0127655.
24. World Health Organization, Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet (London, England). 2004;363(9403):157. https://doi.org/10.1016/S0140-6736(03)15268-3.
25. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. https://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/ [Accessed 17 June 2020]
26. Ashwell M, Gibson S. Waist-to-height ratio as an indicator of 'early health risk': simpler and more predictive than using a 'matrix' based on BMI and waist circumference. BMJ Open. 2016;6(3):e010159. https://doi.org/10.1136/bmjopen-2015-010159.
27. Friedewald WT, Levy RI, Fredrikson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
28. 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. 2001;285:2486–97. https://doi.org/10.1001/jama.285.19.2486.
29. Luhar S, Mallinson PAC, Clarke L, Kinra S. Trends in the socioeconomic patterning of overweight/obesity in India: a repeated cross-sectional study using nationally representative data. BMJ Open. 2018;8(10):e023935. https://doi.org/10.1136/bmjopen-2018-023935.
30. Luhar S, Mallinson PAC, Clarke L, Kinra S. Do trends in the prevalence of overweight by socio-economic position differ between India's most and least economically developed states? BMC Public Health. 2019;19(1):783. https://doi.org/10.1186/s12889-019-7155-9.
31. National Nutrition Monitoring Bureau. Diet and nutritional status of tribal population and prevalence of hypertension among adults—report on second repeat survey. NNMB Technical Reports 2009 National Institute of Nutrition: Hyderabad, India. https://www.nin.res.in/downloads/NNMBTribalReport.pdf. [Accessed 8 June 2020]
32. Lumsden MA, Hor K. Impact of obesity on the health of women in midlife. Obstet Gynecol. 2015;17(3):201–8. https://doi.org/10.1111/tog.12199.
33. Yadav S, Mishra J, Murry B, Saraswathy KN. A study on reproductive trajectories of Bhil women of Rajasthan, India. Voice of Intellectual Man-An International Journal. 2018;8(2):141–50. https://doi.org/10.5958/2319-4308.2018.00025.7.
34. Kelly M. The nutrition transition in developing Asia: dietary change, drivers and health impacts. In: Eating, drinking: surviving. Cham: Springer; 2016. p. 83–90. https://doi.org/10.1007/978-3-319-42468-2_9.
35. Misra A, Singhal N, Sivakumar B, Bhagat N, Jaiswal A, Khurana L. Nutrition transition in India: secular trends in dietary intake and their relationship to diet-related non-communicable diseases. Journal of diabetes. 2011;3(4):278–92. https://doi.org/10.1111/j.1753-0407.2011.00139.x.
36. Devi NK, Longkumer I, Chandel S, Mondal PR, Saraswathy KN. No effect of high physical activity on body mass index among Bhil tribal population in India. Online J Health Allied Sci. 2018;17(2):2 https://www.ojhas.org/issue66/2018-2-2.html.
37. Gupta R, Rao RS, Misra A, Sharma SK. Recent trends in epidemiology of dyslipidemias in India. Indian Heart J. 2017;69(3):382–92. https://doi.org/10.1016/j.ihj.2017.02.020.

38. Ismail IM, Azeez K, Antomy A, Kunnummal SV. Metabolic syndrome and its associated factors among the adult population residing in Kannavam tribal area of Kannur District, Kerala. Tropical Journal of Medical Research. 2016;19(1):36. https://doi.org/10.4103/1119-0388.172060.
39. Bhardwaj AK, Kumar D, Raina SK, Bansal P, Bhushan S, Chander V. Community based assessment of biochemical risk factors for cardiovascular diseases in rural and tribal area of Himalayan Region India. Biochem Res. 2013;2013:1–6. https://doi.org/10.1155/2013/696845.
40. Delisle H, Ntandou G, Sodjinou R, Couillard C, Després JP. At-risk serum cholesterol profile at both ends of the nutrition spectrum in West African adults? The Benin study. Nutrients. 2013;5(4):1366–83. https://doi.org/10.3390/nu5041366.
41. Kshatriya GK, Acharya SK. Gender disparities in the prevalence of undernutrition and the higher risk among the young women of Indian tribes. PLoS One. 2016;11(7):e0158308. https://doi.org/10.1371/journal.pone.0158308.
42. Misra A, Wasir JS, Vikram NK. Carbohydrate diets, postprandial hyperlipidaemia, abdominal obesity & Asian Indians: a recipe for atherogenic disaster. Indian J Med Res. 2005;121(1):5.
43. Bays HE, Toth PP, Kris-Etherton PM, Abate N, Aronne LJ, Brown WV, et al. Obesity, adiposity, and dyslipidemia: a consensus statement from the National Lipid Association. J Clin Lipidol. 2013;7(4):304–83. https://doi.org/10.1016/j.jacl.2013.04.001.
44. Zhang FL, Xing YQ, Wu YH, Liu HY, Luo Y, Sun MS, et al. The prevalence, awareness, treatment, and control of dyslipidemia in northeast China: a population-based cross-sectional survey. Lipids Health Dis. 2017;16(1):61. https://doi.org/10.1186/s12944-017-0453-2.
45. Opoku S, Gan Y, Fu W, Chen D, Addo-Yobo E, Trofimovitch D, et al. Prevalence and risk factors for dyslipidemia among adults in rural and urban China: findings from the China National Stroke Screening and prevention project (CNSSPP). BMC Public Health. 2019;19(1):1500. https://doi.org/10.1186/s12889-019-7827-5.
46. Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. Obesity and dyslipidemia. Metabolism. 2019;92:71–81. https://doi.org/10.1016/j.metabol.2018.11.005.
47. Kaur P, Rao TV, Sankarasubbaiyan S, Narayanan AM, Ezhil R, Rao SR, et al. Prevalence and distribution of cardiovascular risk factors in an urban industrial population in south India: a cross-sectional study. JAPI. 2007;55:771–6.
48. Mohan V, Mathur P, Deepa R, Deepa M, Shukla DK, Menon GR, et al. Urban rural differences in prevalence of self-reported diabetes in India—the WHO–ICMR Indian NCD risk factor surveillance. Diabetes Res Clin Pract. 2008;80(1):159–68. https://doi.org/10.1016/j.diabres.2007.11.018.
49. Deepa M, Farooq S, Deepa R, Manjula D, Mohan V. Prevalence and significance of generalized and central body obesity in an urban Asian Indian population in Chennai, India (CURES: 47). Eur J Clin Nutr. 2009;63(2):259–67. https://doi.org/10.1038/sj.ejcn.1602920.
50. Bhardwaj S, Misra A, Misra R, Goel K, Bhatt SP, Rastogi K, et al. High prevalence of abdominal, intra-abdominal and subcutaneous adiposity and clustering of risk factors among urban Asian Indians in North India. PLoS One. 2011;6(9):e24362. https://doi.org/10.1371/journal.pone.0024362.
51. Misra PJ, Mini GK, Thankappan KR. Risk factor profile for non-communicable diseases among Mishing tribes in Assam, India: results from a WHO STEPs survey. Indian J Med Res. 2014;140(3):370. https://www.ijmr.org.in/text.asp?2014/140/3/370/143788–8.
52. Sajeev P, Soman B. Prevalence of noncommunicable disease risk factors among the Kani tribe in Thiruvananthapuram district, Kerala. Indian Heart J. 2018;70(5):598–603. https://doi.org/10.1016/j.ihj.2018.01.022.
53. Sawant AM, Shetty D, Mankeshwar R, Ashavaid TF. Prevalence of dyslipidemia in young adult Indian population. Japi. 2008;56(2):99–102.
54. Joshi R, Taksande B, Kalantri SP, Jajoo UN, Gupta R. Prevalence of cardiovascular risk factors among rural population of elderly in Wardha district. J Cardiovasc Dis Res. 2013;4(2):140–6. https://doi.org/10.1016/j.jcdr.2013.03.002.
55. Guptha S, Gupta R, Deedwania P, Bhansali A, Maheshwari A, Gupta A, et al. Cholesterol lipoproteins and prevalence of dyslipidemias in urban Asian Indians: a cross sectional study. Indian Heart J. 2014;66(3):280–8. https://doi.org/10.1016/j.ihj.2014.03.005.
56. Gupta A, Gupta R, Sharma KK, Lodha S, Achari V, Asirvatham AJ, et al. Prevalence of diabetes and cardiovascular risk factors in middle-class urban participants in India. BMJ Open Diabetes Res Care. 2014;2(1):e000048. https://doi.org/10.1136/bmjdrc-2014-000048.
57. Gupta R, Sharma M, Goyal NK, Bansal P, Lodha S, Sharma KK. Gender differences in 7 years trends in cholesterol lipoproteins and lipids in India: insights from a hospital database. Indian J Endocrinol Metab. 2016;20(2):211–8. https://doi.org/10.4103/2230-8210.176362.
58. Ajay Raj S, Sivakumar K, Sujatha K. Prevalence of dyslipidemia in South Indian adults: an urban-rural comparison. Int J Community Med Public Health. 2016;3(8):2201. https://doi.org/10.18203/2394-6040.ijcmph20162571.
59. Bartlett J, Predazzi IM, Williams SM, Bush WS, Kim Y, Havas S, et al. Is isolated low high-density lipoprotein cholesterol a cardiovascular disease risk factor? New insights from the Framingham offspring study. Circ Cardiovasc Qual Outcomes. 2016;9(3):206–12. https://doi.org/10.1161/CIRCOUTCOMES.115.002436.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):126–137
https://doi.org/10.1007/s13410-021-00952-6

ORIGINAL ARTICLE

Dietary supplementation of mustard oil reduces blood glucose levels by triggering insulin receptor signaling pathway

P. Anjali Devi[1] · V. Pandiyan[1] · T. M. A. Senthil Kumar[2] · G. V. P. P. S. Ravi Kumar[3] · K. Padmanath[1]

Received: 24 July 2020 / Accepted: 5 April 2021 / Published online: 26 April 2021

Abstract

Background Dietary fatty acids can alter membrane fatty acid composition with the consequent change in the action of various receptors. Incorporation of mustard oil was found to increase insulin secretion, reduce blood glucose levels, and increase the expression of glucose transporter gene 4 (*Glut4*).

Methods Three-week-old male Wistar rats were fed with 8% lipid-inclusive isocaloric mash diet. There were non-diabetic control (NDC) and diabetic control (DC) groups fed with ghee, and similarly non-diabetic (NDT) and diabetic treatment (DT) groups fed with mustard oil. Streptozotocin (STZ) was administered intraperitoneally once at a dose rate of 40 mg/kg bodyweight for the induction of diabetes. Blood glucose was estimated using glucometer periodically. Lipids were extracted from mustard oil and in tissue samples, and fatty acid estimation was done using gas chromatography (GC). Gene expression of 84 genes related to diabetes was measured in muscle tissue using Qiagen™ RT^2 polymerase chain reaction (PCR) profiler array. The real-time PCR data obtained as threshold cycle (Ct) values were analyzed using Ingenuity Pathway Analysis® (IPA®) software.

Results After induction of diabetes by day 30, the average glucose levels were above 500 mg/dL in diabetic groups, but for the mustard oil treatment group, they were reduced to 337 mg/dL by the 60 days of treatment. Significantly higher levels of unsaturated fatty acids particularly linoleic acid and linolenic acid were found in mustard oil treatment groups. Insulin receptor signaling was prominent in both ghee-fed normal and mustard oil–fed diabetic treatment groups. Glucose was found to be the major upstream regulator in all the groups except for ghee-fed diabetic control group.

Conclusions Mustard oil inclusion in the diet reduces blood glucose levels by increased insulin receptor signaling, thereby partially reversing diabetic state in experimentally induced diabetic rats.

Keywords Mustard oil · Blood glucose level · Relative gene expression · Pathway analysis · Fatty acid profile · Gas chromatography · Polymerase chain reaction (PCR) profiler array

Introduction

Diabetes mellitus is a chronic metabolic disorder with a direct impact on the health and economy of the affected individual. Dietary intervention is always being part of a therapeutic regimen in the treatment of diabetes. The fatty acid composition of the diet is known to influence the fatty acid composition of skeletal muscle phospholipids [1–3].

✉ K. Padmanath
padmanath.k@tanuvas.ac.in

P. Anjali Devi
anjuvet6690@gmail.com

V. Pandiyan
mypands@rediffmail.com

T. M. A. Senthil Kumar
tmaskumar@yahoo.com

G. V. P. P. S. Ravi Kumar
gandham71@gmail.com

[1] Department of Veterinary Biochemistry, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai 600007, India

[2] Vaccine Research Centre-Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai 600051, India

[3] National Institute of Animal Biotechnology, 37/Survey No.37, Extended Q City Road, Opposite HP Petrol Pump & Mysamma Temple, Gowli doddy, Hyderabad, Telangana 500075, India

Diet plays a significant role in improving insulin sensitivity and reducing the risk of diabetes and its complications [4, 5]. Dietary fat can have huge implications on the fatty acid composition of the cell membrane and its functions like membrane fluidity, ion permeability, insulin receptor binding, and interaction of glucose transporters with second messengers [6–8]. Membranes enriched with the unsaturated fatty acids have shown to increase the number of insulin receptors and their affinity to insulin [9, 10], bind more insulin [1, 11] and improve insulin action [12–15].

Oils constitute an important source of lipids and the energy in the human diet which provides a wide variety of unsaturated fatty acids [16]. Though there is no particular oil which is identified as an elixir to cure diabetes, incorporation of oils like peanut oil [17], avocado oil [18], sunflower oil [19], sesame oil [20], *Nigella sativa* oil [21], olive oil [22, 23] fish oil [24, 25], and mustard oil [26] has shown beneficial effects in patients with diabetes by improving glycemic control. Supplementation of oils like palm oil [27], soya bean oil, and olive oil [28] in the diet reduces blood glucose levels by increasing insulin secretion. Oils like extra virgin olive oil in the diet reverse insulin resistance [22]. Oils in the diet also cause β-cell regeneration [26, 29], and also their performance [22]. Dietary supplementation of oils rich in unsaturated fatty acids influenced glucose transporter 4 (*Glut4*) gene expression and insulin receptor signaling in muscles [24, 25, 30, 31].

Our previous study [26] has shown that blood glucose levels were decreased by dietary incorporation of mustard oil. When mustard oil was added to the diet, β-cell regeneration, increased insulin secretion, and upregulated *Glut4* expression in muscle cells were also observed. However, the mechanism by which dietary supplementation with mustard oil lowered blood glucose levels was not known. We had two key questions that arose after the study. The first question concerned the underlying molecular mechanisms responsible for increased insulin secretion, and the second question concerned the associated cellular biochemical pathways that resulted in increased *Glut4* expression and decreased blood glucose levels. In the previous research, although the regeneration mechanism was not apparent, insulin secretion would have been increased by regenerated β-cells. This answered our first question partially. Hence, to find an answer to the second question, the present study was undertaken. Generally, in the absence of diet or exercise, much of the glucose transporter protein (GLUT4) is sequestered in storage vesicles in the cell. GLUT4 storage vesicles undergo exocytosis during exercise or when insulin is available to transmit GLUT4 to the plasma membrane, which then carries out glucose transport [32, 33]. There were missing links in our findings, i.e., increased insulin secretion, increased *Glut4* expression, and decreased blood glucose levels. The rationale behind the present study was to find the connection between them.

Materials and methods

Study design, animals, and diets

Male Wistar albino rats of 5 weeks of age were procured from Laboratory Animal Medicine Unit, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India. They were housed in polypropylene cages on clean corncob beddings and were maintained under a controlled temperature of 20 ± 2 °C in 12-h light to dark cycles and were given access to feed and purified water. Rats were acclimatized for 2 weeks before the treatment. The rats were randomly divided into four treatment groups with eight animals in each group. The experimental rats were fed with isocaloric mash diets formulated and procured from Central Feed Technology Unit, TANUVAS, Kattupakkam, Kanchipuram District, India. Among the four groups, two groups were induced with experimentally diabetes. Before induction of diabetes mellitus, the blood glucose level was assessed by ACCU CHEK® Active glucometer to rule out spontaneous diabetes in the rats. Animals, showing normal blood glucose levels between 80 and 110 mg/dL, were selected and were fasted overnight. To 80 mL of distilled water, 0.52 g of trisodium citrate and 0.677 g of citric acid were added and mixed well; then, pH 4.5 was maintained and the volume was made up to 100 mL with distilled water to prepare 0.1 M citrate buffer. Streptozotocin (STZ) at the dose rate of 40 mg/kg b. wt. was dissolved in 0.1 M cold citrate buffer (pH 4.5) was given intraperitoneally once to induce diabetes. These animals were allowed access to 5% glucose solution overnight, to prevent hypoglycemia by massive pancreatic insulin release due to the destruction of pancreatic β-cells. The diabetes was confirmed by measuring blood glucose concentration 72 h after STZ injection. The rats with a fasting blood glucose level above 250 mg/dL were used for the experiment. The fourth day after STZ injection was considered day 1 of the trial; the experiment was continued for 60 days.

At the start of the trial, there were four groups of rats: the non-diabetic control (NDC), diabetic control (DC) groups fed with ghee, non-diabetic treatment (NDT), and diabetic treatment (DT) groups treated with mustard oil. Diets had similar composition except for the source of fat, which were ghee and mustard oil given at 8% inclusion level. Table 1 presents the percentage composition of various ingredients in the experimental diet. Twenty grams of feed was fed to each rat per day, throughout the study period. When the experimental diet was changed, there was an average reduction of 20% in the feed intake per day in both ghee and mustard oil–fed rats for a week. Then, the feed intake resumed to the levels before the diet change and that continued until the end of the study. The blood glucose level of all the rats from groups I to IV was

assessed by ACCU CHEK® Active glucometer on the 0th, 30th, and 60th days. Similarly, bodyweight was also measured on the 0th, 30th, and 60th days. Rats were sacrificed on the 60th day, and samples were collected for analysis.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Madras Veterinary College, Chennai, India, and animals were taken care of according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Chemicals and supplements

Cold-pressed mustard oil was purchased from Parambhariyam™ cold-pressed oil store, Perungalathur, Chennai, 600063, India. The oil was extracted in the mill inside the store using a motorized oil press machine made up of the wood of the *Albizia lebbeck* tree. Seeds of Indian mustard (*Brassica juncea*) were used to extract oil. The source of the mustard was unknown, as that was purchased from the wholesale market. The oil produced was unrefined and without any additives. The oil was purchased on the same day of production and was used within 10 days of purchase. Ghee was purchased from a dairy plant, Department of Livestock Products Technology (Dairy Science), Madras Veterinary College, Chennai, 600007. The ghee was used within a week of purchase. The milk used for producing ghee was procured from Instructional Livestock Farm Complex, Madhavaram Milk Colony, Chennai, 600051. Analytical-grade STZ was purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra, India. Reagents for ribonucleic acid (RNA) isolation, complementary deoxyribonucleic acid (cDNA) synthesis, and real-time polymerase chain reaction (RT-PCR) were purchased from Qiagen Inc., India.

Collection of muscle sample

Animals were sacrificed at the end (60th day) of the experiment. Muscle tissue from the right thigh muscle was collected and used for fatty acid estimation. A portion of the same muscle was collected in RNAlater™ solution and stored at −20 °C until used for gene expression studies.

Measurement of fatty acids

Lipids were extracted from mustard oil and tissue samples [34]. An SP2380 column (Merck) with 30 m × 0.25 mm × 0.2-μm film thickness was used. Gas chromatography (GC) was done using Chemito™–GC 8610 with the following conditions. The oven temperature was held at 160 °C for 3 min and increased at a rate of 5 °C per min up to 220 °C and held at 220 °C for 7 min. The injector and detector temperature was set at 250 °C. The carrier gas flow rate for nitrogen was 4 to 5 mL/min and zero air-300 mL/min with a split of 50:1. For standard calibration, a fatty acid methyl ester (FAME) mix of 0.1 μL obtained from Supelco® was injected into the column, fixing the temperature and flow rate of gases and choosing the optimum range and sensitivity. The standard FAME was calibrated, stored in a file, and retained whenever the unknown samples were injected.

Differential mRNA expression of diabetes-related genes in muscle tissue

Gene expression of muscle tissue homogenates was determined by using RT^2 PCR profiler array™. RNA isolation was performed using Qiagen™ RNeasy® mini kit. cDNA was synthesized using Qiagen™ RT^2 first strand kit. The quantification of cDNA was done by Nanodrop™ spectrophotometer. Real-time PCR was performed using Bio-Rad iCycler, CFX96 thermal cycler, using RT^2 PCR profiler array™ plates with code PARN-023ZA-2 purchased from Qiagen™. All the above procedures were performed as per the instructions of the manufacturer. This RT^2 PCR profiler array™ is a 96-well plate with built-in primers to monitor the expression of 84 genes related to diabetes, plus 5 housekeeping genes (Actb, B2m, Hprt1, Ldha, and Rplp1). This array also includes control elements for data normalization, genomic DNA contamination detection, RNA sample quality, and general PCR performance. The threshold cycle (Ct) values for each gene obtained were fed into GeneGlobe™ Data Analysis Center, a web-based software tool provided by Qiagen™ Inc. This software performs normalization to the housekeeping genes (HKG) by calculating the ΔCt for each gene of interest (GOI) in the plate (Ct value of GOI-Ct value of HKG). Any Ct

Table 1 Percentage composition of various ingredients in the experimental diet

Ingredients	Ghee included diet (%)	Mustard oil included diet (%)
Maize	70	70
Soya bean meal	10	10
Fish meal	10	10
Ghee	8	-
Mustard oil	-	8
Mineral mixture	1.5	1.5
Salt	0.5	0.5
Total	100	100
ME kcal/kg	3475	3475
Crude protein (%)	16.7	16.7

Table 2 Effect of mustard oil on bodyweight in control and experimental groups of rats (g)

Groups	0th day	30th day	60th day	*F* value
NDC	$218.00^{aA} \pm 6.14$	$288.33^{bB} \pm 10.75$	$265.83^{cB} \pm 5.49$	21.12**
NDT	$205.67^{aA} \pm 6.45$	$277.67^{bA} \pm 12.12$	$254.17^{cA} \pm 9.48$	1.06^{NS}
DC	$207.83^{aB} \pm 12.23$	$155.17^{aA} \pm 10.97$	$145.67^{aA} \pm 10.59$	8.702**
DT	$193.83^{aA} \pm 8.80$	$162.00^{aA} \pm 5.63$	$169.33^{bA} \pm 4.59$	0.05^{NS}
F value (between groups)	1.284*	50.057**	57.171**	

*Significant ($p < 0.05$); **highly significant ($p < 0.01$)

Means bearing different superscripts in a column differ significantly between groups

value > 35 was a negative call. The web-based software package automatically performs all ΔΔCt-based fold-change calculations from the uploaded raw threshold cycle data based on the widely used and agreed upon ΔΔCt method [35]. The software also allows for the ability to define the best reference genes for normalization with guidance and recommendations.

Functional analysis using Ingenuity Pathway Analysis

The pathway analysis including canonical pathways was performed using the Ingenuity Pathway Analysis (IPA) tool™ software provided by Qiagen™ and licensed to Indian Veterinary Research Institute, Izatnagar, Bareilly, India. Ingenuity Pathway Analysis™ is an all-in-one, web-based software application that enables analysis, integration, and understanding of data from gene expression, micro RNA (miRNA), and single-nucleotide polymorphism (SNP) microarrays, as well as metabolomics, proteomics, and RNAseq experiments. The results of the IPA™ were compared, and the involvement of a particular pathway was chosen [36].

Results

Effect of mustard oil on bodyweight

The changes in the bodyweight of rats in the four different groups are mentioned in Table 2. Rats in NDC and NDT groups showed an increase in the bodyweight irrespective of the lipid type fed. There was a significant decrease in the bodyweight of the DC rats. Rats belonging to the DT group showed a significant decrease in bodyweight by the 30th day of the trial, whereas a significant increase was observed by the 60th day of the trial.

Effect of mustard oil on blood glucose level

The changes in the blood glucose levels of rats in the four different groups are mentioned in Table 3. After the induction of diabetes, a highly significant increase in blood glucose levels was observed in the rats of DC during the entire period of study. This group showed an elevated blood glucose level which was more than fivefold higher during the 30th day and 60th day when compared to blood glucose level at the start of the trial. In the DT group, though there was a significant increase in blood glucose level observed on the 30th day, there was a significant reduction of blood glucose level during day 60 when compared to blood glucose level at the start of the trial.

Fatty acid composition of mustard oil and its effects on muscle tissue of experimental rats

The fatty acid composition of mustard oil was analyzed, and the values obtained are presented in Table 4. The effects of mustard oil in muscle tissue of the control and the experimental group of rats are presented in Table 5. The rats in the DT group showed a highly significant ($p < 0.01$) reduction in

Table 3 Effect of mustard oil on blood glucose level in control and experimental groups of rats (mg/dL)

Groups	0th day	30th day	60th day	*F* value
NDC	$91.17^{abA} \pm 1.99$	$108.33^{aB} \pm 3.84$	$104.17^{aB} \pm 3.38$	7.991**
NDT	$104.00^{bB} \pm 3.29$	$116.17^{aC} \pm 4.16$	$93.33^{aA} \pm 1.89$	12.364**
DC	$105.67^{bA} \pm 7.59$	$535.33^{bB} \pm 31.32$	$576.67^{cB} \pm 17.26$	152.687**
DT	$84.67^{aA} \pm 4.57$	$565.00^{bC} \pm 12.64$	$337.50^{bB} \pm 34.15$	128.561**
F value (between groups)	4.417**	218.522**	141.551**	

**Highly significant ($p < 0.01$)

Means bearing different superscripts in a column differ significantly between groups

Table 4 Fatty acid level in mustard oil (%)

Fatty acid	Percentage
Myristic acid	0.24
Palmitic acid	27.97
Stearic acid	14.55
Oleic acid	27.71
Linoleic acid	13.29
Linolenic acid	0.27
Arachidic acid	0.23
Behenic acid	6.89
Ecosapentaenoic acid	0.61
Docosahexaenoic acid	2.17
Palmitoleic acid	1.87
Others	4.13

saturated fatty acids such as myristic acid, palmitic acid, and stearic acid when compared to DC. The rats in the DT group showed a highly significant ($p < 0.01$) increase in monounsaturated fatty acids such as oleic acid and palmitoleic acid when compared to the DC group. The rats in the DT group showed a highly significant ($p < 0.01$) increase in polyunsaturated fatty acids (PUFA) such as linoleic acid, linolenic acid, and eicosapentaenoic acid when compared to the DC group.

Real-time PCR analysis of various genes related to diabetes

In this study, the relative gene expression of various genes related to diabetes was studied in all the groups. The obtained Ct values using real-time PCR was fed into a web-based software tool provided by Qiagen™ Inc., and relative gene expression of other groups to normal control was analyzed and is presented in Table 6. The heat map of the canonical pathway analysis of all groups is shown in Fig. 1. Among the target genes related to diabetes, more than 80% of genes were upregulated showing twofold differences compared to the normal control irrespective of the treatment. The differentially expressed genes were involved in carbohydrate, amino acid metabolism, immunity and defense, lipid, fatty acid, steroid metabolism, and signal transduction.

Ingenuity Pathway Analysis®

Among the pathways and molecules analyzed by the IPA®, the top five canonical pathways and upstream regulatory molecules were chosen for comparative analysis. Among the canonical pathways, insulin receptor signaling pathway was found to be prominent in both the NDC and DT groups (Table 7). Among the top five upstream regulators found in this analysis, glucose was present as an upstream regulator in all the groups except DC (Table 8).

Discussion

Dietary inclusion of various vegetable oils tends to influence blood glucose levels in animals and human beings [20]. Unsaturated fatty acids have a greater impact on metabolic disorders especially on diabetes mellitus, cardiovascular disease, etc. Hence, the present study was undertaken to assess the influence of supplementation of mustard oil on blood glucose level, the fatty acid composition of muscle tissue, and on the expression of genes related to diabetes in a way to elucidate the molecular mechanisms behind the reduction of blood glucose level. This is the first report on the examination

Table 5 Effect of mustard oil on muscle tissue fatty acids in experimental groups (%)

Fatty acid	NDC	NDT	DC	DT	*F* value
Myristic acid (14:0)	$2.11^{d} \pm 0.08$	$1.07^{b} \pm 0.02$	$2.11^{c} \pm 0.06$	$0.68^{a} \pm 0.01$	753.05**
Palmitic acid (16:0)	$26.70^{c} \pm 0.71$	$19.68^{b} \pm 0.54$	$25.87^{c} \pm 0.77$	$16.10^{a} \pm 0.63$	57.50**
Stearic acid (18:0)	$4.86^{b} \pm 0.46$	$4.12^{b} \pm 0.48$	$10.18^{b} \pm 0.93$	$5.83^{a} \pm 0.43$	19.83**
Oleic acid (18:1)	$34.55^{b} \pm 0.99$	$34.30^{b} \pm 0.37$	$28.80^{a} \pm 1.67$	$32.39^{b} \pm 0.64$	6.50**
Linoleic acid (18:2)	$18.61^{a} \pm 0.53$	$25.62^{c} \pm 0.53$	$21.07^{b} \pm 0.86$	$26.54^{c} \pm 0.31$	39.68**
Linolenic acid (18:3)	$0.54^{a} \pm 0.04$	$1.20^{b} \pm 0.20$	$0.81^{ab} \pm 0.23$	$2.47^{c} \pm 0.14$	25.60**
Arachidic acid (20:0)	$0.15^{a} \pm 0.02$	$0.33^{b} \pm 0.01$	$0.13^{a} \pm 0.01$	$0.42^{c} \pm 0.05$	25.08**
Behenic acid (22:0)	$2.69^{a} \pm 0.26$	$5.88^{bc} \pm 0.43$	$4.84^{b} \pm 0.64$	$7.04^{c} \pm 0.34$	17.64**
Ecosapentaenoic acid (20:5)	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.07^{ab} \pm 0.01$	$0.09^{b} \pm 0.01$	3.14*
Docosahexaenoic acid (22:6)	$1.54^{a} \pm 0.09$	$1.56^{a} \pm 0.16$	$3.57^{b} \pm 0.54$	$1.87^{a} \pm 0.08$	11.11**
Palmitoleic acid (16:1)	$5.87^{c} \pm 0.39$	$3.36^{b} \pm 0.27$	$1.26^{a} \pm 0.14$	$1.32^{a} \pm 0.14$	72.22**
Others	$0.65^{a} \pm 0.01$	$2.54^{b} \pm 0.38$	$1.37^{a} \pm 0.29$	$5.08^{c} \pm 0.37$	41.36**

*Significant ($p < 0.05$); **highly significant ($p < 0.01$)

Means bearing different superscripts in a column differ significantly between groups

Table 6 Real-time PCR analysis of various genes related to diabetes (Ct)

	Code	Gene	NDC	NDT	DC	DT
1	NM_012544	Ace	24.32	22.80	-	22.59
2	NM_016987	Acly	24.13	23.36	-	23.01
3	NM_017191	Adra1a	24.32	22.06	22.18	22.63
4	NM-013108	Adrb3	24.27	22.70	21.94	22.31
5	NM_134432	Agt	23.90	22.66	21.81	22.05
6	NM_017093	Akt2	23.86	22.80	22.21	22.53
7	NM_012909	Aqp2	24.08	23.76	22.01	22.38
8	NM_03116	Ccl5	23.95	23.36	22.38	23.14
9	NM_021866	Ccr2	24.06	22.37	21.42	22.36
10	NM_013121	Cd28	28.49	26.57	26.49	27.16
11	NM_013755	Ceacam1	24.61	22.92	21.89	22.89
12	NM_012524	Cebpa	24.65	20.72	22.32	22.68
13	NM_031674	Ctla4	24.48	23.37	-	22.65
14	NM_012789	Dpp4	24.40	22.91	-	23.08
15	NM_022199	Dusp4	24.23	21.91	21.76	22.27
16	NM_053535	Enpp1	24.16	22.99	22.20	22.31
17	NM_012558	Fbp1	-	-	-	-
18	NM_001101680	Foxc2	24.04	23.06	21.78	22.48
19	NM_012560	Foxg1	24.18	25.38	22.12	22.32
20	NM_001108250	Foxp3	25.05	23.77	22.59	23.45
21	NM_013098	G6pc	24.69	23.06	22.54	22.81
22	NM_012707	Gcg	24.06	22.57	21.45	22.18
23	NM_172092	Gcgr	26.17	22.94	23.19	23.44
24	NM_012565	Gck	25.05	21.42	22.04	22.44
25	NM_012728	Glp1r	24.72	21.40	-	22.92
26	NM_022215	Gpd1	24.31	23.22	-	22.82
27	NM_032080	Gsk3b	24.12	21.72	21.63	22.38
28	NM_012580	Hmox1	24.36	22.67	22.29	22.70
29	NM_013103	Hnf1b	24.39	23.13	22.22	22.49
30	NM_022180	Hnf4a	23.79	22.32	21.99	22.24
31	NM_012967	Icam1	23.93	23.24	22.07	22.71
32	NM_013159	Ide	24.26	23.22	21.73	22.94
33	NM_138880	Ifng	24.62	22.67	22.56	23.30
34	NM_012817	Igfbp5	24.23	23.15	22.87	22.93
35	NM_053355	Ikbkb	25.41	22.81	22.36	22.72
36	NM_012854	Il10	25.28	22.06	21.94	22.43
37	NM_022611	Il12b	25.20	23.31	-	23.03
38	NM_133380	Il4r	24.80	23.98	-	22.48
39	NM_012589	Il6	24.10	22.52	21.99	22.63
40	NM_022955	Inppl1	24.20	-	21.74	22.27
41	NM_019129	Ins1	23.45	21.62	20.86	21.67
42	NM_012969	Irs1	24.38	22.57	21.46	22.42
43	NM_001168633	Irs2	24.78	23.34	22.48	23.14
44	NM_031020	Mapk14	24.69	23.25	22.69	22.77
45	NM_053829	Mapk8	24.38	22.89	22.12	22.47
46	NM_019218	Neurod1	24.64	22.85	22.22	22.83
47	NM_001276711	Nfkb1	23.51	22.44	21.12	22.17
48	NM_013093	Nkx2-1	26.05	22.29	22.12	22.92
49	NM_021838	Nos3	24.72	23.44	-	22.60

Table 6 (continued)

	Code	Gene	NDC	NDT	DC	DT
50	NM_001100708	Nrf1	25.36	21.27	-	23.65
51	NM_021748	Nsf	24.34	23.17	22.42	22.94
52	NM_013063	Parp1	24.73	22.92	22.34	23.13
53	NM_022852	Pdx1	25.05	23.59	22.36	23.06
54	NM_001108978	Pik3cd	24.23	24.12	22.71	23.27
55	NM_013005	Pik3r1	25.70	23.18	23.08	23.90
56	NM_013196	Ppara	25.05	20.90	21.93	22.78
57	NM_013124	Pparg	25.23	22.01	21.64	22.00
58	NM_031347	Ppargc1a	24.24	22.02	22.28	22.66
59	NM_012637	Ptpn1	24.17	21.23	21.70	22.18
60	NM_022268	Pygl	24.30	21.54	21.55	22.40
61	NM_013019	Rab4a	23.44	20.57	23.11	22.26
62	NM_144741	Retn	24.71	22.44	23.19	23.13
63	NM_019177	Sell	24.63	21.19	23.19	23.16
64	NM_012620	Serpine1	24.37	20.08	-	22.49
65	NM_019347	Slc14a2	24.61	21.06	22.50	22.74
66	NM_012751	Slc2a4	24.06	20.13	21.59	22.03
67	NM_022689	Snap23	23.97	21.20	22.97	22.83
68	NM_030991	Snap25	39.88	-	36.55	40.17
69	NM_017051	Sod2	23.39	20.54	20.96	21.14
70	NM_001276707	Srebf1	24.66	21.06	21.96	22.71
71	NM_031125	Stx4	25.21	21.31	22.36	22.76
72	NM_013038	Stxbp1	24.46	21.84	22.02	22.27
73	NM_031126	Stxbp2	24.57	21.28	23.02	22.61
74	NM_001107038	Stxbp4	26.70	24.48	24.25	25.09
75	NM_021578	Tgfb1	23.98	20.92	22.43	22.53
76	NM_012675	Tnf	24.38	21.11	22.46	22.77
77	NM_013091	Tnfrsf1a	24.64	20.89	22.20	22.58
78	NM_130426	Tnfrsf1b	24.18	19.96	21.93	22.06
79	NM_144755	Trib3	24.63	20.19	22.04	22.51
80	NM_019354	Ucp2	24.04	20.36	22.36	22.42
81	NM_012663	Vamp2	24.37	21.18	22.00	22.75
82	NM_057097	Vamp3	24.06	21.27	21.63	22.37
83	NM_031631	Vapa	23.99	21.30	21.94	22.94
84	NM_031836	Vegfa	24.61	22.67	22.67	22.94
85	NM_031144	Actb	23.69	21.54	23.02	22.85
86	NM_012512	B2m	21.78	21.26	22.30	22.16
87	NM_012583	Hprt1	23.09	21.05	22.14	23.24
88	NM_017025	Ldha	22.29	20.50	20.87	21.32
89	NM_001007604	Rplp1	21.66	20.04	21.22	21.88
90	U26919	RGDC	22.65	20.25	21.10	21.52
91	SA_00104	RTC	23.93	27.47	25.54	26.60
92	SA_00104	RTC	23.68	27.89	25.82	26.46
93	SA_00104	RTC	24.47	28.00	25.54	26.17
94	SA_00103	PPC	19.57	19.65	19.68	19.83
95	SA_00103	PPC	19.26	20.27	20.03	20.06
96	SA_00103	PPC	19.52	20.28	19.97	20.36

◀ **Fig. 1** Heat map of Ingenuity Pathway Analysis® of canonical pathways. Normal control is non-diabetic control (NDC), diabetes normal is diabetic control with the (DC) groups fed with ghee, normal treatment is non-diabetic treatment (NDT), and diabetic treated is diabetic treatment (DT) groups that were fed with mustard oil

of pathways focused on the influence of mustard oil diet on STZ-induced diabetic as well as non-diabetic rats with PCR profiler array.

The increase in bodyweight of rats in the NDC and NDT groups could be attributed to normal growth and metabolism that take place in the animals. But the marginal decrease in blood glucose levels in the NDT group is due to the effect of mustard oil. The significant increase in blood glucose levels and decreased bodyweight of DC rats during the 30th day and 60th day may be attributed to the low insulin levels caused by the destruction of β-cells by streptozotocin [37]. There was a significant decrease in bodyweight in DC rats which may be due to muscle wasting and loss of tissue proteins due to excessive metabolism of tissue fats and proteins for gluconeogenesis in streptozotocin-induced diabetic rats [38–40].

Among DT rats, there was a significant increase in blood glucose level during day 30 which is due to the destruction of β-cells by STZ induction, while the significant reduction of blood glucose level observed during day 60 is attributed to the mustard oil inclusion in the diet. This indicates that mustard oil inclusion in diet requires a stipulated period of time to bring fatty acid changes at the membrane level. Christopher et al. [41] have observed that changes in fatty acid composition in the cell membrane are more in animals fed with a particular diet for 12 weeks. Our earlier experiments have also proved that the inclusion of mustard oil in the diet drastically reduced blood glucose level, the altered fatty acid composition of muscle, increased insulin secretion by regeneration of β-cells of the pancreas, and the increased expression of *Glut4* [26]. The glucose-lowering effect of mustard oil observed in our experiment is due to increased β-cell regeneration, insulin secretion, and peripheral utilization of glucose [42]. The increase in bodyweight by day 60 is due to the anabolic effect exerted by insulin on protein metabolism which had stimulated protein synthesis and reduced protein degradation [43]. Insulin also suppresses adipose tissue lipolysis which may result in an inhibition of muscular tissue proteolysis and lipolysis [44]. Hence, the increase in bodyweight by day 60 may be attributed to reduced proteolysis and lipolysis in mustard oil–treated diabetic rats promoted by insulin.

This is the first report of a study utilizing pathway-focused PCR based-arrays to examine mustard oil–induced gene expression in non-diabetic and diabetic rats. Genome-wide microarray analysis is a hybridization-based method that works well for moderate to relatively abundant transcripts but is not ideal for detecting genes expressed at low levels. On the other hand, PCR is an amplification-based method and because of

Table 7 Summary of analysis from Ingenuity Pathway Analysis® of canonical pathways

Top canonical pathway	NDC	NDT	DC	DT
Glucocorticoid receptor signaling	6.9% (20/288)	6.9% (20/288)	6.6% (19/288)	6.9% (20/288)
Dendritic cell maturation	8.9% (17/192)	8.9% (17/192)	-	-
Type II diabetes mellitus signaling	11.7% (15/128)	11.7% (15/128)	11.7% (15/128)	11.7% (15/128)
Insulin receptor signaling	10.6% (15/141)	12.8% (17/133)	-	10.6% (15/141)
HMGB1 signaling	12.8% (17/133)	-	11.3% (15/133)	-
Role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis	-	6.1% (19/311)	6.1% (19/311)	-
Role of osteoblasts, osteoclasts, and chondrocytes in rheumatoid arthritis	-	-	7.3% (17/233)	-
Dendritic cell maturation	-	-	-	8.9% (17/192)

this, PCR arrays could technically be far more sensitive with a greater dynamic range than microarrays [45]. The relative gene expression of the DC, NDT, and DT groups were analyzed with respect to the NDC group. In order to get a precise overview of the underlying molecular mechanisms which had caused this anabolic effect and the pathways involved, a pathway analysis was performed.

Among the pathways and molecules analyzed by the Ingenuity Pathway Analysis®, the top five pathways and upstream regulatory molecules were chosen for comparative analysis. In the DC group, insulin receptor signaling is not at detectable levels. But the insulin receptor signaling pathway is found to be prominent in the NDC, NDT, and DT groups suggesting the action of insulin and the responsiveness of the muscle tissues to insulin. Insulin is essential to bind to insulin receptors to trigger insulin receptor signaling. In DC due to low insulin levels, this pathway is not pronounced. In NDC, there is a normal insulin secretion and function; hence, the insulin receptor signaling is prominent. Activation of the insulin receptor signaling pathway in the DT group had resulted in reduced blood glucose levels, and improved bodyweight suggests that there is increased insulin action (Fig. 2). Insulin signals the cells of insulin-sensitive peripheral tissues, primarily skeletal muscle, to increase its uptake of glucose through translocation by exocytosis of the insulin-sensitive glucose transporter protein (GLUT4) from intracellular vesicles to the plasma membrane. Under conditions of low insulin, most GLUT4 is sequestered in intracellular vesicles in muscle and fat cells. Glucose transporter 4 (GLUT4) is mainly expressed in insulin- sensitive cells such as adipose tissue and skeletal muscle cells and cardiomyocytes [46]. The main function of this protein is to facilitate glucose uptake into these cells and maintain control of blood glucose levels. GLUT4 protein in the basal state is stored in intracellular vesicles and their translocation to the plasma membrane occurs mainly by insulin action [47] or through an insulin-independent pathway during muscle contraction by exercise. Mustard oil supplementation had increased insulin levels in STZ-induced diabetic rats [26]. Hence, the reduction

Table 8 Top upstream regulators obtained from Ingenuity Pathway analysis®

Upstream regulator	NDC	NDT	DC	DT
TNF	2.35E−35	4.30E−36	2.07E−29	2.35E−35
Rosiglitazone	2.55E−35	9.87E−36	2.03E−31	2.55E−35
D-Glucose	3.14E−34 a	3.26E−33 a	-	3.14E−34 a
PPARG	5.70E−34 a	2.22E−34 a	3.12E−30 a	5.70E−34 a
streptozotocin	2.37E−33	1.15E−33	1.17E−31	2.37E−33
ins1	-	-	2.22E−30 a	-

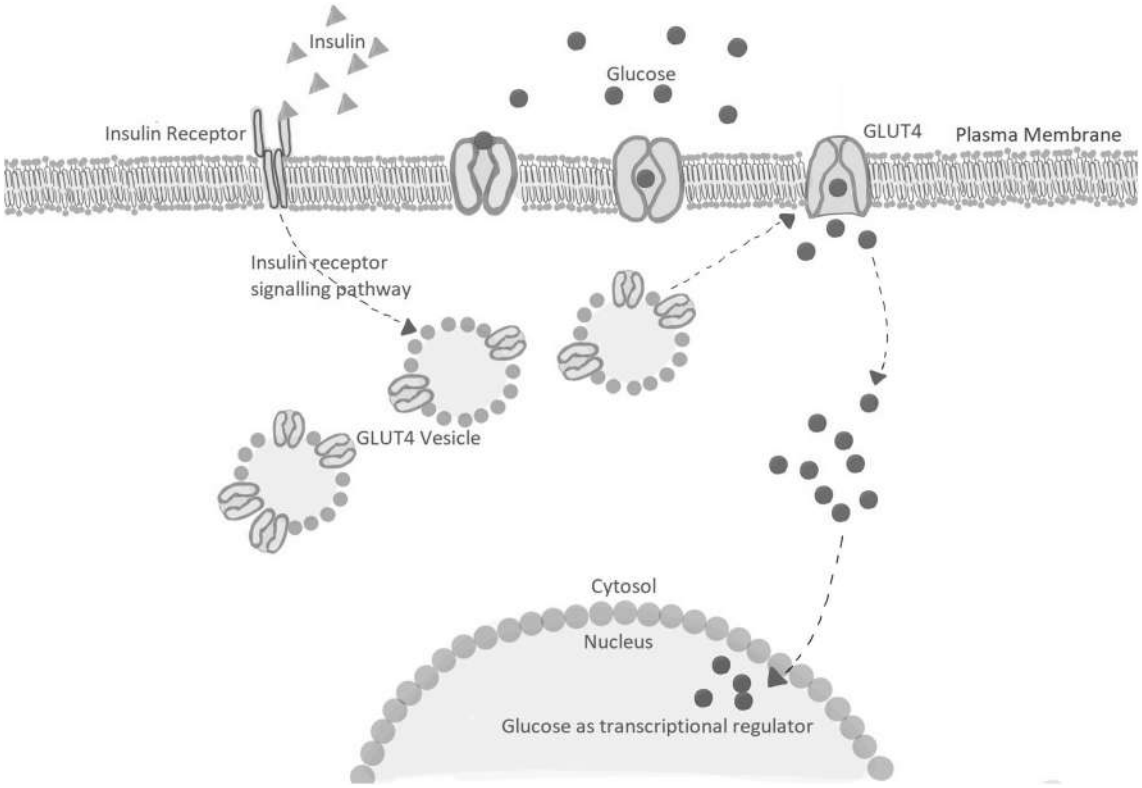


Fig. 2 Influence of mustard oil supplementation on insulin receptor signaling pathway. Dietary mustard oil supplementation increases insulin secretion. Activation of insulin receptor by insulin triggers insulin receptor signaling that initiates a cascade of events. This eventually causes translocation of GLUT4 containing vesicles to plasma membrane. The presence of GLUT4 at plasma membrane facilitates glucose entry into cell. Glucose then enters nucleus and serves as transcriptional regulator in non-diabetic rats and diabetic rats treated with mustard oil, as evidenced by results

in blood glucose levels and increased bodyweight in the DT group are due to the prominence of insulin receptor signaling triggered by increased levels of insulin.

From the comparison of top upstream regulators obtained from the results of the Ingenuity Pathway Analysis®, it was noticed that glucose was found to be the upstream regulator in all the groups, except for the DC group. In DC, due to low insulin levels, the glucose uptake by the cells was limited, whereas in the case of other groups, the glucose uptake was more when compared to DC. This is supported by the fact that the glucose level was very high in DC and the levels were drastically reduced in the DT group. This indicates partial restoration of glucose uptake by the cells in rats of the DT group due to insulin action and insulin receptor signaling, due to which glucose had entered the muscle cells and served as an upstream regulator in this group.

Insulin not only promotes the translocation but also increases the gene expression of *Glut4*. *Glut4* messenger RNA (mRNA) expression is downregulated in states of relative insulin deficiency such as streptozotocin-induced diabetes and chronic fasting; hence, insulin is an important factor in the gene expression of *Glut4* [48], suggesting that insulin acts as a positive regulator of *Glut*4 gene expression.

Conclusion

We understand that mustard oil in the diet decreases blood glucose levels by triggering insulin receptor signaling by means of increased insulin secretion due to β-cell regeneration. But the mechanism by which mustard oil causes β-cell regeneration and insulin secretion in diabetic rats is yet to be known. Finding the molecular mechanisms behind mustard oil–mediated β-cell regeneration will pave way for devising novel dietary strategies to treat diabetes mellitus. Focusing on influence of different dietary oils and dietary components over β-cell health and insulin levels will help us to prepare customized diets for diabetic patients. Hence, from the results of the present experiment, it is concluded that dietary mustard oil supplementation has decreased blood glucose levels by triggering the insulin receptor signaling pathway.

References

1. Liu S, Baracos VE, Quinney HA, Clandinin MT, et al. Dietary omega-3 and polyunsaturated fatty acids modify fatty acyl composition and insulin binding in skeletal-muscle sarcolemma. Biochem J. 1994;299:831–7.

2. Ayre KJ, Hulbert AJ. Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. J Nutr. 1996;126: 653–62.
3. Olomu JM, Baracos VE. Prostaglandin synthesis and fatty acid composition of phospholipids and triglycerides in skeletal muscle of chicks fed combinations of flaxseed oil and animal tallow. Lipids. 1991;26:743–9.
4. Mann JI. Nutrition recommendations for the treatment and prevention of type 2 diabetes and the metabolic syndrome: an evidence-based review. Nutr Rev. 2006;64:422–7.
5. Kinsell LW, Walker G, Michaels GD, Olson FE, Coelho M, McBride Y, et al. Dietary fats and the diabetic patient. N Engl J Med. 1959;261:431–4.
6. Abbott SK, Else PL, Hulbert AJ, et al. Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n−3 and n−6 PUFA. Br J Nutr. 2010;103:522–9.
7. Ginsberg BH, Brown TJ, Simon I, Spector AA, et al. Effect of the membrane lipid environment on the properties of insulin receptors. Diabetes. 1981;30:773–80.
8. Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, et al. Dietary fats and insulin action. Diabetologia. 1996a;39: 621–31.
9. Russo LG. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol. 2009;77(6):937–46.
10. Gould RJ, Ginsberg BH, Spector AA, et al. Lipid effects on the binding properties of a reconstituted insulin receptor. J Biol Chem. 1982;257(1):477–84.
11. Cheema SK, Clandinin MT. Diet- and diabetes-induced change in insulin binding to the nuclear membrane in spontaneously diabetic rats is associated with change in the fatty acid composition of phosphatidylinositol. J Nutr Biochem. 2001;12:213–8. https://doi.org/10.1016/S0955-2863(00)00135-2.
12. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV, et al. The regulation between insulin sensitivity and fatty acid composition of skeletal muscle phospholipids. N Engl J Med. 1993;328(4):238–44.
13. Storlien LH, Pan DA, Kriketos AD, Connor JO, Caterson ID, Cooney GJ, et al. Skeletal muscle membrane lipids and insulin resistance. Lipids. 1996b;31:S261–5.
14. Simonikova P, Wein S, Gasperikova D, Ukropec J, Certik M, Klimes I, et al. Comparison of the extrapancreatic action of γ-linolenic acid and n-3 PUFAs in the fat diet-induced insulin resistance. Endocr Regul. 2002;36:143–9.
15. Paniagua JA, de la Sacristana AG, Sánchez E, Romero I, Vidal-Puig A, Berral FJ, et al. A MUFA-rich diet improves posprandial glucose, lipid and GLP-1 responses in insulin-resistant subjects. J Am Coll Nutr. 2007;26(5):434–44.
16. Kumar A, Sharma A, Upadhyaya KC, et al. Vegetable oil: nutritional and industrial perspective. Curr Genomics. 2016;17(3):230–40.
17. Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Charakborty G, Toney JH, et al. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-a both in vitro and in vivo systems. Lipids Health Dis. 2009;26:8–25.
18. Perez-Rosales R, Villanueva-Rodrıguez S, Cosıo-Ramırez R, et al. El aceite de aguacate y sus propiedades nutricionales (Avocado oil and its nutritional properties). e-Gnosis. 2015;3:10.
19. Rama T, Padmanath K, Valli C, Pandian V, et al. Influence of sunflower oil supplementation in streptozotocin induced diabetic rats. Res J Pharm, Biol Chem Sci. 2018;9(2):510–21.
20. Dinesh Kumar B, Mukherjee S, Pradhan R, Mitra A, Chakraborty C. Effects of edible oils in type 2 diabetes mellitus. J Clin Diagn Res. 2009;3:1389–94.
21. Hamdan A, Haji Idrus R, Mokhtar MH. Effects of *Nigella sativa* on type-2 diabetes mellitus: a systematic review. Int J Environ Res Public Health. 2019;16(24):4911. https://doi.org/10.3390/ijerph16244911.
22. Jurado-Ruiz E, Álvarez-Amor L, Varela LM, Berná G, Parra-Camacho MS, María J, et al. Extra virgin olive oil diet intervention improves insulin resistance and islet performance in diet-induced diabetes in mice. Sci Rep. 2019;9:1311.
23. Rasmussen OW, Thomsen CH, Hansen KW, Winther E, Hermansen K, et al. Favourable effect of olive oil in patients with non-insulin-dependent diabetes. The effect on blood pressure, blood glucose and lipid levels of a high-fat diet rich in monounsaturated fat compared with a carbohydrate-rich diet. Ugeskr Laeger. 1995;157(8):1028–32.
24. Girón MD, Salto R, Hortelano P, Periago JL, Vargas AM, Suárez MD, et al. Increased diaphragm expression of GLUT4 in control and streptozotocin-diabetic rats by fish oil-supplemented diets. Lipids. 1999;34:801–7. https://doi.org/10.1007/s11745-999-0426-0.
25. Peyron-Caso E, Fluteau-Nadler S, Kabir M, Guerre-Millo M, Quignard-Boulangé A, Slama G, et al. Regulation of glucose transport and transporter 4 (GLUT-4) in muscle and adipocytes of sucrose-fed rats: effects of N-3 poly- and monounsaturated fatty acids. Horm Metab Res. 2002;34:360–6. https://doi.org/10.1055/s-2002-33467.
26. Sukanya V, Pandiyan V, Vijayarani K, Padmanath K, et al. A study on insulin levels and the expression of *Glut4* in streptozotocin (STZ) induced diabetic rats treated with mustard oil diet. Indian J Clin Biochem. 2019. https://doi.org/10.1007/s12291-019-00852-x.
27. Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O, et al. High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. Metabolism. 1996;45(12):1539–46.
28. Picinato MC, Curi R, Machado UF, Carpinelli AR, et al. Soybean- and olive-oils-enriched diets increase insulin secretion to glucose stimulus in isolated pancreatic rat islets. Physiol Behav. 1998;65: 289–94. https://doi.org/10.1016/s0031-9384(98)00157-7.
29. Maidin NQH, Ahmad N. Protective and antidiabetic effects of virgin coconut oil (Vco) on blood glucose concentrations in alloxan induced diabetic rats. Int J Pharm Pharm Sci. 2013;7(10):57–60.
30. Poletto AC, Anhe GF, Eichler P, Takahashi HK, Furuya DT, Okamoto MM, et al. Soybean and sunflower oil-induced insulin resistance correlates with impaired GLUT4 protein expression and translocation specifically in white adipose tissue. Cell Biochem Funct. 2010;28(2):114–21.
31. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. Br J Nutr. 2000;83(1):S59–66.
32. Shepherd PR, Kahn BB. Glucose transporters and insulin action–implications for insulin resistance and diabetes mellitus. N Engl J Med. 1999;341(4):248–57.
33. Bryant NJ, Govers R, James DE, et al. Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol. 2002;3(4): 267–77.
34. Folch J, Lees M, Sloane-Stanley GH, et al. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226(1):497–509.
35. Pfaffl MW. Mathematical modelling of prefermenters-I. Model development and verification. Nucleic Acids Res. 2001. https://doi.org/10.1016/S0043-1354(98)00516-8.
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25(4):402–8.
37. Ming Z, Xiao-Yan L, Jing L, Zhi-Gang X, Li C, et al. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2009:1–9.

38. Kitukale MD, Chandewar AV. An overview on some recent herbs having antidiabetic potential. Res J Pharm, Biol Chem Sci. 2014;5(6):190.
39. Jayasri MA, Radha A, Mathew TL, et al. A Amylase and a glucosidase inhibitory activity of Costus pictus in the management of diabetes. J Herb Med Toxicol. 2009;3:91–4.
40. Guyton AC, Hall JE. Textbook of medical physiology. 10th ed. Philadelphia: Saunders WB; 2000. p. 810–8.
41. Gerling CJ, Mukai K, Chabowski A, Heigenhauser GJF, Holloway GP, Spriet LL, et al. Incorporation of omega-3 fatty acids into human skeletal muscle sarcolemmal and mitochondrial membranes following 12 weeks of fish oil supplementation. Front Physiol. 2019. https://doi.org/10.3389/fphys.2019.00348.
42. Kumar V, Ahmed D, Gupta PS, Anwar F, Mujeeb M, et al. Antidiabetic, anti-oxidant and antihyperlipidemic activities of Melastoma malabathricum Linn leaves in streptozotocin induced diabetic rats. BMC Compl Altern Med. 2013;13(222):1–19.
43. Murray RR, Granner DK, Mayes PA, Rodwell VW, et al. Harper's biochemistry. 25th ed. Stamford: Appleton and Lange; 1999. p. 610–7.
44. Campbell PJ, Carlson MG, Hill JO, Nurjhan N, et al. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. Am J Phys. 1992;263(6):E1063–9.
45. Boone DR, Micci MA, Taglialatela IG, Hellmich JL, Weisz HA, Bi M, et al. Pathway-focused PCR array profiling of enriched populations of laser capture microdissected hippocampal cells after traumatic brain injury. PLoS ONE. 2015;10(5):0127287.
46. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001;409(6821):729–33.
47. Carvalho E, Schellhorn SE, Zabolotny JM, Tozzo SME, Peroni OD, Houseknecht KL, et al. GLUT4 overexpression or deficiency in adipocytes of transgenic mice alters the composition of GLUT4 vesicles and the subcellular localization of GLUT4 and insulin-responsive aminopeptidase. J Biol Chem. 2004;279(20):21598–605.
48. Charron MJ, Katz EB, Olson AL, et al. GLUT4 gene regulation and manipulation. J Biol Chem. 1999;274(6):3253–6.

International Journal of Diabetes in Developing Countries (January–March) 42(1):138–146
https://doi.org/10.1007/s13410-021-00960-6

ORIGINAL ARTICLE

Effect of vitamin D on arterial stiffness in type 2 diabetes patients with intermediate chronic kidney disease

Sadishkumar Kamalanathan[1] · Saibal Das[2] · Akila Srinivasan[2] · Nishanthi Anandabaskar[3] · Jayaprakash Sahoo[1] · Sandhiya Selvarajan[2]

Received: 2 January 2021 / Accepted: 22 May 2021 /Published online: 15 June 2021

Abstract

Purpose To evaluate the association between serum 25-hydroxy vitamin D level and arterial stiffness in type 2 diabetes patients with intermediate chronic kidney disease (CKD).

Methods The serum 25-hydroxy vitamin D level and various parameters of vascular functions were assessed in type 2 diabetes patients with intermediate CKD (stages 3a and 3b). The carotid-femoral pulse wave velocity (cfPWV) among vitamin D-deficient, -insufficient, and normal patients was compared.

Results One hundred and twenty-one patients were included. The cfPWV in the vitamin D-deficient group (1190.32 ± 256.95 cm/s) was significantly higher ($p < 0.05$) than that in the vitamin D-insufficient (1053.74 ± 138.96 cm/s) and normal (1038.45 ± 171.08 cm/s) groups. Similarly, the aortic augmentation pressure (13.29 ± 5.18 mm Hg) in the vitamin D-deficient group was significantly higher ($p < 0.05$) than that in the vitamin D-insufficient (10.01 ± 5.12 mm Hg) and normal groups (9.27 ± 4.97 mm Hg). Furthermore, the augmentation index was $24.66 \pm 8.84\%$ in the vitamin D-deficient ($p < 0.05$) as compared to $20.11 \pm 8.12\%$ in the vitamin D-insufficient group and $20.36 \pm 6.14\%$ in the normal group. Other vascular parameters were not significantly different among the groups.

Conclusion Different parameters of arterial stiffness, such as cfPWV, aortic augmentation pressure, and augmentation index, were significantly higher in the vitamin D-deficient group as compared to those in the vitamin D-insufficient and normal groups among type 2 diabetes patients with intermediate CKD.

Keywords Arterial stiffness · Chronic kidney disease (CKD) · Carotid-femoral pulse wave velocity (cfPWV) · 25-Hydroxy vitamin D · Type 2 diabetes

Introduction

Chronic kidney disease (CKD) is characterized by impaired renal structure and/or function present for at least 3 months. It is estimated to be 8–16% worldwide and has been showing a progressively increasing trend globally owing to the expanding population of type 2 diabetes mellitus (DM) with associated comorbidities like hypertension and diabetic nephropathy [1]. In India, the prevalence of CKD is found to be 17.2%, of which nearly 6% is constituted by CKD stage 3 or above [2]. Around 40–60% of CKD in India is reportedly contributed by diabetes and hypertension with diabetic nephropathy being the paramount cause [3]. Patients with early diabetic nephropathy or those progressing towards end-stage renal disease tend to have multiple cardiovascular comorbidities primarily due to inflammation and endothelial dysfunction [4, 5].

Arterial stiffness is a pathophysiological index of endothelial dysfunction, which is used as a surrogate marker for assessing adverse cardiovascular outcomes in diabetes patients with CKD [6]. It is measured non-invasively using applanation tonometry or devices using oscillometric principle for assessing various indices of vascular functions, such as carotid-femoral pulse wave velocity (cfPWV), brachial-ankle PWV (baPWV), arterial stiffness

✉ Sandhiya Selvarajan
sandhiyaselvarajan@gmail.com

[1] Department of Endocrinology, JIPMER, Puducherry 605 006, India

[2] Department of Clinical Pharmacology, JIPMER, Puducherry 605 006, India

[3] Department of Pharmacology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry 605 107, India

index, central blood pressure, and augmentation index. Among these indices, cfPWV is considered to be the gold standard owing to its robust association with the severity of coronary artery disease [7]. Additionally, studies have found a correlation between cfPWV and albuminuria as well as stages of CKD [8]. Besides, arterial stiffness in non-diabetic patients has been shown to increase gradually from stage 2 to 5 of CKD [9]. Studies reveal that pulse wave velocity is influenced by the stages of CKD as the biological milieu varies in each stage [10].

Arterial stiffness has been found to have an inverse association between endothelial dysfunction and vitamin D level in the healthy population [11]. Correspondingly, insufficient vitamin D levels are associated with enhanced oxidative stress and endothelial dysfunction in overly obese children [12]. Though vitamin D deficiency has been described in the majority of CKD patients, conflicting evidence exists concerning the association of serum 25(OH)D level and vascular health across diverse ethnicities [13–18]. Hence, this study was performed to evaluate the association between serum 25(OH)D level and parameters of vascular functions in type 2 DM patients with intermediate CKD.

Patients and methods

Ethics statement

This study was approved by the Institutional Ethics Committee (Human Studies) and carried out in a tertiary care institute in India. Written informed consent was obtained from all the participants before enrollment.

Study design

This was a cross-sectional observational study.

Patients

Patients of either gender aged between 18 and 65 years with type 2 DM and intermediate CKD (stages 3a (estimated glomerular filtration rate (eGFR), 45–59 ml/min/1.73 m^2) and 3b (eGFR, 30–44 ml/min/1.73 m^2) with or without hypertension and receiving stable treatment for the previous 3 or more months attending Endocrinology OPD were included. The exclusion criteria were patients on vitamin D-based replacement therapy within the past 6 months prior to screening, serious comorbidities, pregnant women, and lactating mothers.

Estimation of parameters

The demographic characteristics of the enrolled patients were recorded and 6 ml of venous blood was collected from each patient to estimate serum 25(OH)D level, FPG (fasting plasma glucose) and PPG (postprandial plasma glucose), HbA_{1C}, blood urea, serum creatinine, and electrolytes. Serum 25(OH)D level was measured using chemiluminescence immunoassay (Advia Centaur XP Immunoassay System, Siemens, Germany). eGFR was calculated using Modification of Diet in Renal Disease (MDRD) study formula ($eGFR = 186 \times serum\ Cr\ 1.154 \times age\ 0.203 \times 1.212$ (if patient is black) $\times 0.742$ (if female)) [19].

The parameters of vascular functions, namely cfPWV and other arterial stiffness parameters (baPWV, arterial stiffness index, ankle-brachial index, aortic systolic and diastolic pressures, aortic augmentation pressure, and augmentation index), were measured using an automated non-invasive cardiovascular analysis system PeriScope™ (Genesis Medical Systems, Hyderabad, India), functioning under the oscillometry principle. It has four blood pressure cuffs (tied over both the arms and above both the ankles) and four electrocardiogram leads (to measure lead I and lead II waveforms). The instrument concomitantly measures both blood pressures and electrocardiogram waveforms to calculate various parameters of arterial stiffness [20, 21]. Before measuring arterial stiffness, it was ensured that the participants have not consumed food at least 2 h preceding the procedure. They were asked to rest in the supine position for at least 10 min ahead of commencing the test and were instructed not to move, sleep, or talk during the procedure to circumvent erroneous results. For each participant, two successive readings were taken and an average of the arterial stiffness parameters was used for analysis.

Statistical analyses

The continuous variables were represented as mean ± SD and the categorical variables were represented by frequency (%). Normality was checked by the Kolmogorov–Smirnov test. The parameters of vascular functions among patients with normal, insufficient, and deficient vitamin D levels were compared using one-way ANOVA followed by Tukey's post hoc test. A post hoc analysis was also performed between patients who had comorbidities and those who did not have, and between patients who were on concomitant medications and those who were not. All analyses were performed with SPSS version 19 (IBM, NY, USA). Different parameters of vascular functions were compared between the genders by independent t-test. A p-value of < 0.05 was considered statistically significant.

Results

One hundred and twenty-one type 2 DM patients with intermediate CKD were included as per eligibility criteria from April 2018 to November 2019. The baseline characteristics of the enrolled patients are as given in Table 1. Each patient was on 3 ± 2 drugs. Among the recruited patients, 91 (75.2%) were deficient (serum 25(OH) D < 20 ng/ml), 19 (15.7%) were insufficient (25(OH) D = 21–29 ng/ml), and 11 (9.1%) were normal (25(OH) D > 30 ng/ml). The mean value of serum 25(OH)D level in the overall population was found to be 17.38 ± 5.87 ng/ml while that in the deficient group was 11.18 ± 6.17 ng/ml. There was no significant difference in the serum 25(OH)D level between males and females. The biochemical parameters did not vary among the patients with normal, insufficient, and deficient serum 25(OH)D levels (Table 2). There were no significant differences in the biochemical parameters in each group (according to their serum 25(OH)D status) of patients when categorized according to comorbidities and concomitant medication intake.

The cfPWV in the 25(OH)D-deficient group (1190.32 ± 256.95 cm/s) was significantly higher ($p < 0.05$) compared to the insufficient (1053.74 ± 138.96 cm/s) and normal (1038.45 ± 171.08 cm/s) groups (Fig. 1). Similarly, both aortic augmentation pressure and augmentation index were significantly higher in the 25(OH)D-deficient group ($p < 0.05$) in comparison to the insufficient and normal

Table 1 Demographic characteristics of the participants (n = 121)

Demographic characteristics		Overall population (n = 121)	Vitamin D-deficient group (n = 91)	Vitamin D-insufficient group (n = 19)	Normal vitamin D group (n = 11)
Age (years)		53.57 ± 8.09	52.17 ± 7.49	54.10 ± 9.11	53.57 ± 8.82
Male (%)		44 (36.4)	26 (28.6)	14 (73.7)	4 (36.4)
Body mass index (kg/m^2)		26.61 ± 4.83	27.81 ± 3.88	26.25 ± 5.12	26.64 ± 4.07
Occupational status (%)	Housewife	60 (49.6)	45 (49.45)	10 (52.6)	5 (45.5)
	Farmer	25 (20.7)	12 (13.2)	8 (42.1)	5 (45.5)
	Laborer	9 (7.4)	5 (5.5)	3 (15.8)	1 (9.1)
	Retired	6 (4.9)	4 (4.4)	1 (5.3)	1 (9.1)
	Other	21 (17.4)	12 (13.2)	4 (21.1)	5 (45.5)
Non-vegetarian diet (%)		95 (78.5)	75 (82.4)	15 (78.9)	5 (45.5)
Smokers (%)		21 (17.4)	12 (13.2)	6 (31.6)	3 (27.3)
Alcoholics (%)		14 (11.6)	5 (5.5)	5 (26.3)	4 (36.4)
Family history of diabetes (%)		75 (61.9)	55 (60.4)	15 (78.9)	5 (45.5)
Duration of diabetes (years)		7.39 ± 4.38	7.14 ± 5.11	7.69 ± 3.01	7.50 ± 4.00
Medications for diabetes (%)	Metformin	40 (33.1)	20 (21.9)	15 (78.9)	5 (45.5)
	Sulfonylurea	72 (59.5)	50 (54.9)	15 (79.9)	7 (63.6)
	Other OHA	30 (24.8)	12 (13.2)	12 (63.2)	6 (54.5)
	Insulin	35 (28.9)	20 (21.9)	10 (52.6)	5 (45.5)
Other comorbidities (%)	Hypertension	48 (39.7)	25 (27.5)	18 (94.7)	5 (45.5)
	CAD	24 (19.8)	10 (10.9)	10 (52.6)	4 (36.4)
	Hypothyroidism	14 (11.6)	7 (7.7)	5 (26.3)	2 (18.2)
	Others	26 (21.5)	12 (13.2)	8 (42.1)	6 (54.5)
Co-medications (%)	ACEI/ARB	86 (71.1)	65 (71.4)	15 (79.9)	6 (54.5)
	CCB	17 (14.1)	8 (8.8)	5 (26.3)	4 (36.4)
	Beta blocker	43 (35.5)	18 (19.8)	18 (94.7)	7 (63.6)
	Aspirin	24 (19.8)	10 (10.9)	10 (52.6)	4 (36.4)
	Statin	66 (54.5)	40 (43.9)	18 (94.7)	8 (72.7)
	L-Thyroxine	15 (12.4)	8 (8.8)	6 (31.6)	1 (9.1)
	Furosemide	14 (11.6)	5 (5.5)	6 (31.6)	3 (27.3)
	Others	32 (26.4)	15 (16.5)	13 (68.4)	4 (36.4)

All data are represented by the mean ± SD or frequency (%)

ACE, angiotensin-converting enzyme inhibitor; *ARB*, angiotensin receptor blocker; *CAD*, coronary artery disease; *CCB*, calcium channel blocker; *OHA*, oral hypoglycemic agent

Table 2 Biochemical parameters of the study participants (n = 121)

Parameters	Overall population (n = 121)	Vitamin D-deficient group (n = 91)	Vitamin D-insufficient group (n = 19)	Normal vitamin D group (n = 11)
Serum 25(OH)D (ng/ml)	17.38 ± 5.87	11.18 ± 6.17*#	23.22 ± 4.17	36.23 ± 5.07
UACR (µg/mg)	10.69 ± 34.20	11.90 ± 35.20	10.58 ± 36.11	10.11 ± 30.22
eGFR (ml/min/1.73 m^2)	56.35 ± 12.47	57.47 ± 11.74	54.44 ± 10.47	54.77 ± 10.89
HbA1C (%)	8.53 ± 2.18	8.66 ± 1.99	8.19 ± 2.18	8.20 ± 2.48
Blood urea (mg/dl)	35.60 ± 18.78	34.58 ± 16.11	36.77 ± 17.12	35.02 ± 17.88
Serum creatinine (mg/dl)	1.83 ± 0.34	1.89 ± 0.33	1.87 ± 0.27	1.84 ± 0.39
Albumin (g/dl)	4.25 ± 2.74	4.65 ± 1.99	4.32 ± 3.74	4.73 ± 2.09
Sodium (meq/l)	134.58 ± 15.49	136.82 ± 13.72	135.58 ± 16.49	134.80 ± 15.01
Potassium (meq/l)	4.47 ± 0.63	4.45 ± 0.59	4.44 ± 0.60	4.48 ± 0.77
Calcium (mg/dl)	8.91 ± 0.58	8.90 ± 0.60	8.87 ± 0.45	8.98 ± 0.59
Phosphate (mg/dl)	3.55 ± 0.68	3.56 ± 0.13	3.55 ± 0.75	3.54 ± 0.18

All data are represented by the mean ± SD

eGFR, estimated glomerular filtration rate; *UACR*, urine albumin creatinine ratio

*As compared to the vitamin D-insufficient group ($p < 0.05$ by one-way ANOVA)

#As compared to the normal vitamin D group ($p < 0.05$ by one-way ANOVA)

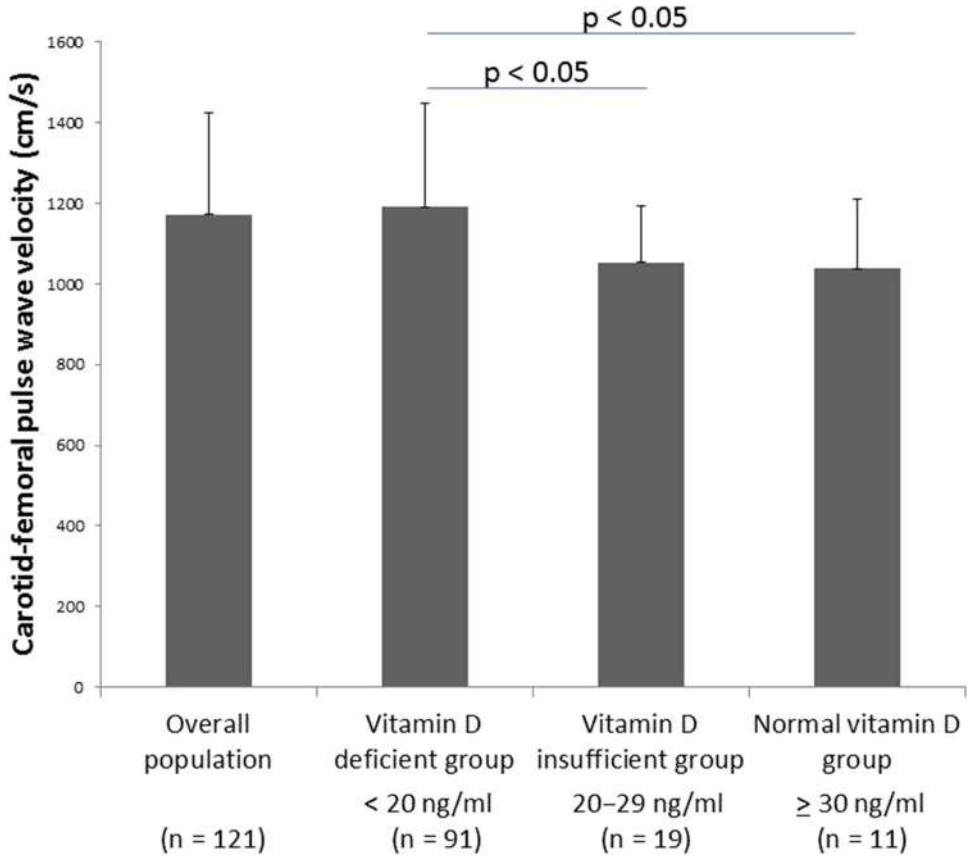


Fig. 1 Association between carotid-femoral pulse wave velocity and serum 25-hydroxy vitamin D levels in type 2 diabetes patients with intermediate chronic kidney disease (n = 121). One-way ANOVA followed by Tukey's post hoc test was performed

groups (Table 3). The parameters of vascular functions did not show gender variations across the groups. Other vascular function parameters (baPWV, arterial stiffness index, ankle-brachial index, aortic systolic, and diastolic pressures) measured in the study did not show any difference based on serum 25(OH)D level. There were no significant differences in the parameters of vascular functions between vitamin D-insufficient and normal groups. There were no significant

Table 3 Parameters of vascular functions in type 2 DM patients with intermediate CKD based on serum 25(OH)D levels (n = 121)

Parameters	Overall population (n = 121)	Vitamin D-deficient group (n = 91)	Vitamin D-insufficient group (n = 19)	Normal vitamin D group (n = 11)
Heart rate (per minute)	82.48 ± 11.97	81.68 ± 12.56	80.44 ± 10.14	81.81 ± 12.73
Systolic blood pressure (mm Hg)	135.37 ± 20.10	136.18 ± 22.11	134.12 ± 24.04	133.34 ± 18.12
Diastolic blood pressure (mm Hg)	76.57 ± 8.73	76.59 ± 12.34	76.52 ± 9.01	74.78 ± 7.30
Pulse pressure (mm Hg)	58.80 ± 15.84	59.50 ± 13.42	57.10 ± 13.42	59.18 ± 14.41
Carotid-femoral pulse wave velocity (cm/s)	1172.50 ± 250.26	1190.32 ± 256.95*#	1053.74 ± 138.96	1038.45 ± 171.08
Right brachial-ankle pulse wave velocity (cm/s)	1643.39 ± 615.58	1639.11 ± 620.18	1650.84 ± 598.55	1647.78 ± 701.82
Left brachial-ankle pulse wave velocity (cm/s)	1586.11 ± 299.65	1590.12 ± 301.65	1597.11 ± 304.54	1580.12 ± 307.51
Right brachial arterial stiffness index (mm Hg)	34.25 ± 13.19	36.58 ± 15.19	34.78 ± 9.87	36.74 ± 18.12
Left brachial arterial stiffness index (mm Hg)	33.83 ± 12.64	32.87 ± 10.85	34.58 ± 10.61	34.42 ± 10.64
Right ankle arterial stiffness index (mm Hg)	43.38 ± 14.69	42.81 ± 10.90	40.81 ± 10.97	41.38 ± 10.89
Left ankle arterial stiffness index (mm Hg)	41.85 ± 14.51	42.12 ± 10.78	40.14 ± 10.57	42.78 ± 8.58
Right ankle-brachial index	1.09 ± 0.93	1.09 ± 0.97	1.09 ± 0.90	1.09 ± 0.92
Left ankle-brachial index	1.06 ± 0.10	1.06 ± 0.18	1.06 ± 0.09	1.06 ± 0.13
Aortic systolic blood pressure (mm Hg)	117.79 ± 19.43	118.82 ± 20.33	116.99 ± 29.33	115.58 ± 15.30
Aortic diastolic blood pressure (mm Hg)	76.43 ± 9.03	75.25 ± 10.13	77.83 ± 7.03	74.58 ± 9.54
Aortic pulse pressure (mm Hg)	41.36 ± 12.88	42.06 ± 10.82	40.36 ± 14.85	41.39 ± 12.83
Aortic augmentation pressure (mm Hg)	11.22 ± 6.17	13.29 ± 5.18*#	10.01 ± 5.12	9.27 ± 4.97
Augmentation index (%)	22.45 ± 7.94	24.66 ± 8.84*#	20.11 ± 8.12	20.36 ± 6.14
Augmentation index at 75% of heart rate (%)	22.45 ± 7.75	23.15 ± 6.75	22.50 ± 8.75	22.11 ± 9.15

*As compared to the vitamin D-insufficient group. #As compared to the normal vitamin D group ($p < 0.05$ by one-way ANOVA)

All data are represented by the mean ± SD ($p < 0.05$ by one-way ANOVA)

differences in the parameters of vascular functions in each group (according to their serum 25(OH)D status) of patients when categorized according to comorbidities and concomitant medication intake.

Discussion

In the present study, 75% of the patients with type 2 DM and intermediate CKD were found to be vitamin D-deficient. The proportion of diabetes patients with vitamin D deficiency is similar to a South Indian study by Anandabaskar et al. [22]. We found a significantly higher cfPWV (1190.32 ± 256.95 cm/s) in the vitamin D-deficient group compared to the vitamin D-insufficient and normal groups. To some extent, this is slightly higher as related to cfPWV of 992 ± 66 cm/s and around 1004.07 cm/s seen in CKD stages 1–3 and stage 3 of non-diabetic patients respectively [23]. This could be attributed to the combined effect of CKD and diabetes on exacerbating arterial stiffness. One of the studies done in patients with type 2 diabetes and CKD has found that the extent of CKD-associated increase in arterial stiffness varies among the arterial regions with a maximum increase seen in the heart-carotid and heart-femoral regions [24].

The aortic augmentation pressure and augmentation index were significantly higher in the vitamin D-deficient group as compared to vitamin D-insufficient and normal groups. These findings are similar to a study done in healthy Caucasians, African Americans, and Hispanics (n = 554) with vitamin D insufficiency showing a significant association between serum 25(OH)D levels and pulse wave velocity and augmentation index [11]. Furthermore, a study done in around 560 participants from the Czech Republic found that serum 25(OH)D concentration was inversely related to aortic PWV [25]. Correspondingly, another study performed on 302 Korean adults has reported a negative correlation between serum 25(OH)D levels and baPWV [26]. A Korean study done in 305 type 2 diabetes patients found that vitamin D deficiency is common in type 2 diabetes with a significant inverse association between 25(OH)D levels and arterial stiffness [27].

Though our study did not show any gender difference, a study done in CKD patients from Turkey (N = 101) found that majority of the CKD patients were having vitamin D deficiency with a female predominance. Besides, the same study has shown a significantly higher augmentation index in CKD patients with vitamin D deficiency [28]. In subjects with DM, impaired glucose tolerance is associated with enhanced arterial stiffness, thus resulting in increased cardiovascular risk [29]. Surprisingly, studies have shown

Table 4 Comparison of the present study findings with other studies

Study	Participants	Study design	Salient findings
Present study	Stage 3a and 3b CKD	Observational study	Carotid-femoral pulse wave velocity, aortic augmentation pressure, and augmentation index in the vitamin D-deficient group were significantly higher as compared to those in the vitamin D-insufficient and normal vitamin D groups
Studies with similar findings			
Al Mheid et al. [11]	Healthy participants	Observational study	Vitamin D insufficiency was associated with an increased pulse wave velocity, augmentation index, and endothelial dysfunction
Mayer et al. [25]	General population	Cross-sectional study	Inverse association between 25(OH)D level and aortic pulse wave velocity
Lee et al. [27]	Patients with type 2 diabetes	Cross-sectional study	Inverse association between 25(OH)D level and arterial stiffness in type 2 diabetes patients
Gialluria et al. [15]	Healthy volunteers	Cross-sectional study	Inverse association between 25(OH)D level and arterial stiffness
Lieberman et al. [16]	Young patients with and without type 1 DM	Observational study	Serum 25(OH)D level had an inverse association with pulse wave velocity in adolescents with type 1 diabetes
Jha et al. [17]	Youth with type 2 DM, obese controls without type 2 DM, and lean controls without type 2 DM	Cross-sectional study	Inverse relationship between 25(OH)D level and measures of arterial stiffness in lean controls and obese adolescents with type 2 DM
Lee et al. [26]	Korean healthy adults	Observational study	Significant association between 25-hydroxy vitamin D level and brachial-ankle pulse wave velocity
Anandabaskar et al. [22]	Type 2 DM	Interventional study (supplementation of 60,000 IU vitamin D per week for 8 weeks)	Reduced carotid-femoral pulse wave velocity, right and left brachial-ankle pulse wave velocity, and reduced oxidative stress
Akdam et al. [28]	Patients with CKD	Cross-sectional study	Negative correlation between vitamin D levels and augmentation index at heart rate of 75/min
Lupoli et al. [52]	Individuals with vitamin D deficiency, vitamin D insufficiency, and controls	Meta-analysis	An association was found between Vitamin D deficiency, insufficiency, and subclinical atherosclerosis (measured by common carotid artery intima-media thickness)
Studies with contradictory findings			
Ng et al. [53]	Patients with CKD stage 3–4	Observational study	No association between 25(OH)D level and carotid atherosclerosis
Park et al. [18]	Patients with CKD	Observational study	No association of 25(OH)D level with blood pressure or arterial stiffness

CKD, chronic kidney disease; *DM*, diabetes mellitus

FPG to be associated with arterial stiffness even in normal subjects [30]. In subjects with CKD, vascular calcifications and increased arterial stiffness contribute to cardiovascular complications and mortality [31]. Additionally, a study performed on CKD patients (n = 102) from Taiwan has shown a stepwise increase in pulse wave velocity as regards the stages of CKD [32]. Arterial stiffness, especially in the aorta, and premature vascular aging have been reported with CKD [33, 34]. Studies suggest that arterial stiffness, especially pulse wave velocity, should be measured during the initial stages of CKD for early intervention and better prognosis [35]. Similarly, in a study done to evaluate the association between arterial stiffness and reduction in kidney functions (n = 3666), arterial stiffness was found to be an independent risk factor for declining renal function. Furthermore, the authors of that study had proposed that arterial stiffness may be used as a potential target for delaying deterioration in renal function [36].

Increased PWV is associated with atherosclerosis and a subsequent increase in the risk of cardiovascular diseases, such as stroke [37, 38]. Higher PWV is considered to be an important parameter to envisage mortality in patients with end-stage renal disease [39]. Among the various parameters of arterial stiffness, cfPWV is considered the gold standard [40]. An increase in cfPWV by 100 cm/s is associated with higher cardiovascular events and mortality [41]. Moreover, increased cfPWV was found to be significantly associated with cerebral white matter lesions in diabetes patients which could be a potential threat for developing neurological complications, such as stroke [42]. According to the Chronic Renal Insufficiency Cohort (CRIC) Study, done in non-dialysis CKD patients (n = 2795), aortic PWV was considered to be an independent predictor of death and CKD progression [43]. Hence, assessing cfPWV especially in diabetes patients with associated hypertension, CKD, etc., could be used to identify those with a high risk for developing cardiovascular complications.

Apart from cfPWV, arterial augmentation pressure and augmentation index are used as surrogate markers of arterial stiffness to envisage cardiovascular complications [44]. While augmentation pressure goes on increasing in the population steadily even above 55 years of age, the augmentation index attains a plateau by this age. Hence, augmentation pressure is considered to be a more apt marker for measuring arterial stiffness in elderly persons while the augmentation index gives information about the status of arterial stiffness in younger people [45, 46]. The augmentation index is increased in individuals with diabetes as well as impaired glucose tolerance [29]. A study from Germany has found higher augmentation pressure and augmentation index in type 2 diabetes patients equivalent to that of non-diabetic patients with cardiovascular diseases [44]. Additionally, increased augmentation index has been found to be an indicator of all-cause mortality and cardiovascular events only in men, although it was unrelated to the same in women [47].

Vitamin D has been shown to have a beneficial effect on vascular function by protecting the endothelial cells from the detrimental effects of advanced glycation end products as well as through a negative regulatory effect on the renin-angiotensin system [48, 49]. Experimental studies have demonstrated the effectiveness of vitamin D on inhibition of vascular smooth muscle cell proliferation, thus proposing the potential use of vitamin D in the treatment of atherosclerosis [50, 51]. A meta-analysis performed to assess the effect of vitamin D deficiency on common carotid artery intima-media thickness and prevalence of carotid plaques has found that vitamin D deficiency and insufficiency are significantly associated with subclinical atherosclerosis, thus indicating an imminent risk for cardiovascular complications [52]. On the contrary, a study by Park et al. showed that 25(OH)D level was not associated with blood pressure and arterial stiffness, especially baPWV in Korean patients with CKD [18]. Another study by Ng et al. has demonstrated no association between serum 25(OH)D levels and carotid atherosclerosis as assessed by common carotid artery intima-media thickness in diabetes patients with CKD stages 3 and 4 as shown in Table 4 [53].

The inclusion of a homogenous study population of type 2 DM and intermediate CKD from South India is the strength of our study. However, it has the limitations of having a lesser sample size and the absence of a healthy control group in the study.

In conclusion, among type 2 diabetes mellitus patients with intermediate CKD, carotid-femoral pulse wave velocity, aortic augmentation pressure, and augmentation index are significantly higher in those with vitamin D deficiency as compared to the insufficient and normal groups. Nevertheless, the inverse association between serum 25(OH) D level and arterial stiffness in patients with a higher risk of cardiovascular complications, such as those with diabetes and CKD, needs to be confirmed by a randomized controlled trial in a larger population.

Acknowledgements We thank Ms. Sarayu, Mr. Isaac, and Ms. Jayamatha for assisting in patient recruitment. We also thank Mr. Vengatraman and Mrs. Saraswathi, for the estimation of serum 25-hydroxy vitamin D levels.

Author contribution Conceptualization: SS and SK. Data collection: SD. Data analysis: SD and AS. Interpretation of results: SS, SD, JS, and SK. Providing expert inputs: SS, JS, and SK. Drafting the manuscript: SD, AS, and NA.

Funding A research grant was obtained from the Endocrine society of Tamil Nadu and Pondicherry Research for this work.

Data availability The data related to this study can be made available from the corresponding author on reasonable request.

Declarations

Ethics approval The study protocol was approved by the Institutional Ethics Committee.

Consent to participate All patients provided written informed consent for participation in this study.

Consent for publication All patients provided written informed consent for publication of the study results.

Competing interests The authors declare no competing interests.

References

1. Kim S, Lim CS, Han DC, Kim GS, Chin HJ, Kim S-J, et al. The prevalence of chronic kidney disease (CKD) and the associated factors to CKD in urban Korea: a population-based cross-sectional epidemiologic study. J Korean Med Sci. 2009;24:S11–21.
2. Singh AK, Farag YM, Mittal BV, Subramanian KK, Reddy SR, Acharya VN, et al. Epidemiology and risk factors of chronic kidney disease in India–results from the SEEK (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrol. 2013;14:114.
3. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, et al. What do we know about chronic kidney disease in India: first report of the Indian CKD registry. BMC Nephrol. 2012;13:10.
4. Bansal N, Katz R, Robinson-Cohen C, Odden MC, Dalrymple L, Shlipak MG, et al. Absolute rates of heart failure, coronary heart disease, and stroke in chronic kidney disease: an analysis of 3 community-based cohort studies. JAMA Cardiol. 2017;2:314–8.
5. Ninomiya T, Perkovic V, de Galan BE, Zoungas S, Pillai A, Jardine M, et al. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am SocNephrol. 2009;20:1813–21.
6. Shirwany NA, Zou MH. Arterial stiffness: a brief review. Acta Pharmacol Sin. 2010;31:1267–76.
7. Hofmann B, Riemer M, Erbs C, Plehn A, Navarrete Santos A, Wienke A, Silber RE, Simm A. Carotid to femoral pulse wave velocity reflects the extent of coronary artery disease. J Clin Hypertens. 2014;16:629–33.
8. Upadhyay A, Hwang SJ, Mitchell GF, Vasan RS, Vita JA, Stantchev PI, Meigs JB, Larson MG, Levy D, Benjamin EJ, Fox CS. Arterial stiffness in mild-to-moderate CKD. J Am Soc Nephrol. 2009;20:2044–53.
9. Mastanvalli B, Kumar KP, Madhav D, Reddy PV, Vali SM. Evaluation of arterial stiffness in nondiabetic chronic kidney disease patients. Saudi J Kidney Dis Transpl. 2017;28:61–7.
10. Lioufas N, Hawley CM, Cameron JD, Toussaint ND. Chronic kidney disease and pulse wave velocity: a narrative review. Int J Hypertens. 2019;2019:9189362.
11. Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am CollCardiol. 2011;58:186–92.
12. Codoñer-Franch P, Tavárez-Alonso S, Simó-Jordá R, Laporta-Martín P, Carratalá-Calvo A, Alonso-Iglesias E. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. J Pediatrics. 2012;161:848–54.
13. Mehrotra R, Kermah D, Budoff M, Salusky IB, Mao SS, Gao YL, et al. Hypovitaminosis D in chronic kidney disease. Clin J Am SocNephrol. 2008;3:1144–51.
14. Feng M, Lv J, Huang FT, Huang R, Qiu Q, Tang Y, et al. Vitamin D deficiency in patients with stages 1 and 2 chronic kidney disease in Southern China. Niger J Clin Pract. 2018;21:1639–44.
15. Giallauria F, Milaneschi Y, Tanaka T, Maggio M, Canepa M, Elango P, et al. Arterial stiffness and vitamin D levels: the Baltimore longitudinal study of aging. J Clin Endocrinol Metab. 2012;97:3717–23.
16. Lieberman R, Wadwa RP, Nguyen N, Bishop FK, Reinick C, Snell-Bergeon JK, et al. The association between vitamin D and vascular stiffness in adolescents with and without type 1 diabetes. PLoS One. 2013;8:e77272.
17. Jha P, Dolan LM, Khoury PR, Urbina EM, Kimball TR, Shah AS. Low serum vitamin D levels are associated with increased arterial stiffness in youth with type 2 diabetes. Diabetes Care. 2015;38:1551–7.
18. Park KM, Jun HH, Bae J, Choi YB, Yang DH, Jeong HY, et al. 25-hydroxyvitamin D levels was not associated with blood pressure and arterial stiffness in patients with chronic kidney disease. Electrolyte Blood Press. 2017;15:27–36.
19. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Int Med. 2006;145:247–54.
20. Naidu MU, Reddy BM, Yashmaina S, Patnaik AN, Rani PU. Validity and reproducibility of arterial pulse wave velocity measurement using new device with oscillometric technique: a pilot study. Biomed Eng Online. 2005;4:49.
21. Naidu MUR, Reddy CP. Non-invasive measurement of aortic pressure in patients: comparing pulse wave analysis and applanation tonometry. Indian J Pharmacol. 2012;44:230–3.
22. Anandabaskar N, Selvarajan S, Dkhar SA, Kamalanathan S, Tamilarasu K, Bobby Z. Effect of vitamin D supplementation on vascular functions and oxidative stress in type 2 diabetic patients with vitamin D deficiency. Indian J Endocrinol Metab. 2017;21:555–63.
23. Kola S, George M, Srinivasamurthy SK, Selvarajan S, Tamilarasu K, Swaminathan RP, et al. Assessment of arterial stiffness index in hypertensive patients in relation to their treatment status attending a tertiary care center in South India. Pharmacol Pharm. 2014;5:413–8.
24. Kimoto E, Shoji T, Shinohara K, Hatsuda S, Mori K, Fukumoto S, et al. Regional arterial stiffness in patients with type 2 diabetes and chronic kidney disease. J Am Soc Nephrol. 2006;17:2245–52.
25. Mayer O, Filipovský J, Seidlerová J, Vaněk J, Dolejšová M, Vrzalová J, et al. The association between low 25-hydroxyvitamin D and increased aortic stiffness. J Hum Hypertens. 2012;26:650–5.
26. Lee JH, Suh HS. Association of serum 25-hydroxy-vitamin D concentration and arterial stiffness among Korean adults in single center. J Bone Metab. 2017;24:51–8.
27. Lee JI, Oh SJ, Ha WC, Kwon HS, Sohn TS, Son HS, Cha BY. Serum 25-hydroxyvitamin D concentration and arterial stiffness among type 2 diabetes. Diabetes Res Clin Pract. 2012;95:42–7.
28. Akdam H, Alp A. Arterial stiffness and 25-hydroxyvitamin D levels in chronic kidney disease patients. Rev Assoc Méd Bras. 2017;63:910–6.
29. Schram MT, Henry RM, van Djik RA, Kostense PJ, Dekker JM, Nijpels G, et al. Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes. Hypertension. 2004;43:176–81.

30. Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. Am J Hypertens. 2002;15:1101–8.
31. Covic A, Gusbeth-Tatomir P, Goldsmith DJ. Arterial stiffness in renal patients: an update. Am J Kidney Dis. 2005;45:965–77.
32. Wang MC, Tsai WC, Chen JY, Huang JJ. Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. Am J Kidney Dis. 2005;45:494–501.
33. Briet M, Bozec E, Laurent S, Fassot C, London GM, Jacquot C, et al. Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. Kidney Int. 2006;69:350–7.
34. Sarafidis PA, Loutradis C, Karpetas A, Tzanis G, Piperidou A, Koutroumpas G, et al. Ambulatory pulse wave velocity is a stronger predictor of cardiovascular events and all-cause mortality than office and ambulatory blood pressure in hemodialysis patients. Hypertension. 2017;70:148–57.
35. London GM. Arterial stiffness in chronic kidney disease and end-stage renal disease. Blood Purif. 2018;45:154–8.
36. Sedaghat S, Mattace-Raso FU, Hoorn EJ, Uitterlinden AG, Hofman A, Ikram MA, et al. Arterial stiffness and decline in kidney function. Clin J Am Soc Nephrol. 2015;10:2190–7.
37. Mackenzie IS, Wilkinson IB, Cockcroft JR. Assessment of arterial stiffness in clinical practice. QJM. 2002;95:67–74.
38. Kubozono T, Ohishi M. Prognostic significance of regional arterial stiffness for stroke in hypertension. Pulse. 2015;3:98–105.
39. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation. 1999;99:2434–9.
40. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27:2588–605.
41. Zhong Q, Hu MJ, Cui YJ, Liang L, Zhou MM, Yang YW, et al. Carotid–femoral pulse wave velocity in the prediction of cardiovascular events and mortality: an updated systematic review and meta-analysis. Angiology. 2018;69:617–29.
42. Laugesen E, Høyem P, Stausbøl-Grøn B, Mikkelsen A, Thrysøe S, Erlandsen M, et al. Carotid-femoral pulse wave velocity is associated with cerebral white matter lesions in type 2 diabetes. Diabetes Care. 2013;1(36):722–8.
43. Townsend RR, Anderson AH, Chirinos JA, Feldman HI, Grunwald JE, Nessel L, et al. Association of pulse wave velocity with chronic kidney disease progression and mortality: findings from the CRIC study (chronic renal insufficiency cohort). Hypertension. 2018;71:1101–7.
44. Wilhelm B, Klein J, Friedrich C, Forst S, Pfützner A, Kann PH, et al. Increased arterial augmentation and augmentation index as surrogate parameters for arteriosclerosis in subjects with diabetes mellitus and nondiabetic subjects with cardiovascular disease. J Diabetes Sci Technol. 2007;1:260–3.
45. Fantin F, Mattocks A, Bulpitt CJ, Banya W, Rajkumar C. Is augmentation index a good measure of vascular stiffness in the elderly? Age ageing. 2007;36:43–8.
46. McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR, Acct Investigators. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). J Am CollCardiol. 2005;46:1753–1760.
47. Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. High aortic augmentation index predicts mortality and cardiovascular events in men from a general population, but not in women. Eur J PrevCardiol. 2013;20:1005–12.
48. Talmor Y, Golan E, Benchetrit S, Bernheim J, Klein O, Green J, et al. Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. Am J Physiol Renal Physiol. 2008;294:F1059–64.
49. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1, 25-Dihydroxyvitamin D 3 is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110:229–38.
50. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1, 25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension. 1989;13:954–9.
51. Chen S, Law CS, Gardner DG. Vitamin D-dependent suppression of endothelin-induced vascular smooth muscle cell proliferation through inhibition of CDK2 activity. J Steroid BiochemMolBiol. 2010;118:135–41.
52. Lupoli R, Vaccaro A, Ambrosino P, Poggio P, Amato M, Di Minno MN. Impact of vitamin D deficiency on subclinical carotid atherosclerosis: a pooled analysis of cohort studies. J Clin Endocrinol Metab. 2017;102:2146–53.
53. Ng YM, Lim SK, Kang PS, Kadir KA, Tai MS. Association between serum 25-hydroxyvitamin D levels and carotid atherosclerosis in chronic kidney disease patients. BMC Nephrol. 2016;17:151.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):147–160
https://doi.org/10.1007/s13410-021-00954-4

ORIGINAL ARTICLE

Add-on therapy with dapagliflozin in routine outpatient care of type 2 diabetes patients from Turkey: a retrospective cohort study on HbA1c, body weight, and blood pressure outcomes

Derun Taner Ertugrul[1] · Erdal Kan[2] · Cigdem Bahadir Tura[3] · Haci Bayram Tugtekin[4] · Hayati Ayakta[5] · Mehmet Celebioglu[6] · Ceren Yılmaz[7] · Onur Utebay[7] · Ilhan Yetkin[8] · The DAPATURK Study Group

Received: 20 November 2020 / Accepted: 5 April 2021 / Published online: 4 May 2021

Abstract

Background The efficacy of dapagliflozin has been less extensively studied in the real-life context, limiting the comparability of observational real-world evidence with results from dapagliflozin clinical trials. This study aimed to evaluate HbA1c, blood pressure (BP), and weight outcomes in dapagliflozin-treated patients with type 2 diabetes (T2D) treated in a real-world setting.

Methods A total of 1683 T2D patients (mean (SD) age: 54.6 years (9.1), 56.6% were females) initiating dapagliflozin were included in this multicenter retrospective observational cohort study. Data on patient demographics, comorbidities, duration of diabetes, and concomitant antidiabetic treatment were recorded. Change in glycated hemoglobin (HbA1c), body weight, body mass index (BMI), and BP levels with dapagliflozin treatment and dapagliflozin discontinuation rates were evaluated based on baseline, 3rd-month, and 6th-month data.

Results At the end of 6-month dapagliflozin treatment (discontinued in 4.3% of patients), HbA1c levels were ≤7% in 30.1% of patients, BP was ≤140/90 mmHg in 95.9% of patients, and >5% weight loss was evident in 38.0% of patients. Less than 5-year diabetes duration was associated with significantly higher rate of achieving HbA1c ≤7% ($p = 0.032$), BP ≤140/90 mmHg ($p = 0.001$), and BP ≤130/80 mmHg ($p < 0.001$) targets at the 6th month ($p = 0.032$) as compared with longer diabetes duration.

Conclusion In conclusion, this nationwide observational study in T2D patients treated with dapagliflozin in Turkey provided real-life evidence on significant and clinically meaningful reductions in HbA1c, body weight, and BP within 6-month therapy.

This paper was presented in a poster session at the 40th Turkish Congress of Endocrine and Metabolic Diseases which was held on May 9-13, 2018 in Antalya, Turkey.

✉ Derun Taner Ertugrul
dertaner@gmail.com

Erdal Kan
drerdalakan@yahoo.com

Cigdem Bahadir Tura
cigdemtura@hotmail.com

Haci Bayram Tugtekin
drhaci2705@gmail.com

Hayati Ayakta
hayatiayakta@gmail.com

Mehmet Celebioglu
doktorcelebioglu@hotmail.com

Ceren Yılmaz
ceren.yilmaz@astrazeneca.com

Onur Utebay
Onur.Utebay@astrazeneca.com

Ilhan Yetkin
ilhanyetkinster@gmail.com

[1] Clinic of Endocrinology, Kecioren Training and Research Hospital, Pinarbasi, 06000 Kecioren, Ankara, Turkey

[2] Clinic of Endocrinology, Samsun Medicana Hospital, Samsun, Turkey

[3] Clinic of Endocrinology, Tokat State Hospital, Tokat, Turkey

[4] Clinic of Internal Medicine, Umraniye Training and Research Hospital, Istanbul, Turkey

[5] Clinic of Endocrinology, Manisa Grand Medical Hospital, Manisa, Turkey

[6] Clinic of Internal Medicine, Eskisehir Anadolu Hospital, Eskişehir, Turkey

[7] AstraZeneca Pharmaceuticals, Istanbul, Turkey

[8] Department of Endocrinology and Metabolism, Gazi University Faculty of Medicine, Ankara, Turkey

Keywords Type 2 diabetes · Dapagliflozin · Second-line therapy · Glycemic control · Body weight · Blood pressure

Introduction

In accordance with the global estimates on rapidly increasing prevalence of the diabetes worldwide [1], type 2 diabetes (T2D) remains a serious health issue in Turkey with an average prevalence of 13.7% (over 6.5 million people) as reported in the Turkish Diabetes Hypertension Obesity and Endocrine Disease Prevalence Study (TURDEP-II) [2].

Owing to a causal link between dysglycemia, diabetes-related complications, and premature deaths, achievement of optimal glycemic control is considered to be of critical importance in T2D management [1, 3–6]. According to current guidelines, standard treatment for T2D involves the use of metformin, in conjunction with lifestyle changes, as first-line glucose-lowering therapy, and treatment intensification in case of failure to achieve glycemic control [7]. For patients with clinical cardiovascular disease, treatment intensification is recommended to be based on addition of a sodium–glucose cotransporter-2 (SGLT2) inhibitor or a glucagon-like peptide-1 (GLP-1) receptor agonist with proven cardiovascular benefit [7].

SGLT2 inhibitors comprise a novel class of antidiabetic agents with unique insulin-independent mode of action in lowering blood glucose levels (i.e., blocking the reabsorption of filtered glucose in the kidneys) which also enable their combination with other glucose-lowering agents with different mechanistic pathways [8–13].

Dapagliflozin is a highly selective SGLT2 inhibitor approved by the FDA in January 2014 for use as monotherapy or in combination with other antidiabetic therapies in adult patients [8, 14, 15]. Dapagliflozin (Forziga®, also known as Forxiga® or Farxiga®) is the first SGLT-2 inhibitor approved in Turkey in 2015 and reimbursed as restricted to internal medicine and endocrinology prescriptions since July 2016 [16].

The efficacy of dapagliflozin on reduction of HbA1c, body weight, body fat, and blood pressure (BP) as monotherapy [17] or in combination with other oral antidiabetic drugs (OADs) has been shown in numerous randomized controlled trials (RCTs) [10, 14, 15, 18–25]. However, the efficacy of dapagliflozin has been less extensively studied in the real-life context, limiting the comparability of observational real-world evidence with results from dapagliflozin clinical trials [11–13, 26–29].

Scientific societies and health care professionals have repeatedly expressed the need to collect and publish real-world evidence on SGLT-2 inhibitor use across the country [13, 29, 30], whereas to our knowledge, no studies to date investigated the outcomes related to dapagliflozin treatment in patients with T2D in Turkey.

Hence, this retrospective cohort study, providing data on SGLT-2 inhibitors for the first time in Turkish patients, was designed to investigate add-on therapy with dapagliflozin in routine outpatient care of T2D patients from Turkey in terms of patient profile and HbA1c, weight, body mass index (BMI), and BP outcomes in relation to clinical variables and concomitant antidiabetic medications in a real-life clinical setting.

Methods

Study population

A total of 1683 T2D patients initiating dapagliflozin were included in this multicenter retrospective observational cohort study (ClinicalTrials.gov Identifier: NCT03407196) conducted over three visits (baseline, 3rd month, and 6th month) between July 2016 and August 2017 across 79 internal medicine and endocrinology clinics from Turkey.

T2D patients who received first prescription for dapagliflozin between July 2016 and August 2017, aged ≥18 and ≤75 years, and registered in that center for at least 6 months prior to index date (the date of the first prescription for dapagliflozin in patient medical records) were included in the study. Presence of type 1 diabetes, gestational diabetes, EGFR < 30 ml/min, and another interventional clinical trial participation between index date and enrollment date were the exclusion criteria of the study. The selection of sites and investigators was made in accordance with the national situation regarding dapagliflozin prescription (reimbursement, public or private insurance) and the proportion of the different types of centers (internal medicine, endocrinology clinics, different types of hospitals, geographical distribution) and investigators (different types of specialists) involved in dapagliflozin-treated T2D patient management to achieve representativeness of dapagliflozin-treated T2D patients across Turkey.

The study was approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization of Good Clinical Practice, and the local regulations for clinical research.

Study parameters

Data on patient demographics (age, gender), smoking status, type of outpatient clinic, comorbidities, duration of diabetes, microvascular complications, and concomitant antidiabetic treatment including oral antidiabetic drugs (OADs) and injectable drugs including insulin, glucagon-like peptide-1 receptor agonists (GLP1RA) or insulin + GLP1RA were recorded in

each patient. Change in HbA1c, body weight, BMI, and systolic and diastolic BP levels, rates of HbA1c target achievement under dapagliflozin treatment, and dapagliflozin discontinuation rates were evaluated based on baseline, 3rd-month, and 6th-month data and analyzed with respect to diabetes duration and concomitant antidiabetic regimen.

Persistence to dapagliflozin was evaluated based on the time from initial prescription to end of period covered by the final prescription in patient records. A patient was considered to have discontinued treatment if there was a period of greater than 30 days without coverage following the period covered by the final prescription.

Statistical analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). Chi-square (χ^2) test and Mantel-Haenszel test were used for the comparison of categorical data, while numerical data were analyzed using Students' *t* test and one-way ANOVA and post hoc Tukey test. Change over time was evaluated by paired Students' *t* test. Data were expressed as "mean (standard deviation (SD))", minimum-maximum, percent (%), and 95% confidence interval (CI) where appropriate. $p < 0.05$ was considered statistically significant.

Results

Baseline demographic and clinical characteristics (*n* = 1683)

Overall, mean (SD) patient age was 54.6 years (9.1; 73.3% aged 45–64 years), 56.6% of patients were females, and average diabetes duration was 8.7 years. Neuropathy was the most common microvascular complication (31.1%), while hypertension (57.3%) and hyperlipidemia (55.4%) were the two most common comorbidities (Table 1).

Antidiabetic treatments during the study period

Dapagliflozin was discontinued in 26 (2.2%) and 72 (4.3) patients in the 3rd and 6th months of study, respectively. Concomitant antidiabetics at baseline, 3rd-month, and 6th-month visits involved OAD in 52.2%, 50.2%, and 52.8% of patients, and OAD ± injectable drug in 47.8%, 49.7%, and 46.3% of patients, respectively. Most commonly prescribed OADs were metformin (88.1%, 86.0%, and 86.7%, respectively) and DPP4i (51.8%, 52.0%, and 52.3%, respectively). At baseline, 3rd-month, and 6th-month visits, respectively, insulin (overall: 36.6%, 38.5%, and 35.4%) was the most commonly used injectable drug, while most of the insulin-

Table 1 Baseline demographic and clinical characteristics (*n* = 1683)

Age (year)	
Mean (SD)	54.6 (9.1)
Median (min-max)	54.2 (20.1–83.4)
<45, *n* (%)	209 (12.6)
45–64, *n* (%)	1212 (73.3)
≥65, *n* (%)	233 (14.1)
Gender (*n* (%))	
Female	952 (56.6)
Male	731 (43.4)
Clinic (*n* (%))	
Endocrinology	861 (51.2)
Internal medicine	780 (46.3)
Nephrology	42 (2.5)
Duration of diabetes (years)	
Mean (SD)	8.7 (5.9)
Median (min-max)	7.0 (1.0–34.0)
≤5 years (*n* (%))	465 (36.1)
5.1–10 years (*n* (%))	447 (34.7)
10.1–15 years (*n* (%))	206 (16.0)
>15 years (*n* (%))	170 (13.2)
Missing	395
Smoking status (*n* (%))	
Non-smoker	1195 (77.6)
Active smoker	231 (15.0)
Former smoker	113 (7.3)
Missing	144
Comorbidities (*n* (%))	
Hypertension	928 (57.3)
Hyperlipidemia	878 (55.4)
Myocardial infarction	132 (9.0)
Depression	121 (8.4)
Unstable angina	81 (5.7)
Heart failure	54 (3.7)
Arrhythmia	45 (3.1)
Peripheral artery disease	28 (2.0)
Cancer	20 (1.4)
Stroke	20 (1.4)
Microvascular complications (*n* (%))	
Neuropathy	444 (31.1)
Retinopathy	190 (14.0)
Nephropathy	140 (10.0)
HbA1c level (%)	
≤7%	162 (10.0)
≤7.5%	321 (20.0)
Blood pressure (mmHg)	
≤140/90	1000 (80.8)
≤130/80	661 (53.4)

treated patients were receiving basal insulin (33.0%, 34.3%, and 31.6%, respectively) (Table 2).

HbA1c, body weight, and BP outcome and treatment dose under dapagliflozin treatment

A significant decrease in HbA1c levels was noted from 9.0% (SD 1.8, 75 mmol/mol) at baseline to 8.0% (SD 1.3, 64 mmol/mol) in the 3rd month (mean difference −0.95%) and to 7.8% (SD 1.4, 62 mmol/mol) in the 6th month (mean difference −1.22%) of treatment ($p < 0.001$ for each) (Table 3).

A significant decrease in BMI levels was noted from 32.6 kg/m^2 (6.1) at baseline to 31.5 kg/m^2 (18.0) in the 3rd month (mean difference −0.93 kg/m^2) and to 31.0 kg/m^2 (17.6) in the 6th month (mean difference −1.42 kg/m^2) of treatment ($p < 0.001$ for each) (Table 3).

A significant decrease in body weight was noted from 89.2 kg (16.2) at baseline to 86.3 kg (15.2) kg in the 3rd month (mean difference −2.49) and to 85.1 kg (15.3) in the 6th month (mean difference −3.83) of treatment ($p < 0.001$ for each) (Table 3).

A significant decrease in systolic BP was noted from 131.9 mmHg (15.0) at baseline to 126.1 mmHg (11.9) in the 3rd month (mean difference −6.68) and to 124.1 mmHg (11.3) in the 6th month (mean difference −7.78) of treatment ($p < 0.001$ for each). A significant decrease in diastolic BP was noted from 81.2 mmHg (9.4) at baseline to 77.6 mmHg (7.8) in the 3rd month (mean difference −4.16) and to 76.8 mmHg (7.7) in the 6th month (mean difference −4.29) of treatment ($p < 0.001$ for each) (Table 3).

A significant reduction from baseline insulin dose (31.2 U/day (14.5)) was noted that involved total (mean −3.39

Table 2 Antidiabetic treatments during the study period

	Baseline (n = 1683)	3rd month (n = 1618)	6th month (n = 1683)
Dapagliflozin treatment (n (%))			
Yes	1683 (100.0)	1142 (97.8)	1596 (94.8)
Discontinued	0 (0.0)	26 (2.2)	72 (4.3)
Antidiabetic regimen (n (%))			
OAD only	878 (52.2)	586 (50.2)	888 (52.8)
OAD ± injectable drug	805 (47.8)	580 (49.7)	780 (46.3)
Injectable drug only	0 (0.0)	2 (0.2)	6 (0.4)
Type of OAD (n (%))			
Metformin	1482 (88.1)	1004 (86.0)	1459 (86.7)
DPP4i	872 (51.8)	607 (52.0)	880 (52.3)
Sulphonylurea	556 (33.0)	340 (29.1)	500 (29.7)
Thiazolidinedione	207 (12.3)	145 (12.4)	195 (11.6)
Glinide	114 (6.8)	85 (7.3)	110 (6.5)
Alpha glucosidase inhibitor	42 (2.5)	20 (1.7)	27 (1.6)
Type of injectable therapy (n (%))			
None	878 (52.2)	586 (50.2)	897 (53.3)
Insulin	616 (36.6)	450 (38.5)	596 (35.4)
GLP1A	109 (6.5)	90 (7.7)	126 (7.5)
Insulin + GLP1A	80 (4.8)	42 (3.6)	64 (3.8)
Type of insulin therapy (n (%))			
None	987 (58.6)	676 (57.9)	1023 (60.8)
Basal insulin	555 (33.0)	401 (34.3)	532 (31.6)
Bolus insulin	283 (16.8)	188 (16.1)	249 (14.8)
Mixed insulin	135 (8.0)	84 (7.2)	118 (7.0)
Insulin dose (mean (SD))	56.1 (37.8)	51.6 (36.0)	51.3 (35.3)
Bolus insulin	50.4 (25.4)	48.4 (26.7)	48.3 (24.7)
Basal insulin	31.2 (14.5)	29.8 (13.9)	28.9 (13.1)
Mixed insulin	55.5 (26.3)	51.8 (23.0)	54.4 (28.0)

DPP4i, dipeptidyl peptidase-4 inhibitor; *OAD*, oral antidiabetic drug; *CI*, confidence inteirval; *LB*, lower bound; *UB*, upper bound

Table 3 HbA1c, body weight, and BP outcome and treatment dose under dapagliflozin treatment

	Baseline	3rd month	6th month	*p* value
HbA1c (%)				
n	1603	926	1455	**<0.001**
Mean (SD)	9.0 (1.8)	8.0 (1.3)	7.8 (1.4)	
Change, mean (SD; 95% CI UB, LB)	From baseline	−0.95 (1.39;0.86,1.05)	−1.22 (1.55; 1.14, 1.30)	
	From 3rd month	–	−0.37 (0.90; 0.31, 0.43)	
BMI (kg/m^2)				
n	1249	851	1186	**<0.001**
Mean (SD)	32.6(6.1)	31.5 (18.0)	31.0 (17.6)	
Change, mean (SD; 95% CI UB, LB)	From baseline	−0.93 (0.84, 1.03)	−1.42 (1.3, 1.5)	
	From 3rd month	–	−0.61 (0.5, 0.7)	
Body weight (kg)				
n	1273	866	1215	**<0.001**
Mean (SD)	89.2 (16.2)	86.3 (15.2)	85.1 (15.3)	
Change, mean (SD; 95% CI UB, LB)	From baseline	−2.49 (3.15; 2.28, 2.70)	−3.83 (4.20; 3.53, 4.13)	
	From 3rd month	–	−1.67 (5.29; 1.39, 1.95)	
Systolic BP (mmHg)				
n	1238	826	1166	**<0.001**
Mean (SD)	131.9 (15.0)	126.1 (11.9)	124.1 (11.3)	
Change, mean (SD; 95% CI UB, LB)	From baseline	−6.68 (5.93, 7.43)	−7.78 (7.08, 8.47)	
	From 3rd month	–	−2.18 (1.63, 2.73)	
Diastolic BP (mmHg)				
n	1238	826	1166	**<0.001**
Mean (SD)	81.2 (9.4)	77.6 (7.8)	76.8 (7.7)	
Change, mean (SD; 95% CI UB, LB)	From baseline	−4.16 (3.60, 4.73)	−4.29 (3.80, 4.78)	
	From 3rd month	–	−0.90 (0.45, 1.34)	
Insulin dose (U/day)				
Total				
n	616	476	621	
Mean (SD)	56.1 (37.8)	51.6 (36.0)	51.3 (35.3)	**<0.001**
Change from baseline, mean (SD; 95% CI UB, LB)		−3.39 (1.75, 5.03)	−4.68 (3.11, 6.25)	
Bolus				
n	283	178	229	
Mean (SD)	50.4 (25.4)	48.4 (26.7)	48.3 (24.7)	0.107[a]
Change from baseline, mean (SD; 95% CI UB, LB)		−1.74 (−0.38, 3.85)	−2.41 (0.47, 4.36)	**0.015**[b]
Basal				
n	555	383	488	
Mean (SD)	31.2 (14.5)	29.8 (13.9)	28.9 (13.1)	**0.007**[a]
Change from baseline, mean (SD; 95% CI UB, LB)		−1.17 (0.32, 2.03)	−2.04 (1.13, 2.95)	**<0.001**[b]
Mixed				
n	135	74	104	
Mean (SD)	55.5 (26.3)	51.8 (23.0)	54.4 (28.0)	0.234[a]
Change from baseline, mean (SD; 95% CI UB, LB)		−1.11 (−0.73, 2.95)	−0.75 (−1.39, 2.89)	0.488[b]
Number of OADs				
n	1683	1168	1683	
Mean (SD)	2.93 (0.87)	2.86 (0.88)	2.82 (0.93)	**<0.001**
Change from baseline, mean (SD; 95% CI UB, LB)		−0.07 (0.04, 0.10)	−0.11 (0.08, 0.14)	

CI, confidence interval; *LB*, lower bound; *UB*, upper bound; *BP*, blood pressure

Values in bold indicate statistical significance ($p < 0.05$)

[a] Paired Student's *t* test baseline vs. 3rd month

[b] Paired Student's *t* test baseline vs. 6th month

U/day, $p < 0.001$) and basal (mean −1.17 U/day, $p = 0.007$) insulin doses at the 3rd month and total (mean −4.68 U/day, $p < 0.001$), bolus (mean −2.41 U/day, $p = 0.015$), and basal (mean −2.04 U/day, $p < 0.001$) insulin doses at 6th-month visits. Similarly, a significant reduction was noted in the number of OADs from baseline value (mean (SD) 2.93 (0.87) at the 3rd-month (mean −0.007, $p < 0.001$) and 6th-month (mean −0.11, $p < 0.001$) visits (Table 3).

HbA1c levels during study period according to diabetes duration and concomitant regimens

No significant difference was noted in mean (SD) HbA1c levels at baseline, 3rd month, and 6th month, as well as in change from baseline at follow-up visits with respect to diabetes duration (Table 4).

HbA1c levels were significantly higher in patients with OAD ± injectable drug vs. OAD treatment at baseline (mean 9.3% (SD 1.8, 78 mmol/mol) vs. 8.8% (SD 1.7, 73 mmol/mol), $p = 0.001$), 3rd month (mean 8.2% (SD 1.3, 66 mmol/mol) vs. 7.8% (SD 1.3, 62 mmol/mol), $p < 0.001$), and 6th month (mean 8.1% (SD 1.4, 65 mmol/mol) vs. 7.6% (SD 1.3, 60 mmol/mol), $p < 0.001$) visits along with similar difference from baseline HbA1c levels during follow-up visits between the two treatment groups (Table 4).

Body weight during study period according to diabetes duration and concomitant treatments

Diabetes duration of 10.1 to 15 years was associated with highest body weight values at baseline ($p = 0.011$), 3rd-month ($p = 0.031$), and 6th-month ($p = 0.006$) visits as compared with shorter diabetes duration or diabetes for >15 years, while no significant change was noted in change from baseline at follow-up visits with respect to diabetes duration (Table 4).

Body weight was significantly higher in patients with OAD ± injectable treatment vs. OAD treatment at baseline (92.0 kg (16.8) vs. 86.1 kg (15.8), $p < 0.001$) and remained higher in OAD ± injectable treatment group at follow-up visits ($p < 0.001$ for each), despite significantly larger difference from baseline in this group as compared with OAD treatment at both 3rd-month (−2.79 kg (−3.09, −2.49) vs. −2.14 kg (−2.43, −1.85), $p = 0.002$) and 6th-month (−4.34 kg (−4.73, −3.95) vs. −3.30 kg (−3.75, −2.85), $p < 0.001$) visits (Table 4).

BP during study period according to diabetes duration and concomitant regimens

No significant difference was noted in baseline systolic BP levels with respect to diabetes duration, while less than 5-year diabetes duration was associated with significantly lower systolic BP levels at both 3rd-month and 6th-month visits ($p < 0.001$ for each). No significant difference was noted in diastolic BP levels with respect to diabetes duration at baseline or during follow-up visits (Table 5).

No significant difference was noted in systolic and diastolic BP levels with respect to concomitant treatments or HbA1c levels at baseline or during follow-up visits (Table 5).

Target HbA1c, body weight, and BP achievement rates with respect to diabetes duration and concomitant regimens

Rate of target HbA1c (≤7%) achievement increased from 10.0% at baseline to 21.7% and 30.1% at the 3rd- and 6th-month visits, respectively (Tables 1 and 6). Rate of target HbA1c (≤7.5%) achievement increased from 20.0% at baseline to 38.0% and 49.3% at the 3rd- and 6th-month visits, respectively (Tables 1 and 6).

An increase was noted in rate of patients with target BP levels of ≤140/90 mmHg and ≤130/80 mmHg from baseline (80.8% and 53.4%, respectively) to 3rd month (92.4% and 70.7%, respectively) and 6th month (95.9% and 77.3%, respectively) (Tables 1 and 6).

The >5% weight loss target was achieved by 17.5% and 38.0% of patients at 3rd-month and 6th-month visits, respectively (Table 6).

At baseline, HbA1c levels were similar (mean (SD) 9.1% (1.8) vs. 9.0% (1.7), $p = 0.293$), whereas body weight was significantly lower (86.0 kg (15.8) vs. 93.1 kg (15.9), $p = 0.000$) and systolic BP (132.8 mmHg (15.5) vs. 130.7 mmHg (14.4), $p = 0.012$) and diastolic BP (132.8 mmHg (15.5) vs. 130.7 mmHg (14.4), $p = 0.012$) levels were significantly higher in females vs. males. After 6-month of dapagliflozin treatment, higher rate of target HbA1c achievement was noted in male vs. female patients (55.5 vs. 44.6%, $p = 0.000$), along with no significant difference in target BP and body weight achievement rates under dapagliflozin therapy according to gender (Table 6). Less than 5-year diabetes duration was associated with significantly higher rate of achieving HbA1c ≤7% ($p = 0.032$), BP ≤140/90 mmHg ($p = 0.001$), and BP ≤130/80 mmHg ($p < 0.001$) targets at the 6th month ($p = 0.032$) as compared with longer diabetes duration. Diabetes duration had no significant impact on target weight loss achievement (Table 6).

Concomitant OAD therapy vs. OAD ± injectable therapy was associated with significantly higher achievement rates for HbA1c ≤7% (26.3 vs. 17.3%, $p = 0.001$ at 3rd month; 45.6 vs. 30.8% at 6th month, $p < 0.001$) and HbA1c ≤7.5% (37.1 vs. 22.8% at 3rd month; 56.9 vs. 41.4% at 6th month, $p < 0.001$ for each) targets during follow-up visits (Table 6).

OAD ± injectable therapy vs. OAD therapy was associated with higher achievement rate of >5% weight loss target (20.2

Table 4 Change in HbA1c levels and body weight during follow-up with respect to diabetes duration and concomitant regimen study variables

	Baseline (n = 1683)			3rd month (n = 1618)					6th month (n = 1683)						
	n	Mean (SD)	p value	n	Mean (SD)	p value	Change from baseline		n	Mean (SD)	p value	Change from baseline		Change from 3rd month	
							Mean (95% CI LB, UB)	p value				Mean (95% CI LB, UB)	p value	Mean (95% CI LB, UB)	p value
HbA1c level (%)															
Diabetes duration															
≤5 years	457	9.0 (1.8)	0.336[a]	258	7.9 (1.3)	0.131[a]	−0.95 (−1.13, −0.77)	0.158[a]	417	7.7 (1.4)	0.336[a]	−1.36 (−1.52, −1.20)	0.078[a]	−0.41 (−0.53, −0.30)	0.233[a]
5.1–10 years	441	9.1 (1.7)		285	8.0 (1.3)		−1.02 (−1.16, −0.87)		410	7.9 (1.4)		−1.24 (−1.38, −1.10)		−0.32 (−0.42, −0.21)	
10.1–15 years	202	8.9 (1.6)		118	8.2 (1.3)		−0.69 (−0.93, −0.45)		195	7.8 (1.1)		−1.15 (−1.35, −0.94)		−0.45 (−0.61, −0.29)	
>15 years	164	8.9 (1.6)		112	8.0 (1.3)		−0.88 (−1.14, −0.63)		153	7.9 (1.3)		−1.01 (−1.23, −0.78)		−0.24 (−0.43, −0.05)	
Concomitant antidiabetics															
OADs	835	8.8 (1.7)	**<0.001**[b]	452	7.8 (1.3)	**<0.001**[b]	−0.91 (−1.04, −0.78)	0.347[a]	743	7.6 (1.3)	**<0.001**[b]	−1.22 (−1.33, −1.10)	0.997[b]	−0.41 (−0.50, −0.32)	0.232[b]
OAD ± injectable	768	9.3 (1.8)		474	8.2 (1.3)		−1.00 (−1.13, −0.87)		712	8.1 (1.4)		−1.22 (−1.33, −1.10)		−0.34 (−0.42, −0.25)	
Body weight (kg)															
Diabetes duration															
≤5 years	398	87.2 (15.8)	**0.011**[b]	266	84.4 (14.7)	**0.031**[b]	−2.27 (−2.64, −1.90)	0.683[b]	386	83.3 (15.0)	**0.006**[b]	−3.67 (−4.13, −3.21)	0.995[b]	−1.80 (−2.13, −1.47)	0.696[b]
5.1–10 years	403	89.8 (16.5)		277	87.1 (14.8)		−2.57 (−2.95, −2.19)		384	85.8 (15.4)		−3.63 (−4.29, −2.97)		−1.39 (−2.12, −0.66)	
10.1–15 years	188	91.5 (15.5)		122	88.5 (15.3)		−2.33 (−2.90, −1.76)		176	87.9 (14.2)		−3.73 (−4.37, −3.09)		−1.79 (−2.26, −1.31)	
>15 years	155	90.2 (16.4)		118	88.0 (16.2)		−2.27 (−2.88, −1.67)		146	85.8 (15.4)		−3.74 (−4.54, −2.95)		−1.69 (−2.35, −1.04)	
Concomitant antidiabetics															
OADs	622	86.1 (15.1)	**<0.001**[b]	405	84.1 (14.6)	**<0.001**[b]	−2.14 (−2.43, −1.85)	**0.002**[b]	591	82.7 (14.5)	**<0.001**[b]	−3.30 (−3.75, −2.85)	**<0.001**[b]	−1.40 (−1.90, −0.89)	0.073[b]
OAD ± injectable	651	92.0 (16.8)		461	88.2 (15.5)		−2.79 (−3.09, −2.49)		624	87.4 (15.7)		−4.34 (−4.73, −3.95)		−1.91 (−2.20, −1.62)	
HbA1c (%)															
>7%	1118	88.4 (15.9)	**<0.001**[b]	772	85.7 (14.7)	**<0.001**[b]	−2.41 (−2.63, −2.20)	0.109[a]	1077	84.6 (15.1)	**0.003**[b]	−3.65 (−3.97, −3.34)	**0.002**[b]	−1.55 (−1.85, −1.25)	**0.008**[b]
≤7%	126	94.7 (18.1)		81	92.7 (18.7)		−2.99 (−3.74, −2.24)		119	88.9 (16.2)		−5.26 (−6.29, −4.23)		−2.88 (−3.84, −1.92)	
>7.5%	991	88.2 (16.0)	**<0.001**[b]	675	85.5 (14.7)	**<0.001**[b]	−2.45 (−2.68, −2.22)	0.729	955	84.4 (15.0)	**0.002**[b]	−1.58 (−1.92, −1.25)	0.253	−3.68 (−4.02, −3.34)	0.096
≤7.5%	253	92.4 (16.9)		178	89.7 (16.8)		−2.54 (−3.02, −2.05)		241	87.7 (15.9)		−2.00 (−2.49, −1.50)		−4.32 (−4.95,-3.69)	

OAD, oral antidiabetic drug; *CI*, confidence interval; *LB*, lower bound; *UB*, upper bound

Values in bold indicate statistical significance ($p < 0.05$)

[a] One-way ANOVA–Tukey HSD test

[b] Student's t test

Table 5 Change in BP levels during follow-up with respect to diabetes duration and concomitant regimens

	Baseline (n = 1683)			3rd month (n = 1618)			6th month (n = 1683)		
	n	Mean (SD)	p value	n	Mean (SD)	p value	n	Mean (SD)	p value
Systolic BP (mmHg)									
Diabetes duration									
≤5 years	402	129.8 (15.0)	0.007[b]	263	123.7 (10.6)	**0.001**[b]	383	122.0 (9.9)	**<0.001**[b]
5.1–10 years	387	131.5 (14.4)		262	127.0 (12.3)		377	125.0 (12.4)	
10.1–15 years	179	132.8 (15.1)		114	126.0 (11.4)		161	125.6 (12.1)	
>15 years	147	134.3 (14.7)		112	128.3 (14.1)		136	125.7 (11.8)	
Concomitant antidiabetics									
OADs	609	131.7 (15.1)	0.724[a]	393	126.8 (12.4)	0.150[a]	577	124.1 (10.9)	0.877[a]
OAD ± injectable	629	132.0 (15.0)		433	125.6 (11.5)		589	124.0 (11.6)	
HbA1c (%)									
>7%	1098	131.9 (14.9)	0.755[a]	745	126.1 (11.8)	0.622[a]	1046	124.1 (11.3)	0.350[a]
≤7%	113	131.4 (17.1)		71	126.8 (13.1)		105	123.0 (10.0)	
>7.5%	974	132.2 (14.6)	0.093[a]	652	126.4 (11.8)	0.278[a]	932	124.2 (11.4)	0.237[a]
≤7.5%	237	130.4 (17.0)		164	125. (12.6)		219	123.2 (10.4)	
Diastolic BP (mmHg)									
Diabetes duration									
≤5 years	402	80.5 (9.3)	0.483[b]	263	77.2 (8.1)	0.717[b]	383	76.0 (7.5)	**0.026**[b]
5.1–10 years	387	81.1 (8.7)		262	78.0 (7.5)		377	77.2 (7.8)	
10.1–15 years	179	81.8 (9.4)		114	77.5 (7.4)		161	78.0 (8.0)	
>15 years	147	80.9 (10.8)		112	77.5 (8.2)		136	76.5 (7.8)	
Concomitant antidiabetics									
OADs	609	81.1 (9.5)	0.750[a]	393	77.8 (7.9)	0.328[a]	577	76.9 (7.6)	0.616[a]
OAD ± injectable	629	81.2 (9.3)		433	77.3 (7.7)		589	76.7 (7.7)	
HbA1c (%)									
>7%	1098	81.1 (9.3)	0.976[a]	745	77.5 (7.9)	0.208[a]	1046	76.7 (7.7)	0.889[a]
≤7%	113	81.2 (9.7)		71	78.7 (6.7)		105	76.6 (7.5)	
>7.5%	974	81.3 (9.3)	0.179[a]	652	77.7 (7.9)	0.422[a]	932	76.7 (7.8)	0.996[a]
≤7.5%	237	80.4 (9.6)		164	77.1 (7.2)		219	76.7 (7.2)	

[a] Student's t test

[b] Oneway ANOVA–Tukey HSD test

Values in bold indicate statistical significance ($p < 0.05$)

vs. 14.4%, p = 0.024) at the 3rd month visit, while type of concomitant regimen had no significant impact on target BP achievement during follow-up visits (Table 6).

Discussion

Representing the first nationwide data on real-life dapagliflozin treatment in Turkish T2D patients, our findings revealed that first prescription for dapagliflozin in T2D patients was associated with significant decrease in HbA1c (−1.22% on average), BMI (−1.42 kg/m^2 on average), body weight (−3.83 kg on average), and systolic (−7.78 mmHg on average) and diastolic (−4.29 mmHg on average) BP levels from baseline to the 6th month of treatment. At the end of 6-month dapagliflozin treatment (discontinued in 4.3% of patients), HbA1c levels were ≤7% in 30.1% of patients, BP was ≤140/90 mmHg in 95.9% of patients, and >5% weight loss was evident in 38.0% of patients.

Results from past clinical trials showed that patients receiving dapagliflozin in combination with other glucose-lowering agents (OADs or insulin + OADs) had HbA1c reductions in the range of −0.4 to −1.2% [14, 15, 18, 20–24], weight loss reduction in the range of −0.69 to −3.2 kg at 1 year [14, 15, 18, 21–24], and a SBP reduction of −2.6 to 4.28 mmHg and a DBP reduction of −1.2 to 1.6 mmHg [20, 25, 31].

Observational data on the effects of dapagliflozin initiation among T2D patients in primary and secondary hospital care settings revealed reductions in HbA1c (0.89-1.16%), weight (1.8-4.6 kg) and BP (systolic 1.6-5.5 mmHg; diastolic 0.3-3.4 mmHg) after addition of dapagliflozin [13, 27, 28]. In addition, target HbA1c (≤7.0%) levels were noted in 27.0% of

Table 6 Target HbA1c, body weight, and BP achievement rates with respect to diabetes duration and concomitant regimens

	Patients with target HbA1c (*n* (%))						Patients with >5% weight loss				Patients with target BP (*n* (%))					
	3rd month			6th month			3rd month		6th month		3rd month			6th month		
	n	≤7%	≤7.5%	*n*	≤7%	≤7.5%	*n*	*n* (%)	*n*	*n* (%)	*n*	≤140/90	≤130/80	*n*	≤140/90	≤130/80
Overall	926	201 (21.7)	352 (38.0)	1455	438 (30.1)	718 (49.3)	864	151 (17.5)	1206	458 (38.0)	826	763 (92.4)	584 (70.7)	1166	1118 (95.9)	901 (77.3)
Gender																
Female	515	102 (19.8)	177 (34.4)	823	216 (26.2)	367 (44.6)	489	83 (17.0)	672	257 (38.2)	461	424 (92.0)	316 (68.5)	638	606 (95.0)	486 (76.2)
Male	411	99 (24.1)	175 (42.6)	632	222 (35.1)	351 (55.5)	375	68 (18.1)	534	201 (37.6)	365	339 (92.9)	268 (73.4)	528	512 (97.0)	415 (78.6)
p value[a]		0.116	**0.011**		**0.000**	**0.000**		0.656		0.830		0.627	0.126		0.089	0.326
Diabetes duration																
≤5 years	258	60 (23.3)	108 (41.9)	417	149 (35.7)	224 (53.7)	265	41 (15.5)	383	143 (37.3)	263	247 (93.9)	195 (74.1)	383	375 (97.9)	321 (83.8)
5.1–10 years	285	62 (21.8)	106 (37.2)	410	113 (27.6)	198 (48.3)	276	50 (18.1)	382	141 (36.9)	262	244 (93.1)	182 (69.5)	377	361 (95.8)	284 (75.3)
10.1–15 years	118	24 (20.3)	39 (33.1)	195	50 (25.6)	92 (47.2)	122	17 (13.9)	175	63 (36.0)	114	104 (91.2)	80 (70.2)	161	149 (92.5)	115 (71.4)
>15 years	112	25 (22.3)	46 (41.1)	153	45 (29.4)	74 (48.4)	118	17 (14.4)	146	47 (32.2)	112	101 (90.2)	72 (64.3)	136	126 (92.6)	98 (72.1)
p value[a]		0.699	0.495		**0.032**	0.136		0.648		0.339		0.159	0.068		**0.001**	**<0.001**
Antidiabetic regimen																
OAD	452	119 (26.3)	206 (45.6)	743	276 (37.1)	423 (56.9)	404	58 (14.4)	589	212 (36.0)	393	359 (91.3)	273 (69.5)	577	555 (96.2)	442 (76.6)
OAD ± injectable	474	82 (17.3)	146 (30.8)	712	162 (22.8)	295 (41.4)	460	93 (20.2)	617	246 (39.9)	433	404 (93.3)	311 (71.8)	589	563 (95.6)	459 (77.9)
p value[b]		**0.001**	**<0.001**		**<0.001**	**<0.001**		**0.024**		0.166		0.291	0.457		0.605	0.589

OAD, oral antidiabetic drug

Values in bold indicate statistical significance ($p < 0.05$)

[a] χ^2 test

[b] Mantel-Haenszel test

patients [27] alongside ≥1% reduction in HbA1c in 42% of patients, ≥5kg weight loss in 15% of patients [29], and reductions in body weight plus HbA1c in 67.7% of patients [11].

Our findings on clinical effectiveness of dapagliflozin in terms of overall change in HbA1c (- 1.2%) and body weight (3.8 kg) over 6-month treatment in daily clinical practice seem broadly consistent with previous dapagliflozin clinical trials [10, 14, 15, 18–25],

In our cohort, average HbA1c reductions obtained under dapagliflozin treatment were similar with respect to diabetes duration and body weight was significantly higher in patients with 10–15 years of diabetes duration at baseline and remained higher during follow-up visits, whereas systolic BP reduction during 6-month therapy was significantly higher in patients with less than 5 years of diabetes duration. However, less than 5-year diabetes duration was associated with significantly higher rate of achieving HbA1c ≤7%, BP ≤140/90 mmHg, and BP ≤130/80 mmHg targets at the 6th month of dapagliflozin treatment as compared with longer diabetes duration.

Notably, in a past study among 3774 T2D patients including either early (first intensification after metformin or sulfonylurea monotherapy, n = 951) or later (second- or higher-order intensification, n = 2823), users of dapagliflozin, shorter disease duration was reported among early users vs. later users along with baseline-adjusted mean reduction of 1.54% vs. 1.02% in HbA1c, 3.31% vs. 4.06% in weight and 2.50 mmHg vs. 2.84 mmHg in systolic BP after 6–12 months of treatment, respectively [32]. Authors noted early versus later dapagliflozin use to be associated with a greater likelihood of adjusted HbA1c reduction of ≥1% (OR: 1.68, 95% CI: 1.15–2.45) [32]. In this regard, improved HbA1c and BP control with dapagliflozin in our patients with <5-year diabetes duration seems to support the likelihood of improved glycemic and BP control with broader and earlier dapagliflozin use [32].

A higher baseline HbA1c, shorter duration of diabetes, and male gender were reported to be independently associated with a greater HbA1c reduction in T2D patients who initiated dapagliflozin [27]. Our findings also revealed greater improvement in HbA1c levels in males vs. females, despite the similar baseline HbA1c values between males and females.

In accordance with higher baseline HbA1c levels in patients under concomitant OAD therapy, rates of achieving HbA1c ≤7% and ≤7.5 targets were significantly higher in this group as compared with OAD ± injectable therapy, while lower rates of achieving >5% weight loss were noted with concomitant OAD vs. with OAD ± injectable therapy. The increase in HbA1c target achievement in our OAD-treated patients at the end of 6-month dapagliflozin treatment supports the efficacy of dapagliflozin shown in patients with T2D inadequately controlled with metformin [18, 20, 33–35]. Notably, in accordance with the efficacy of add-on dapagliflozin in OAD-treated patients in terms of HbA1c levels, body weight and BP levels in our cohort, data from a retrospective real-world study of dapagliflozin vs. other OADs added to metformin in T2D patients revealed association of dapagliflozin + metformin with a greater reduction in HbA1c level (−1.0% vs. −0.7%), body weight (−1.8 kg vs. −0.7 kg), and SBP (−3.6 mmHg vs. −0.1 mmHg), and DBP (−2.0 mmHg vs. −0.6 mmHg) levels from baseline to 12-month follow-up [13].

Dapagliflozin was reported to be associated with placebo-subtracted changes from baseline in systolic BP of −3.6 to 4.28 mmHg [25, 31] and in diastolic BP of −1.2 mmHg in hypertensive patients, while systolic and diastolic BP reduction of −2.6 and −1.2 mmHg, respectively, in non-hypertensive patients with T2D [31]. The efficacy of dapagliflozin on BP reduction in hypertensive patients with T2D was also reported to be higher in those receiving a β-blocker or calcium-channel blocker agent [25].

Accordingly, the reductions obtained in BP with dapagliflozin in the present study (systolic 7.8 mmHg; diastolic 4.3 mmHg) seem consistent with rate (57.0%) of hypertension in the present cohort and support the likelihood of dapagliflozin treatment to optimize BP control in hypertensive T2D patients by providing a diuretic-like effect enhancing the efficacy of concomitant antihypertensive agents [25].

Data from DIVERSITY-CVR study in 340 patients with early-stage T2D on metformin alone or no glucose-lowering agents who were randomized to receive dapagliflozin or sitagliptin for 24 weeks revealed the superiority of dapagliflozin in terms of the achievement ratio of primary endpoint (HbA1c level maintenance < 7.0%, avoidance of hypoglycemia and ≥ 3.0% body weight loss from baseline) as well as in terms of several secondary endpoints that modulate cardiometabolic risk (reducing FPG, insulin, and uric acid, increasing HLD-c, and suppressing the increase in serum creatinine and the decrease in estimated GFR) [36].

Hence our findings support the preferable cardiometabolic effects of dapagliflozin with consistently reported efficacy at improving cardiometabolic risk factors in both randomized trials and large-scale real-life studies, suggesting that SGLT2 inhibitors might be more suitable than DPP-4 inhibitors for preventing cardiovascular events in patients with early-stage but inadequately controlled type 2 diabetes [30, 36–42]. Notably, the association of dapagliflozin therapy with improvement of several cardiometabolic indices and reduction in cardiovascular risk is considered to emphasize that approaches beyond glycemic control (such as body weight reduction) represent important strategies for reducing the risk of cardiovascular events and mortality in T2D patients [36, 43, 44].

Given the consideration of higher rate of HbA1c target achievement and greater reductions in body weight in patients treated with dapagliflozin beyond 6 months [28, 35], our

findings may indicate likelihood of a better efficacy of longer-term dapagliflozin treatment on HbA1c, body weight, and BP reduction in patients with higher baseline levels.

The major strength of this observational study is the inclusion from a database of 1683 T2D patients at 79 centers in Turkey. The large number of centers increases the likelihood that the study is representative of the overall T2D population and hence its generalizability. However, certain limitations to this study should be considered. First, missing or incorrect data, and the fact that data reflects everyday health care and were collected for non-research purposes is a limitation common to retrospective medical records database analyses. Therefore, clinical values are captured as part of routine clinical practice and there is considerable variation in the timing and completeness of measurements. Second, patient records report issued prescriptions only with no information available on whether the patient collected the prescription. Furthermore, prescriptions may not always include dosage information (not well recorded for insulin) which is needed for more accurate estimation of medication adherence/medication possession ratio. Third, first dapagliflozin prescription recorded in the participating centers may not be the patient's first ever as the patient could have initiated treatment outside of that clinic. Fourth, lack of data on control group values and adverse events such as hypoglycemia or renal dysfunction is another limitation which otherwise would extend the knowledge achieved in the current study. Fifth, this is a descriptive study with no comparative arm and any changes observed may not relate to the pharmaceutical intervention alongside likelihood of differences in data quality and accuracy between various hospitals. Therefore, future trials with larger sample size, adequate ethnicity representation, and long-term observation are necessary to confirm the generalizability of the study findings.

In conclusion, this first nationwide observational survey in T2D patients treated with dapagliflozin in Turkey provided real-life evidence on significant and clinically meaningful reductions in HbA1c, body weight, and BP after initiation of dapagliflozin. Association of 6-month dapagliflozin with higher rate of HbA1c target achievement when used as add-on therapy to OAD rather than to OAD ± injectable therapy, in relation to lower baseline HbA1c levels in the former group, and higher HbA1c and BP target achievement when used in patients with less than 5-year diabetes duration seem to emphasize the likelihood of improved glycemic and BP control with broader and earlier dapagliflozin use in T2D patients. Further prospective, longer-term longitudinal studies are warranted to identify appropriate indications and optimal combinations of dapagliflozin with other antidiabetic medications for optimal clinical efficacy of add-on dapagliflozin in T2D patients.

Acknowledgment This work was supported by Astra-Zeneca Turkey. The authors would like to thank Prof. Sule Oktay, MD, PhD and Cagla Ayhan, MD from KAPPA Consultancy Training Research Ltd (Istanbul, Turkey) for providing editorial support.

The DAPATURK Study Group (Centers are listed in order of enrollment contribution)

1. Derun Taner Ertugrul, M.D., Clinic of Endocrinology, Kecioren Training and Research Hospital, Ankara
2. Ilhan Yetkin, M.D., Clinic of Endocrinology, Gazi University Faculty of Medicine, 3. Ankara
3. Erdal Kan, M.D., Clinic of Endocrinology, Medicana International Hospital, Samsun
4. Cigdem Bahadir Tura, M.D., Clinic of Endocrinology, Tokat State Hospital, Tokat
5. Haci Bayram Tugtekin, M.D., Clinic of Internal Medicine, Umraniye Training and Research Hospital, Istanbul
6. Hayati Ayakta, M.D., Clinic of Endocrinology, Private Grandmedical Hospital, Manisa
7. Mehmet Çelebioglu, M.D., Clinic of Internal Medicine, Private Anadolu Hospital, Eskisehir
8. Eren Gurkan, M.D., Clinic of Endocrinology, Mustafa Kemal University Faculty of Medicine, Hatay
9. Kerem Sezer, M.D., Clinic of Endocrinology, Mersin University Faculty of Medicine, Mersin
10. Ramazan Gen, M.D., Clinic of Endocrinology, Mersin University Faculty of Medicine, Mersin
11. Suleyman Ozcaylak, M.D., Clinic of Internal Medicine, Diyarbakir Health Sciences University Gazi Yasargil Training and Research Hospital, Diyarbakir
12. Yildiz Okuturlar, M.D., Clinic of Internal Medicine, Health Sciences University Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul
13. Mehmet Coskun, M.D., Clinic of Internal Medicine, Diyarbakir Health Sciences University Gazi Yasargil Training and Research Hospital, Diyarbakir
14. Nilgun Govec Giynas, M.D., Clinic of Internal Medicine, Ankara Training and Research Hospital, Ankara
15. Harun Aysal, M.D., Clinic of Internal Medicine, Antalya Atatürk State Hospital, Antalya
16. Ayca Serap Erdem, M.D., Clinic of Internal Medicine, Uskudar State Hospital, Istanbul
17. Mustafa Aydemir, M.D., Clinic of Endocrinology, Afyon State Hospital, Afyon
18. Okan Bakiner, M.D., Clinic of Endocrinology, Baskent University Faculty of Medicine, Adana
19. Emre Cicekli, M.D., Clinic of Internal Medicine, Medicana International Hospital, Sivas
20. Deniz Gezer, M.D., Clinic of Internal Medicine, Mersin City Training and Research Hospital, Mersin
21. Ramazan Kaya, M.D., Clinic of Internal Medicine, Tarsus State Hospital, Mersin
22. Levent Kebapcilar, M.D., Clinic of Internal Medicine, Selcuk University Faculty of Medicine, Konya
23. Umit Cinkir, M.D., Clinic of Endocrinology, Medical Park Gaziantep Hospital, Gaziantep
24. Memmune Sena Ulu, M.D., Clinic of Nephrology, Kocatepe University Faculty of Medicine, Afyon
25. Canan Ersoy, M.D., Clinic of Endocrinology, Uludag University Faculty of Medicine, Bursa
26. Mustafa Timur Kagan, M.D., Clinic of Internal Medicine, Elazig Training and Research Hospital, Elazig
27. Berna Dalmis Ekiz, M.D., Clinic of Internal Medicine, Izan Private Health Hospital, Mugla
28. Faruk Kilinc, M.D., Clinic of Endocrinology, Firat University Faculty of Medicine, Elazig

29. Kevser Onbasi, M.D., Clinic of Endocrinology, Dumlupinar University Faculty of Medicine, Kutahya
30. Mahir Cengiz, M.D., Clinic of Internal Medicine, Istanbul University Cerrahpasa Faculty of Medicine, İstanbul
31 Mehmet Celik, M.D., Clinic of Internal Medicine, Antalya Training and Research Hospital, Antalya
32. Metin Guclu, M.D., Clinic of Endocrinology, Health Sciences University Bursa Yüksek Ihtisas Education and Research Hospital, Bursa
33. Metin Sarıkaya, M.D., Clinic of Nephrology, Health Sciences University Antalya Training and Research Hospital, Antalya
34. Ozcan Ozbag, M.D., Clinic of Internal Medicine, Dr.Ersin Arslan Training and Research Hospital, Gaziantep
35. Ramazan Sari, M.D., Clinic of Endocrinology, Akdeniz University Faculty of Medicine, Antalya
36. Rifki Ucler, M.D., Clinic of Endocrinology, Van 100. Yil University Faculty of Medicine, Van
37. Selcuk Sezikli, M.D., Clinic of Internal Medicine, Merzifon State Hospital, Amasya,
38. Mustafa Araz, M.D., Clinic of Endocrinology, Gaziantep University Faculty of Medicine, Gaziantep
39. Erdal Gundogan, M.D., Clinic of Internal Medicine, Bagcilar Training and Research Hospital, Istanbul
40. Erhan Bozkurt, M.D., Clinic of Internal Medicine, Afyonkarahisar Emirdag State Hospital, Afyon
41. Murat Akbas, M.D., Clinic of Internal Medicine, Istanbul Eyupsultan State Hospital, Istanbul
42. Rifat Bozkus, M.D., Clinic of Internal Medicine, Ulus State Hospital, Ankara
43. Baris Akinci, M.D., Clinic of Endocrinology, 9 Eylül University Faculty of Medicine, Izmir
44. Ersen Karakilic, M.D., Clinic of Endocrinology, Canakkale State Hospital, Canakkale
45. Murat Medeni, M.D., Clinic of Internal Medicine, Bornova Turkan Ozilhan State Hospital, Izmir
46. Ozgur Keskek, M.D., Clinic of Endocrinology, Adana City Hospital, Adana
47. Enver Sukru Goncuoglu, M.D., Clinic of Endocrinology, Dr. Nazif Bagriacik Kadikoy State Hospital, Istanbul
48. Sayid Shafi Zuhur, M.D., Clinic of Internal Medicine, Namık Kemal University Faculty of Medicine, Tekirdag
49. Ahmet Ziya Sahin, M.D., Clinic of Internal Medicine, Cukurova Dr.Askim Tufekci State Hospital, Adana
50. Kursat Dal, M.D., Clinic of Endocrinology, Kecioren Training and Research Hospital, Ankara
51. Mehmet Ali Eren, M.D., Clinic of Endocrinology, Harran University Faculty of Medicine, Sanlıurfa
52. Tugba Arkan, M.D., Clinic of Endocrinology, Kocaeli Health Sciences University Derince Training and Research Hospital, Kocaeli
53. Bengur Taskiran, M.D., Clinic of Internal Medicine, Eskisehir Yunus Emre State Hospital, Eskisehir
54. Gokturk Kilinc, M.D., Clinic of Internal Medicine, Fethiye State Hospital, Mugla
55. Emre Bozkirli, M.D., Clinic of Endocrinology, Baskent University Faculty of Medicine, Adana
56. Sabriye Ozkaya Kafesciler, M.D., Clinic of Endocrinology, Manisa State Hospital, Manisa
57. Nuh Baklaci, M.D., Clinic of Endocrinology, Private Antakya Akademi Hospital, Hatay
58. Esra Cil Sen, M.D., Clinic of Endocrinology, SiSli Hamidiye Etfal Training and Research Hospital, Istanbul
59. Sahin Doganay, M.D., Clinic of Endocrinology, Yildirim Beyazit University Yenimahalle Training and Research Hospital, Ankara
60. Caglar Koseoglu, M.D., Clinic of Internal Medicine, Golbasi State Hospital, Ankara
61. Tamer Tetiker, M.D., Clinic of Endocrinology, Cukurova University Faculty of Medicine, Adana
62. Taner Bayraktaroglu, M.D., Clinic of Endocrinology, Zonguldak Bulent Ecevit University Faculty of Medicine, Zonguldak
63. Aytekin Oguz, M.D., Clinic of Internal Medicine, Istanbul Medeniyet University Faculty of Medicine, Istanbul
64. Esra Ataoglu, M.D., Clinic of Internal Medicine, Istanbul Haseki Training and Research Hospital, Istanbul
65. Muhammed Mustafa Demirpence, M.D., Clinic of Endocrinology, Izmir Health Sciences University Tepecik Training and Research Hospital, Izmir
66. Semra Tursun, M.D., Clinic of Internal Medicine, Private Tekden Hospitals, Denizli
67. Inan Anaforoglu, M.D., Clinic of Endocrinology, Medical Park Trabzon Hospital, Trabzon
68. Omur Tabak, M.D., Clinic of Internal Medicine, Istanbul Health Sciences University Kanuni Sultan Suleyman Training and Research Hospital, Istanbul
69. Rifat Emral, M.D., Clinic of Endocrinology, Ankara University Faculty of Medicine, Ankara
70. Kubilay Karsidag, M.D., Clinic of Endocrinology, Istanbul University Istanbul Faculty of Medicine, Istanbul
71. Oguzhan Sitki Dizdar, M.D., Clinic of Internal Medicine, Kayseri Training and Research Hospital, Kayseri
72. Alparslan Kemal Tuzcu, M.D., Clinic of Endocrinology, Dicle University Faculty of Medicine, Diyarbakir
73. Mustafa Caliskan, M.D., Clinic of Internal Medicine, Duzce Atatürk State Hospital, Duzce
74. Pinar Sirmatel, M.D., Clinic of Internal Medicine, Edirne Sultan I. Murat State Hospital, Edirne
75. Yasin Kocaoz, M.D., Clinic of Internal Medicine, Izmir Menemen State Hospital, Izmir
76. Hakan Dogan, M.D., Clinic of Internal Medicine, Health Sciences University Izmir Bozyaka Training and Research Hospital, Izmir
77. Semin Melahat Fenkci, M.D., Clinic of Endocrinology, Pamukkale University Faculty of Medicine, Denizli
78. Ibrahim Sahin, M.D., Clinic of Endocrinology, Inonu University Faculty of Medicine, Malatya
79. Zuleyha Karaca, M.D., Clinic of Endocrinology, Erciyes University Faculty of Medicine, Kayseri

Funding Astra-Zeneca Turkey

Declarations

Ethics approval The study was approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization of Good Clinical Practice, and the local regulations for clinical research.

Consent to participate This is a retrospective observational cohort study.

Consent for publication Permission was obtained from our institutional ethics committee for the use of patient data for publication purposes.

Conflict of interest Ceren Yilmaz, M.D and Onur Utebay, M.D are Astra-Zeneca employees. Other authors declare that they have no conflict of interest.

References

1. International Diabetes Federation. IDF Diabetes Atlas. 9th ed. Brussels, Belgium: International Diabetes Federation; 2019. http://www.diabetesatlas.org. Accessed 3 Feb 2020.
2. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al. TURDEP-II Study Group. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol. 2013;28:169–80.
3. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359:1577–89.
4. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med. 2008;358:2560–72.
5. UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.
6. Khunti K, Godec TR, Medina J, Garcia-Alvarez L, Hiller J, Gomes MB, et al. Patterns of glycaemic control in patients with type 2 diabetes mellitus initiating second-line therapy after metformin monotherapy: Retrospective data for 10 256 individuals from the United Kingdom and Germany. Diabetes Obes Metab. 2018;20:389–99.
7. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2018;61:2461–98.
8. Jung CH, Jang JE, Park JY. A novel therapeutic agent for type 2 diabetes mellitus: SGLT2 inhibitor. Diabetes Metab J. 2014;38:261–73.
9. Nauck MA. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Des Devel Ther. 2014;8:1335–80.
10. Min KW, Ku BJ, Lee JH, Kim MS, Ahn KJ, Lee MK, et al. Addition of Ipragliflozin to metformin treatment in Korean patients with type 2 diabetes mellitus: subgroup analysis of a phase 3 trial. Diabetes Metab J. 2017;41:135–45.
11. Han E, Kim A, Lee SJ, Kim JY, Kim JH, Lee WJ, et al. Characteristics of dapagliflozin responders: A longitudinal, prospective, nationwide dapagliflozin surveillance study in Korea. Diabetes Ther. 2018;9:1689–701.
12. Chow W, Miyasato G, Kokkotos FK, Bailey RA, Buysman EK, Henk HJ. Real-world canagliflozin utilization: Glycemic control among patients with type 2 diabetes mellitus-A multi-database synthesis. Clin Ther. 2016;38:2071–82.
13. Huang H, Bell KF, Gani R, Tugwell CW, Eudicone JM, Krukas-Hampel MR. A retrospective real-world study of dapagliflozin versus other oral antidiabetic drugs added to metformin in patients with type 2 diabetes. Am J Manag Care. 2018;24:132–7.
14. Rosenstock J, Vico M, Wei L, Salsali A, List JF. Effects of dapagliflozin, an SGLT2 inhibitor, on HbA(1c), body weight, and hypoglycemia risk in patients with type 2 diabetes inadequately controlled on pioglitazone monotherapy. Diabetes Care. 2012;35:1473–8.
15. Strojek K, Yoon KH, Hruba V, Elze M, Langkilde AM, Parikh S. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with glimepiride: a randomized, 24-week, double-blind, placebo-controlled trial. Diabetes Obes Metab. 2011;13:928–38.
16. Forziga Turkey-Summary of Product Characteristics (SPC). 2016. https://www.astrazeneca.com/content/dam/az/Country-Sites/Turkey /Medicines/ Forziga-5-mg-KUB.pdf. Accessed 25 Dec 2019.
17. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. Diabetes Care. 2010;33:2217–24.
18. Bailey CJ, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;375:2223–33.
19. Bolinder J, Ljunggren Ö, Johansson L, Wilding J, Langkilde AM, Sjöström CD, et al. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. Diabetes Obes Metab. 2014;16:159–69.
20. Nauck MA, Del Prato S, Meier JJ, Durán-García S, Rohwedder K, Elze M, et al. Dapagliflozin versus glipizide as add-on therapy in patients with type 2 diabetes who have inadequate glycemic control with metformin: a randomized, 52-week, doubleblind, active-controlled noninferiority trial. Diabetes Care. 2011;34:2015–22.
21. Bolinder J, Ljunggren Ö, Kullberg J, Johansson L, JWilding J, Langkilde AM, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012;97:1020–31.
22. Matthaei S, Bowering K, Rohwedder K, Grohl A. Parikh S; Study 05 Group. Dapagliflozin improves glycemic control and reduces body weight as add-on therapy to metformin plus sulfonylurea: a 24-week randomized, double-blind clinical trial. Diabetes Care. 2015;38:365–72.
23. Jabbour SA, Hardy E, Sugg J, Parikh S, Study 10 Group. Dapagliflozin is effective as add-on therapy to sitagliptin with or without metformin: a 24-week, multicenter, randomized, double-blind, placebo-controlled study. Diabetes Care. 2014;37:740–50.
24. Wilding JP, Woo V, Soler NG, Pahor A, Sugg J, Rohwedder K, et al. Dapagliflozin 006 Study Group. Long-term efficacy of dapagliflozin in patients with type 2 diabetes mellitus receiving high doses of insulin: a randomized trial. Ann Intern Med. 2012;156:405–15.
25. Weber MA, Mansfield TA, Cain VA, Iqbal N, Parikh S, Ptaszynska A. Blood pressure and glycaemic effects of dapagliflozin versus placebo in patients with type 2 diabetes on combinationantihypertensive therapy: a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Diabetes Endocrinol. 2016;4:211–20.
26. Scheerer MF, Rist R, Proske O, Meng A, Kostev K. Changes in HbA1c, body weight, and systolic blood pressure in type 2 diabetes patients initiating dapagliflozin therapy: a primary care database study. Diabetes Metab Syndr Obes. 2016;9:337–45.
27. Brown RE, Gupta N, Aronson R. Effect of dapagliflozin on glycemic control, weight, and blood pressure in patients with type 2 diabetes attending a specialist endocrinology practice in Canada: A retrospective cohort analysis. Diabetes Technol Ther. 2017;19:685–91.
28. Wilding J, Bailey C, Rigney U, Blak B, Beekman W, Emmas C. Glycated hemoglobin, body weight and blood pressure in type 2 diabetes patients initiating dapagliflozin treatment in primary care: A retrospective study. Diabetes Ther. 2016;7:695–711.
29. McGovern A, Dutta N, Munro N, Watters K, Feher M. Dapagliflozin: clinical practice compared with pre-registration trial data. Br J Diabetes Vasc Dis. 2014;14:138–43.
30. Nelson AJ, Navar AM. How Real-world Data Augments What We Know About SGLT-2 Inhibitors for Cardiovascular Risk Reduction. ACC Latest in Cardiology, Sep 09, 2020. https://www.acc.org/latest-in-cardiology/articles/2020/09/08/08/23/how-real-world-data-augments-what-we-know-about-sglt-2-inhibitors-for-cv-risk-reduction. Accessed 28 Oct 2020.

31. Sjöström CD, Johansson P, Ptaszynska A, List J, Johnsson E. Dapagliflozin lowers blood pressure in hypertensive and non-hypertensive patients with type 2 diabetes. Diab Vasc Dis Res. 2015;12:352–8.
32. Wilding JPH, Rigney U, Blak BT, Nolan ST, Fenici P, Medina J. Glycaemic, weight, and blood pressure changes associated with early versus later treatment intensification with dapagliflozin in United Kingdom primary care patients with type 2 diabetes mellitus. Diabetes Res Clin Pract. 2019;155:107791.
33. Scorsone A, Saura G, Fleres M, Spano L, Aiello V, Brancato D, et al. Efficacy and renal safety of dapagliflozin in patients with type 2 diabetes mellitus also receiving metformin: A real-life experience. J Diabetes Res. 2018;2018:8501418.
34. Nyström T, Bodegard J, Nathanson D, Thuresson M, Norhammar A, Eriksson JW. Novel oral glucose-lowering drugs are associated with lower risk of all-cause mortality, cardiovascular events and severe hypoglycaemia compared with insulin in patients with type 2 diabetes. Diabetes Obes Metab. 2017;19:831–41.
35. Bailey CJ, Gross JL, Hennicken D, Iqbal N, Mansfield TA, List JF. Dapagliflozin add-on to metformin in type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled 102-week trial. BMC Med. 2013;11:43.
36. Fuchigami A, Shigiyama F, Kitazawa T, Okada Y, Ichijo T, Higa M, et al. Efficacy of dapagliflozin versus sitagliptin on cardiometabolic risk factors in Japanese patients with type 2 diabetes: a prospective, randomized study (DIVERSITY-CVR). Cardiovasc Diabetol. 2020;19:1.
37. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373:2117–28.
38. Persson F, Nystrom T, Jorgensen ME, Carstensen B, Gulseth HL, Thuresson M, et al. Dapagliflozin is associated with lower risk of cardiovascular events and all-cause mortality in people with type 2 diabetes (CVD-REAL Nordic) when compared with dipeptidyl peptidase-4 inhibitor therapy: a multinational observational study. Diabetes Obes Metab. 2018;20:344–51.
39. Patorno E, Pawar A, Franklin JM, Najafzadeh M, Deruaz-Luyet A, Brodovicz KG, et al. Empagliflozin and the risk of heart failure hospitalization in routine clinical care. Circulation. 2019;139:2822–30.
40. Schork A, Saynisch J, Vosseler A, Jaghutriz BA, Heyne N, Peter A, et al. Effect of SGLT2 inhibitors on body composition, fluid status and renin-angiotensin-aldosterone system in type 2 diabetes: a prospective study using bioimpedance spectroscopy. Cardiovasc Diabetol. 2019;18:46.
41. Irace C, Casciaro F, Scavelli FB, Oliverio R, Cutruzzola A, Cortese C, et al. Empagliflozin influences blood viscosity and wall shear stress in subjects with type 2 diabetes mellitus compared with incretin-based therapy. Cardiovasc Diabetol. 2018;17:52.
42. Handelsman Y, Mathieu C, Del Prato S, Johnsson E, Kurlyandskaya R, Iqbal N, et al. Sustained 52-week efficacy and safety of triple therapy with dapagliflozin plus saxagliptin versus dual therapy with sitagliptin added to metformin in patients with uncontrolled type 2 diabetes. Diabetes Obes Metab. 2018;21:883–92.
43. Rosenzweig JL, Bakris GL, Berglund LF, Hivert M-F, Horton ES, Kalyani RR, et al. Primary prevention of ASCVD and T2DM in patients at metabolic risk: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2019;104:3939–85.
44. Reaven PD, Emanuele NV, Wiitala WL, Bahn GD, Reda DJ, McCarren M, et al. Intensive glucose control in patients with type 2 diabetes-15-year follow-up. N Engl J Med. 2019;380:2215–24.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):161–165
https://doi.org/10.1007/s13410-021-00959-z

ORIGINAL ARTICLE

Empirical sulphonylurea in neonatal diabetes: results from a tertiary care center

Inderpal Singh Kochar[1] · Smita Ramachandran[1]

Received: 27 January 2021 / Accepted: 13 May 2021 / Published online: 31 May 2021

Abstract

Background To study the genetic profile of infants with neonatal diabetes and response to empirical glibenclamide.

Method A retrospective study, between 2014 and 2018, data retrieved from the records of infants admitted with neonatal diabetes and with genetic analysis. Started on insulin and given empirical glibenclamide in selected cases to evaluate the effects.

Results Eleven children were diagnosed with NDM and genetic testing was done in all. Two cases were KCJN11, one HNF1B, one INS, one FOXP3, and one EIF2AK3. Four were started on empirical glibenclamide and three cases responded well. No noted severe adverse events.

Conclusion Glibenclamide is a safe drug for treating neonatal diabetes and may be tried empirically in selected cases.

Keywords Neonatal diabetes · Glibenclamide · Insulin

Introduction

Neonatal diabetes (NDM) is a rare condition presenting with hyperglycemia within the first 6 months of life. It can be transient (TND) or permanent (PND) and are usually associated with genetic defects [1]. NDM occurs approximately in 90,000–160,000 live births, and over 20 mutations have been identified [2].

TND constitutes about 50–60% of all NDM and tends to resolve by 12 weeks but may recur later during periods of increased insulin resistance, namely puberty and pregnancy [3].

PND, however, requires lifelong insulin and may be associated with monogenic mutations resulting in abnormal development of pancreatic islet cells, decreased B-cell mass, or B-cell dysfunction [3].

In the neonatal period, it can sometimes be difficult to diagnose NDM as hyperglycemia can result due to host of other causes: prematurity, stress, sepsis, inotropes, caffeine, steroids, and parenteral nutrition [4]. Hence genetic testing becomes very important for both management and prognostication.

However, the results of genetic testing can take several weeks and giving a trial of sulphonylureas may offer some benefits in such cases [5].

In this study we have attempted to evaluate the risk and benefits of starting glibenclamide with insulin in neonatal diabetes before the results of genetic testing.

Materials and methods

A retrospective study was planned, where all the data from children presenting from February 2014 till September 2018, with neonatal diabetes at less than 6 months of age were analyzed. The children were admitted in the Pediatric Endocrinology department of Indraprastha Apollo Hospital.

Neonatal diabetes was diagnosed in children less than 6 months of age at presentation, with polyuria, dehydration, failure to gain weight, and random blood sugar > 200 mg/dl.

All the infants were admitted and started on subcutaneous insulin detemir, at a starting dose of 0.3 u/kg/d, and 2–3 doses of insulin lispro were given, titrated according to the blood sugars

Genetic mutation analysis was done after obtaining informed consent from the parents.

✉ Inderpal Singh Kochar
inderpal_kochar@yahoo.com

✉ Smita Ramachandran
smita_rama25@yahoo.com

[1] Consultant Pediatric and Adolescent Endocrinology, Indraprastha Apollo Hospital, New Delhi, India

The genetic analysis of the probands, and their parents were sent to Royal Devon and Exeter NHS Foundation Trust laboratory, Exeter, UK. All the infants had Sanger sequencing done for the KCNJ11, ABCC8, and INS genes and expanded sequencing was done in one case with FOXP3 mutation.

The study was approved by the institutional review board.

Empirical glibenclamide was given only in infants with the following criteria:

1. Requiring high doses of insulin > 0.7 u/kg/d
2. Persisting hyperglycemia
3. With no associated diarrhea, syndromic features, dermatitis
4. Non-consanguineous parents

The infants were given a trial of oral glibenclamide starting at 0.2 mg/k/d along with the subcutaneous insulin.

Four infants fulfilling these criteria were given a trial of glibenclamide.

The dose of glibenclamide was titrated according to the blood sugar values.

Results

During the study period from 2014 to 2018, 11 children were diagnosed with NDM, and genetic testing was done in all. Two cases were KCJN11, one HNF1B, one INS, one FOXP3, and one EIF2AK3. The results for 5 cases came back negative for any mutations. In this group 7 cases were males and the rest females. All the children were born at full term with birth weight more than 2.5 kg.

The age of presentation of the earliest was at 25 days of life and the oldest was at 4 months of age.

Four infants (case 1, 2, 7,11) were given a trial of empirical glibenclamide after sending the samples for genetic testing. All these infants were receiving insulin at a dose of > 0.7 u/k/d and were not euglycemic on this dose; none of the parents of these cases had consanguineous marriage and had no syndromic features.

Three of the cases (2, 7, 11) responded favorably and insulin was tapered off slowly.

However in case no 1, there was an initial reduction on insulin dose, and we were able to stop insulin, but within a month the child became hyperglycemic and was restarted on insulin. His genetic results revealed no mutations, but the child developed recurrent episodes of diarrhea and dermatitis; at this point an expanded genetic analysis was done which came back positive for FOXP3 mutation and was diagnosed as IPEX syndrome. This child was lost to follow-up.

Of the other three cases started on empirical glibenclamide genetic studies for 2 cases came positive for KCJN11 (case 2, 11), and 1 came back (case 7) negative for any mutation. Case 7 was diagnosed as TDM and was weaned off glibenclamide at 9 months of age and continues to be healthy.

Case 2 presented to us at 6 months of age diagnosed with NDM at 4 months requiring 1.2 u/k/d of insulin but was still having hyperglycemia. He was started empirically on glibenclamide and responded favorably. He was off insulin by 18 days of starting glibenclamide and maintained normal blood sugars at a dose of 0.4 mg/k/d. His genetic mutation was positive for KCJN11.

Case 5 at the beginning required insulin at the dose of 0.8 u/k/d but was not given empirical glibenclamide, as its parents had consanguineous marriage. Her genetic analysis came positive for EIF2AK3 c.287G>A,/c.2511_2514del and was diagnosed as Wolcott-Rallison syndrome.

All the infants tolerated the oral sulphonylurea well, and there were no severe adverse effects. Even in case no 1, in which glibenclamide was not successful, there were no side effects to it.

Discussion

TDM accounts for 50–60% of NDM, and overexpression of genes at chromosome 6q24 is the most common cause of transient neonatal diabetes [6]. They usually present with hyperglycemia within the first few days or weeks of life and mostly without diabetic ketoacidosis (DKA). 6q24 mutation may be associated with umbilical hernias and macroglossia.

This can be due to uniparental disomy or maternal hypomethylation, and insulin is the treatment of choice [7].

Mutations of KCJN11 and ABCC8 are the most common causes of permanent neonatal diabetes and the second most common cause of transient NDM [6, 8]. Both these genes encode for either of the subunits of the ATP-sensitive K channel (KATP) of the pancreatic beta-cells. Activating mutations of these genes result in failure to release insulin despite persisting hyperglycemia resulting in NDM [6].

Sulphonylureas bind to the mutated KATP channels and aid in insulin secretion, thus mitigating the hyperglycemia.

KCNJ11 mutations in PND may be associated with attention deficits, seizures, sleep disruptions and developmental delays, and DEND syndrome. Sulphonylureas have been reported to improve the neurological outcomes in such children when started early in addition to treating hyperglycemia [9].

In a study by Carmody et al [5], they evaluated the data of 118 with genetic defects and 95% of the NDMs were due to ABCC8 or KCJN11. Of these they had empirically started oral glibenclamide in six patients before the genetic results. The genetic results were due to ABCC8, KCJN11, or 6q24. Only in one case the genetic testing failed to identify any cause of NDM, and the trial of sulphonylurea was unsuccessful. All the three cases with 6q24 mutations showed

Table 1 Neonatal diabetes mutations

S.no	Gene	Location	Mutation	Mutation DNA level	Zygosity	Sex	Treatment	Age at presentation	Diagnosis
1	FOXP3	Exon 10	Missense	c.1040G>A	Hemizygous	M	Insulin 1 u/k/d Glibenclamide 0.3 mg/k/d	25 days	IPEX syndrome
2	KCJN11	Exon 1	Missense	c.685G>	Heterozygous	M	Glibenclamide 0.4 mg/k/d	4 months	TND
3	HNF1B	Novel	Missense	p.S19C	Heterozygous	M	Insulin 0.4 u/kg/d	4 months	PNDs
4	No mutation detected	-	-	-	-	M	Insulin 0.4 u/k/d	3 months	TND
5	EIF2AK3	Exon 5,13	Frameshift	c.287G>A,/c.2511_2514del	Heterozygous	F	Insulin 0.8 u/k/d	1.2 months	Wolcott-Rallison syndrome
6	INS	Exon 3	Missense	c.287G>	Heterozygous	F	Insulin 0.7 u/k/d	3 months	PND
7	No mutation detected	-	-	-	-	F	Insulin 0.8 u/k/d glibenclamide 0.4 mg/kg/d	1.2 months	TND
8	No mutation detected	-	-	-	-	M	Insulin 0.5 u/k/d	4 months	TND
9	No mutation detected	-	-	-	-	M	Insulin 0.3 u/k/d	3 months	TND
10	No mutation detected	-	-	-	-	M	Insulin 0.4 u/k/d	3 months	TND
11	KCJN11		p.R201H		Heterozygous	F	Glibenclamide 1 mg/k/d	4 months	PND

remissions on glibenclamide within weeks of initiation; however the duration of start of remission was variable [5].

Glibenclamide as an anti-hyperglycemic drug has been widely used in the adult population, and its side effects have been studied over a long term. The most common gastrointestinal side effects noted in adults were not seen in children; only one study reported a transitory secretory diarrhea on high doses [10].

Another reported side effect is of dental discoloration, is also very uncommon in children, and is treatable with dental cleaning [11].

There have been reports of patients developing hypoglycemia due to glibenclamide in type 2 diabetes in adults, but this has been very rare in children with NDM and the incidence much lesser in comparison to insulin [12].

Insulin that can be given is either as long-acting insulin detemir or glargine (as two daily doses) or short-acting insulin in small doses (may require 3–4 daily doses). Recent studies have shown Continuous subcutaneous insulin infusion (CSII) by insulin pumps offer the advantage of the ability to deliver smaller doses of insulin relative to the multiple daily injections regimen [13].

Glibenclamide is tolerated very well in the NDM age group with very few severe side effects reported [5, 14–15].

Starting the patient on glibenclamide has certain advantages of : ease of administration, cost-effective (in comparison to Insulin), shortens duration of hospital stay, neurodevelopmental benefits in some cases, and a good safety profile [5].

However, there are certain disadvantages to the same, namely titrating the dose to manage hyperglycemia and hypoglycemia in TDM, unknown long-term effects, in ABCC8 and KCJN11 mutations, may need to reintroduce if the first trial fails and may give false sense of security to parents in the absence of genetic results [5].

Attempting empirical treatment in NDM should be done as inpatient management, after having evaluated the child completely for any syndromic features, family history of consanguinity, and known associations with NDM mutations. As revealed in our patient of Wolcott-Rallison who was not given a trial of glibenclamide due to a positive history of consanguinity.

Empirical oral sulphonylurea therapy does not mitigate the importance of gene testing in NDM; it should be used as a modality to shorten inpatient stay duration and probably the dose of insulin, while the genetic reports are awaited.

Genetic testing is imperative for not only diagnosis but also for prognosis and final outcomes on the given treatment (Table 1).

Conclusion

This study reiterates the need to genetically test all cases of NDM, but a trial of sulphonylurea can be attempted in certain cases, but only in supervised tertiary care centers under endocrinologist guidance. There were no adverse events on the children treated empirically, even in the case where glibenclamide was unsuccessful. Larger studies with longer follow-ups are needed to evaluate the entire spectrum of the outcomes.

Acknowledgments The authors would like to thank the Department of Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK.

Author contribution Dr. IPS Kochar substantially contributed to conception and design, interpretation of data, acquired the data, analysis, drafted and revised the work critically. Dr. Smita Ramachandran substantially contributed to conception and design, interpretation of data, acquired the data, analysis, drafted and revised the work critically.

Declarations

Ethics approval The study protocol has been approved by the research institutes committee on human research. The research was conducted ethically in accordance with World Association Declaration Helsinki.

Informed consent Taken

Ethical committee clearance from institutional board
Taken

Conflict of interest The authors declare no competing interests.

References

1. Naylor RN, Greeley SAW, Bell GI, Philipson LH. Genetics and pathophysiology of neonatal diabetes mellitus. J Diabetes Investig. 2011;2:158–69.
2. Letourneau LR, Carmody D, Wroblewski K, Denson AM, Sanyoura M, Naylor RN, et al. Diabetes presentation in infancy: high risk of diabetic ketoacidosis. Diabetes Care. 2017;*40*(10): e147–8. https://doi.org/10.2337/dc17-1145.
3. Aguilar-Bryan L, Bryan J. Neonatal diabetes mellitus. Endocr Rev. 2008;29(3):265–91.
4. Rozance PJ, Hay WW. Neonatal hyperglycemia. Neoreviews 2010;11:e632–9. Available at: http://neoreviews.aappublications.org/lookup/doi/10.1542/neo.11.
5. Carmody D, Bell CD, Hwang JL, Dickens JT, Daniela Sima I, Felipe DL, et al. Sulfonylurea treatment before genetic testing in neonatal diabetes: pros and cons. The Journal of Clinical Endocrinology & Metabolism. 2014;99(12):E2709–14. https://doi.org/10.1210/jc.2014-2494.
6. Flanagan SE, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, et al. Mutations in ATP-sensitive K1 channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. Diabetes. 2007;56:1930–7.

7. Abdollahi A. LOT1 (ZAC1/PLAGL1) and its family members: mechanisms and functions. J Cell Physiol. 2007;210(1):16–25.
8. De Franco E, Flanagan SE, Houghton JA, Lango Allen H, Mackay DJ, Temple IK, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. Lancet. 2015;386:957–63 Available at: http://linkinghub.elsevier.com/ retrieve/pii/S0140673615600988.
9. Mlynarski W, Tarasov AI, Gach A, Girard CA, Pietrzak I, Zubcevic L, et al. Sulfonylurea improves CNS function in a case of intermediate DEND syndrome caused by a mutation in KCNJ11. Nat Clin Pract Neurol. 2007;3:640–5.
10. Codner E, Flanagan S, Ellard S, García H, Hattersley AT. High-dose glibenclamide can replace insulin therapy despite transitory diarrhea in early-onset diabetes caused by a novel R201L Kir6.2 mutation. Diabetes Care. 2005;28:758–9.
11. Kumaraguru J, Flanagan SE, Greeley SA, Nuboer R, Støy J, Philipson LH, et al. Tooth discoloration in patients with neonatal diabetes after transfer onto glibenclamide: a previously unreported side effect. Diabetes Care. 2009;32:1428–30.
12. Codner E, Flanagan SE, Ugarte F, García H, Vidal T, Ellard S, et al. Sulfonylurea treatment in young children with neonatal diabetes: dealing with hyperglycemia, hypoglycemia, and sick days. Diabetes Care. 2007;30:e28–9.
13. Dahl A, Kumar S. Recent advances in neonatal diabetes. Diabetes Metab Syndr Obes. 2020;13:355–64. https://doi.org/10.2147/DMSO.S198932.
14. Ganesh R, Suresh N, Vasanthi T, Ravikumar KG. Neonatal diabetes: a case series. Indian Pediatr. 2017;54:33–6.
15. Kochar IPS, Jindal R. Glibenclamide for neonatal diabetes. Indian pediatric. 2013;50:428–9.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):166–170
https://doi.org/10.1007/s13410-021-00948-2

ORIGINAL ARTICLE

Knowledge, attitude, and practice towards diabetes mellitus among outpatient diabetic elderly persons: a descriptive study

Uchenna Cosmas Ugwu [1] · Osmond Chukwuemeka Ene [1]

Received: 18 November 2020 / Accepted: 25 March 2021 / Published online: 7 April 2021

Abstract

Objective To investigate the knowledge, attitude, and practice (KAP) towards diabetes mellitus (DM) among outpatient diabetic elderly persons attending tertiary healthcare facilities in Nigeria and also to verify the null hypotheses of no significant differences within demographic variables.

Methods The study was based on descriptive research design and was carried out in 36 public tertiary healthcare facilities in Nigeria, from August to December 2019. Using the purposive sampling technique, a total of 360 diabetic elderly persons aged 60 years and above were studied. An adapted KAP questionnaire with 0.86 reliability indexes was used for data collection. Frequency, percentage, and chi square were used for data analysis. All analysis was completed using IBM SPSS version 22.

Results There were 348 (97%) valid copies of the questionnaire used for analysis. The study revealed that the outpatient diabetic elderly persons had high knowledge 303 (87%), positive attitude 265 (76%), and good practice 286 (82%) towards DM. Statistically, there was a significant difference within variables of age (p value=0.001<0.05) and education level (p value=0.018<0.05) while none existed on the variable of gender (p value=0.071>0.05).

Conclusion The participants had high KAP relating to DM. However, the finding of this study is not a complete reflection of the real situation regarding DM among elderly persons in Nigeria. There are overwhelming evidences of high prevalence of DM and thus the need for KAP sustainability and subsequent improvement in the healthcare sector to successfully combat the disease and proffer reliable ways of prevention among the population.

Keywords Diabetes mellitus · Diabetic elderly persons · KAP · Descriptive · Nigeria

Introduction

Globally, diabetes mellitus (DM) is common in the elderly population [1]. It is a contemporary public health problem that is particularly significant among persons aged 65 years and above [2]. DM is reported to have triggered high mortality and morbidity, decline in functional abilities, and high risks of medical and healthcare costs as well as institutionalization [3]. Previous researchers reported that while half of the diabetic elderly population is unaware that they have DM, approximately 20 per cent of them develop the disease by the age of 75 years [4]. In developed regions such as the USA, approximately 30 per cent of persons aged 65 years and above are diabetic [5]. The situation is more worrisome in Nigeria where a systematic review and meta-analysis study reported 5.77 per cent (95% CI 4.3-7.1) as the overall pooled prevalence of DM among adults [6]. This depicts an increase in the prevalence of DM in Nigeria and thus a national issue. Surprisingly, about one-third of elderly persons with diabetes are undiagnosed and thus positioning the diseases as a global growing burden to the public health sector [7]. Further studies indicate that older adults with DM are more vulnerable to other multiple chronic and acute microvascular and cardiovascular complications associated with the disease [3].

Adequate knowledge, positive attitude, and desirable practice towards DM are crucial in order to facilitate early diagnosis, treatment, and management as well as reducing the burden of the disease [2, 3]. It would also be important in delaying the microvascular and cardiovascular complications by achieving optimal glycemic control (i.e., desirable glycated hemoglobin (HbA1c) level, usually below 6.5% by patients with DM) [8].

✉ Uchenna Cosmas Ugwu
uchennacos.ugwu@unn.edu.ng

[1] Department of Human Kinetics and Health Education, Faculty of Education, University of Nigeria, Nsukka, Enugu State, Nigeria

Further researches indicate that persons with sound KAP towards diabetes are mostly diligent with self-care and better control of the disease [9]. Glycemic control can be attained through the adoption of healthy measures including exercise, diet, control, and medical check-ups. From the literature reviewed, there are still limited researches on KAP towards DM among the elderly population in Nigeria. Therefore, the objective of this research was to investigate the KAP towards DM among outpatient diabetic elderly persons attending tertiary healthcare facilities in Nigeria and also to verify the null hypotheses of no significant differences within demographic variables.

Materials and methods

The ethical approval for the study was granted by the Ethical Review Committee of the Department of Human Kinetics and Health Education, Faculty of Education, University of Nigeria, Nsukka. This was done in accordance with the stipulated principles of the American Psychological Association [10] on the ethical standard for conducting health-related researches involving human persons and also with the consideration of ethical regulations and principles of the World Medical Association's Declaration of Helsinki [11]. Written informed consent was received from all the potential respondents before the actual data collection process. No monitory commitment was made to the respondents for participating in the study, and thus, the respondent's personal influence was eliminated.

This study was based on descriptive research design and was carried out in public tertiary healthcare facilities in Nigeria, from August to December 2019. A sample size of 360 outpatient diabetic elderly persons was selected from the 36 public tertiary healthcare facilities. A purposive selection of 10 participants from each of the sampled healthcare facilities was adopted. The choice of the purposive sampling procedure was to eliminate sampling bias and also to ensure that only the outpatient diabetic elderly persons were studied. The eligibility criteria included (a) must be confirmed outpatient diabetic person attending tertiary healthcare facility, (b) must be 60 years and above, and (c) must complete the informed consent form. All the eligible participants received a copy of the KAP questionnaire and responded as deemed appropriate.

The instrument used for data collection was the questionnaire "Knowledge, Attitude, and Practice towards Diabetes Questionnaire (KAPDQ)" with internal consistency of 0.86 indexes. This reliability index was very appropriate for use in this study based on the recommendation of previous researchers who asserted that the research tool must score 0.70 and above before it is adjudged reliable for use in a study [12]. The KAPDQ was adapted from the (a) Knowledge, Attitude, and Practice of Diabetes Mellitus Questionnaire developed for nondiabetic and diabetic persons in Bangladesh [13], (b) Knowledge and Awareness of Diabetes Questionnaire developed for the Chennai Urban Rural Epidemiology Study [14], (c) AusDiab Health Knowledge, Attitudes, and Practices Questionnaire 99/00 [15], and (d) KAP construction guides [16]. With the approval of the Chief Medical Director of the sampled healthcare facilities, the instrument was successfully administered to the study participants during their routine visit to the hospital arena. The KAPDQ was made up of four major parts: A to D. These parts gathered data on the respondents' profile; measured knowledge relating to diagnosis, risk factors, prevention, and complications of DM; assessed the participants' attitude relating to adherence to treatment of DM; and finally measured the participants' practice on preventive strategies, self-care, dietary modifications, and monitoring of blood sugar. The response options of "yes" and "no" indicating correct or incorrect for knowledge, attitude, and practice relating to DM were adopted. For each correct response, one point was ascribed to it and the entire scores were properly calculated. Hence, 50 per cent was set as benchmark for decision based on the recommendation of the World Health Organization regarding World Health statistics scores [17]. Therefore, any score that is above 50 per cent was considered high KAP while scores below 50 per cent implied low KAP relating to DM, respectively.

The data analysis was completed using IBM SPSS version 22 [18]. The profile of the study participants was presented using descriptive statistics. Frequency, percentages, and chi square were used to analyze the data generated on KAP relating to DM. The results of this research were deemed statistically significant at $p \leq 0.05$.

Results

There were 348 (97%) valid copies of the questionnaire used for data analysis. Out of 360 copies that were administered, only 12 copies (3%) were discarded (incomplete responses = 5 copies and not returned =7 copies). The return rate of the questionnaire was very high and quite encouraging. A greater percentage of the participants 185 (53%) were 70 years and above. Only few of them 163 (47%) were between 60 and 69 years. The total number of male participants was higher than the number of females. Thus, about 197 (57%) were males while only 151 (43%) were females. Of them, 216 (62%) had a low education level (LEL) while 132 (38%) had a high education level. By implication, majority of the elderly participants with DM had low education (see Table 1 for details).

The result revealed that the majority of outpatient diabetic elderly persons had high knowledge 303 (87%), positive attitude 265 (76%) and good practice 286 (82%) towards DM. Also, only 45 (13%), 83 (24%), and 62 (18) reported low

Table 1 Profile of the study participants (n=348)

Variables	Parameter	Frequency	Percentage
Age	70 years and above	185	53
	60–69 years	163	47
Gender	Male	197	57
	Female	151	43
Education level	Low education level (PSLC & WASSC)	216	62
	High education level (university degree)	132	38

Keys: *n* sample size; *PSLC* Primary School Leaving Certificate; *WASSC* West African Senior School Certificate

knowledge, negative attitude, and bad practice towards DM, respectively (see Table 2 for details).

The statistically significant differences within demographic variables on KAP relating to DM are presented in Table 3. The odds ratios (OR=1.948≥1) and (OR=1.191≥1) of the demographic variables of gender and education level indicated a positive or strong correlation between KAP towards DM, while age showed a negative or weak correlation (OR=0.752≤1). It was also revealed that the male participants 197 (57%) and those who possessed a low education level 216 (62%) had more KAP towards DM than the females 151 (47%) and those who possessed a high education level 132 (38%). On the contrary, Table 3 further shows that the participants aged 60-69 years 163 (47%) had more KAP than those aged 70 years and above 185 (53%). Statistically, there was a significant difference within variables of age (p value=0.001<0.05) and education level (p value=0.018<0.05) while none existed on the variable of gender (p value=0.071>0.05) (see Table 3 for details).

Discussion

To the best of the authors' knowledge, this is the first descriptive research of this kind to investigate the KAP towards DM among outpatient diabetic elderly persons attending tertiary healthcare facilities in Nigeria. The study revealed that the participants had high knowledge 303 (87%), positive attitude 265 (76%), and good practice 286 (82%) towards DM. These findings were surprising as they do not reflect or translate the real DM situation in Nigeria. Ideally, high knowledge, positive attitude, and good practice towards DM should result in a very low per cent incidence rate of DM particularly among elderly persons. Although this research did not verify the indicators relating to high scores on KAP towards DM by the participants, the high scores could be linked to technological advancement and increased access to the global social and media networks. This finding is in consistence with other results which reported that KAP scores of people living with DM were higher when compared to those who do not [19, 20]. The surprising result may also be attributed to personal life experiences and other coindicators such as lifestyle and exposure which might have played significant roles in personal development and understanding of some chronic age-related diseases such as DM by the participants.

Also, the participants' positive attitude towards the disease could be linked to their desire to manage the condition, live healthily, and cope with the disease. As regards to good practice, the participants might have understood that negative practice and negligence to diets, medical care, and professional attention could result in more complications, deteriorations, and poor quality of health. Although our research was restricted to KAP towards DM, further studies are needed to explore the contributing factors to high knowledge, positive attitude, and good practices among the sufferers of DM as reported in this study.

In contrast, related studies had reported discouraging outcomes on KAP relating to DM among the diverse populations in very low- and middle-income countries of the world [21, 22]. Also, in similar settings to Nigeria, abundant studies reported poor knowledge, negative attitude, and bad practice towards DM [14, 22–24]. The above reports confirmed the assertions that DM is a global epidemic that presents a high percentage of world disability, mortality, and huge medical

Table 2 Showing the status of KAP towards DM by the study participants (n = 348)

Parameter	Correct *f* (%)	Remark	Incorrect *f* (%)	Remark
Knowledge of DM	303 (87%)	High knowledge	45 (13%)	Low knowledge
Attitude towards DM	265 (76%)	Positive attitude	83 (24%)	Negative attitude
Practice relating to DM	286 (82%)	Good practice	62 (18)	Bad practice

Keys: *f* frequency; % percentage; *n* sample size; *()* bracket sign

Table 3 Showing statistically significant differences within demographic variables on KAP towards DM (n = 348)

Variables	Parameter	n (%)	Correct f (%)	Incorrect f (%)	Odds	Odds ratio	p value	Remark
Age	70 years and above	185 (53%)	171 (92%)	14 (8%)	9.19	0.752	0.001	*
	60–69 years	163 (47%)	147 (90%)	16 (10%)	12.21			
Gender	Male	197 (57%)	178 (90%)	19 (10%)	9.37	1.948	0.071	**
	Female	151 (43%)	125 (83%)	26 (17%)	4.81			
Edu. level	LEL (PSLC, WASSC)	216 (62%)	195 (90%)	21 (10%)	9.29	1.191	0.018	*
	HEL (university degree)	132 (38%)	117 (89%)	15 (11%)	7.80			

Keys: *OR* odds ratio; *Edu.* education; *LEL* low education level; *HEL* high education level; *n* sample size; *PSLC* Primary School Leaving Certificate; *%* per cent; *WASSC* West African Senior School Certificate. * Significant difference; ** no significant difference. *f* frequency

expenses [25]. This suggests that possessing adequate KAP relating to DM and its complications should be encouraged. The outcome of this study, no doubt, would serve as a positive step in enhancing early diagnosis, treatment, management, and desired care by individuals as well as making them take absolute charge of their health [26]. Also, it would serve as a veritable tool for creating awareness campaigns and education of the populace on the nature and etiology of DM as well as to promote effective DM preventive and management measures. This is the existing research gap this study filled.

Our study revealed that the majority of the participants had low education levels (primary and secondary education), aged 70 years and above, and were males. Also, gender and education level were found to be positively correlated with KAP towards DM, while none existed with age. Furthermore, the male participants and those with low education levels had more KAP towards DM than the females and those with high education. The study equally reported that the participants aged 60-69 years had more KAP than those aged 70 years and above. These findings present obvious implications. Firstly, DM can be considered one of the age-related diseases since the majority of the sufferers are in the age of 70 years and above. Secondly, it could be affirmed that people who have high education are more vulnerable to DM than their counterparts with low education. Thirdly, the female individuals are more likely to suffer DM more than the male folks who had more KAP regarding DM. These implications are translatable. Thus, elderly individuals with more KAP towards DM would be better informed on early diagnosis, management, and treatment options regarding DM. Although the present study was delimited to establishing the demographic (age, gender, and education) discrepancies on the study phenomenon, there are clear indications that sociodemographics remarkably influenced the occurrences of DM in the population. These findings align with the results of previous researchers who found sociodemographic variables as important determinants in their respective studies [22, 23]. Statistically, there were significant differences within variables of age and education level while none was reported on gender. This is in accordance with other findings that reported statistically significant differences within demographic variables [23, 24].

The current research records some notable limitations. In this study, only the outpatient diabetic elderly persons were purposively sampled and studied. Hence, the findings of the study might not be a true representation of KAP relating to DM among all the diabetic elderly persons since the sampling bias was not comprehensively controlled. Further studies of this kind are recommended to explore the KAP towards DM in both primary and secondary healthcare facilities in Nigeria. Also, future research is needed to explore the major source of information regarding high KAP relating to DM. This study primarily relied on a questionnaire tool for data collection. There is a need to employ other data collection tools such as interview schedule and focus group discussion. These measures would allow the participants to express their views and experiences regarding DM.

Conclusion

The current research presented the KAP towards DM among outpatient diabetic elderly persons attending public tertiary healthcare facilities in Nigeria. The participants had high KAP relating to DM. However, the finding of this study is not a complete reflection of the real situation regarding DM among elderly persons in Nigeria. There are overwhelming evidences of a high prevalence of DM and thus the need for KAP sustainability and subsequent improvement in the healthcare sector to successfully combat the disease and proffer reliable ways of prevention among the populations.

Code availability Not applicable

Author contribution UCU conceived the work and reviewed relevant literature. All the authors acquired, analyzed, and interpreted the data. OCE drafted the work and revised it critically for important intellectual content. All authors approved the version to be published and equally agreed to be accountable for all parts of the work.

Data availability All relevant data and materials are available with the corresponding author and will be provided on request.

Declarations

Ethics approval Approval was obtained from the ethics committee of the University of Nigeria Nsukka. 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 The participants have consented to the submission of the original article to the journal.

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

1. Meneilly GS, Tessier D. Diabetes in elderly adults. J Gerontol Med Sci. 2001;56:M5–M13.
2. Hammami S, Mehri S, Hajem S, Koubaa N, Souid H, Hammami M. Prevalence of diabetes mellitus among non-institutionalized elderly in Monastir City. BMC Endocr Disord. 2012;12:15.
3. Brown AF, Mangione CM, Saliba D, Sarkisian CA, California Healthcare Foundation/American Geriatrics Society Panel on Improving Care for Elders with Diabetes. California Healthcare Foundation/ American Geriatrics Society Panel on Improving Care for Elders with Diabetes. Guidelines for improving the care of the older persons with diabetes mellitus. J Am Geriatr Soc. 2003;51:S265–80.
4. Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. Diabetes Care. 1998;21:518–24.
5. Center for Disease Control and Prevention. National Diabetes Fact Sheet: general information and national estimates on diabetes in the United States. Atlanta, Georgia, U.S. Department of Health and Human Services, Center for Disease Control and Prevention; 2011.
6. Uloko AE, Musa BM, Ramalan MA, Gezawa ID, Puepet FH, Uloko AT, et al. Prevalence and risk factors for diabetes mellitus in Nigeria: a systematic review and meta-analysis. Diabetes Ther. 2018;9(3):1307–16.
7. Kalyani KR, Golden SH, Cefalu W. Diabetes and ageing: unique considerations and goals of care. Diabetes Care. 2017;40:440–3.
8. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of diabetes. Diabetes Care. 2009;32(1):193–203.
9. Powers MA, Bardsley J, Cypress M, Duker P, Funnell MM, Hess Fischl A, et al. Diabetes self-management education and support in type 2 diabetes: a joint position statement of the American Diabetes Association, the American Association of Diabetes Educators, and the academy of nutrition and dietetics. J Acad Nutr Diet. 2015;115(8):1323–34.
10. American Psychological Association. Ethical principles of psychologists and code of conduct. Washington, DC; 2017.
11. World Medical Association. Declaration of Helsinki ethical principles for medical research involving human subjects. JAMA. 2013;310:2191–4.
12. Tavakol M, Dennick R. Making sence of Cronbach's alpha. Int J Med Educ. 2011;2:53–5.
13. Fatema K, Hossain S, Ali L, Natasha K, et al. Knowledge attitude and practice regarding diabetes mellitus among nondiabetic and diabetic study participants in Bangladesh. BMC Public Health. 2017;17:364.
14. Mohan D, Raj D, Shanthirani CS, Datta M, Unwin NC, Kapur A, et al. Awareness and knowledge of diabetes in Chennai–the Chennai urban rural epidemiology study [CURES-9]. J Assoc Physicians India. 2005;53:283–7.
15. National Health Medical Research Council. AusDiab 2005: the Australian Diabetes, Obesity and Lifestyle Study: tracking the accelerating epidemic: its causes and outcomes. Melbourne: International Diabetes Institute; 2006.
16. World Health Organization. Advocacy, communication and social mobilization for TB control: a guide to developing knowledge, attitude and practice surveys. Geneva: WHO; 2008.
17. World Health Organization. WHO library cataloguing-in-publication data. Switzerland: World Health Statistics; 2012.
18. IBM. Corp. IBM SPSS Statistics for Windows, version 22. Armonk: IBM Corp; 2013.
19. Gul N. Knowledge, attitudes and practices of type 2 diabetic patients. J Ayub Med Coll Abbottabad. 2010;22(3):128–31.
20. Raj CP, Angadi M. Hospital-based KAP study on diabetes in Bijapur, Karnataka. Indian J Med Spec. 2011;1(2):80–3.
21. Al-Maskari F, El-Sadig M, Al-Kaabi JM, et al. Knowledge, attitude and practices of diabetic patients in the United Arab Emirates. PLoS One. 2013;8(1):e52857.
22. Islam FMA, Chakrabarti R, Dirani M, Islam MT, Ormsby G, Wahab M, et al. Knowledge, attitudes and practice of diabetes in rural Bangladesh: the Bangladesh population based diabetes and eye study (BPDES). PLoS One. 2014;9(10):e110368.
23. Saleh F, Mumu SJ, Ara F, Begum HA, Ali L. Knowledge and self-care practices regarding diabetes among newly diagnosed type 2 diabetics in Bangladesh: a cross-sectional study. BMC Public Health. 2012;12:1112.
24. Deepa M, Bhansali A, Anjana RM, Pradeepa R, Joshi SR, Joshi PP, et al. Knowledge and awareness of diabetes in urban and rural India: the Indian Council of Medical Research India diabetes study (phase I): Indian Council of Medical Research India diabetes 4. Indian J Endocrinol Metab. 2014;18(3):379–85.
25. 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.
26. Shrivastava SR, Shrivastava PS, Ramasamy J. Role of self-care in management of diabetes mellitus. J Diabetes Metab Disord. 2013;12:14.

International Journal of Diabetes in Developing Countries (January–March) 42(1):171–172
https://doi.org/10.1007/s13410-021-00991-z

LETTER TO THE EDITOR

Triple challenge of diabetes, COVID-19, and mucormycosis

Ashu Rastogi[1] · **Anshu Khamesra**[2]

Received: 24 May 2021 / Accepted: 28 July 2021 / Published online: 13 August 2021

Sir,

India is witnessing a steep surge of COVID-19 along with increasing mortality from SARS-CoV-2 infection in the second wave propounded by an unprecedented outbreak of mucormycosis infections. Although mucormycosis is a rare disease, the prevalence of mucormycosis in India is about 80 times that of developed countries [1]. A systematic review identified 101 reported cases of mucormycosis in COVID-19 worldwide and majority (83%) from India [2]. This is likely the tip of the iceberg as no national surveillance exists for mucormycosis. Countrywide data suggest close to 8880 cases of mucormycosis during the COVID-19 pandemic in India as of 22 May 2021 [3]. This has forced the Indian government to declare mucormycosis a notifiable disease under the Epidemic Diseases Act, 1897, on May 20, 2021.

Has fungal infections come as a surprise in the COVID-19 pandemic? Looking back, the incidence of fungal infection during the SARS-CoV1 infection in 2003 was 14.8–27%, contributing to maximum mortality (25–73.7% of all deaths) [4]. A high probability of increased incidence of fungal infections in COVID-19 in affected or recovered patients is unlikely to be unprecedented considering biological similarities between SARS-CoV-1 and -2, both requiring prolonged hospitalization. Moreover, prolonged supraphysiological doses of glucocorticoids (GCs) predispose patients with compromised immunity (secondary to diabetes) for opportunistic fungal infections including mucormycosis. High-dose steroids are used for severe or critically ill COVID-19 patients that reduces mortality [5]. GCs are continued beyond the second week in patients requiring prolonged ICU stay increasing susceptibility for mucormycosis.

Why is that India is ravaged with mucormycosis in the COVID-19 pandemic, while the rest of the world has only isolated reports of mucormycosis? Is it secondary to swelling number of COVID-19 patients in the second wave in India with the potentially more virulent strain B1.617.2, when the rest of the world is observing a downtrend? Or is it due to healthcare delivery issues including hasty makeshift COVID care facilities with possible unsterilised hospital linen, non-sterile medical devices including humidifiers for inhaled oxygen, usage of industrial oxygen due to shortage of medical oxygen, tropical climate, and agrarian population? Uncontrolled hyperglycemia (mean HbA1c 8.48%) [6] and increasing number of COVID-19 patients on supraphysiological and prolonged course of glucocorticoids (resulting in worsening glycemic control) also predispose to mucormycosis.

An early recognition of symptoms, equitable distribution of healthcare resources including diagnostic facilities (radiological and histopathology), adequate supplies of antifungal drugs, availability of operative facilities, and surgical expertise at peripheral centers are some measures that may ward off this triple challenge.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. J Fungi. 2019;5:26.
2. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. Diab Metabol Syn Clin Res Rev. 2021. https://doi.org/10.1016/j.dsx.2021.05.019. Accessed on 22 May 2021.
3. https://www.deccanherald.com/national/covid-19-crisis-which-states-in-india-have-reported-the-highest-number-of-mucormycosis-cases-988666.html. Accessed on 23 May 2021

✉ Ashu Rastogi
ashuendo@gmail.com

[1] Department of Endocrinology, Post Graduate Institute of Medical Education and Research, 16, Nehru Extension Block, Sector 12, Chandigarh 160012, India

[2] Department of Medicine, Santokh Hospital, Chandigarh 160012, India

4. Yin CH, Wang C, Tang Z, Zhang SW, Wang BS. Clinical analysis of 146 patients with critical severe acute respiratory syndrome in Beijing areas. Clin J Emerg Med. 2004;1:12–4.
5. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in hospitalized patients with COVID-19. N Engl J Med. 2021;384(8):693–704. https://doi.org/10.1056/NEJMoa2021436.
6. https://www.biospectrumindia.com/news/79/17555/average-hba1c-level-registers-marginal-improvement-in-india.html. Accessed on 22 May 2021.

International Journal of Diabetes in Developing Countries (January–March 2022) 42(1):173
https://doi.org/10.1007/s13410-021-00982-0

CORRECTION

Correction to: Barriers to postpartum follow-up of mothers with gestational diabetes mellitus and its implications: a mixed method study

Ninu P. Mathew[1] · Arathi P. Rao[1] · Prakash Narayanan[1]

Published online: 22 July 2021

Correction to: International Journal of Diabetes in Developing Countries (2021) 41:127–135 https://doi.org/10.1007/s13410-020-00853-0

The corresponding Author of this paper is Arathi P. Rao.

The original article can be found online at https://doi.org/10.1007/s13410-020-00853-0.

✉ Arathi P. Rao
arathimph@gmail.com

[1] Prasanna School of Public Health, Manipal Academy of Higher Education, Manipal, Udupi, Karnataka 576104, India

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 2021-2022

Patrons of RSSDI

Dr. H.B. Chandalia, Mumbai
Dr. C. Munichhoodappa, Bengaluru
Dr. Ashok K. Das, Puducherry
Dr. Binode K. Sahay, Hyderabad
Dr. V. Seshiah, Chennai
Dr. P.V Rao, Hyderabad
Dr. Jitendra Singh, New Delhi
Dr. V Mohan, Chennai
Dr. Vinod Kumar, New Delhi

President

Dr. Ch.Vasanth Kumar, Hyderabad

President Elect

Dr. Brij Makkar, New Delhi

Immediate Past President

Dr. Banshi Saboo, Ahmedabad

Secretary

Dr. Sanjay Agarwal, Pune

Vice-President

Dr. Anuj Maheshwari, Lucknow

Vice-President

Dr. Vijay Viswanathan, Chennai

Joint Secretary

Dr. Sujoy Ghosh, Kolkata

Treasurer

Dr. Sunil Gupta, Nagpur

Executive Committee

Dr. C R Anand Moses, Chennai
Dr. Sudhir Bhandari, Jaipur
Dr. J K Sharma, New Delhi
Dr. Bikash Bhattacharjee, Guwahati
Dr. Pratap Jethwani, Rajkot
Dr. L. Sreenivasa Murthy, Bengaluru
Dr. Sanjay Reddy, Bengaluru
Dr. Shalini Jaggi, New Delhi

Co-opted

Dr. Vijay Panikar, Mumbai
Dr. Rakesh Sahay, Hyderabad
Dr. Amit Gupta, Noida

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?
Send in your Research proposals by email to the RSSDI Secy/ Chairman research committee by email/ apply directly on web site.

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 50% 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 should be addressed to:
1. The Secretary , RSSDI
2. Soft copy of the research proposal should be sent to Secretary, RSSDI

When to apply
Proposals will be accepted Twice a year. Once between 1st Jan - 31st April & then July 1st to 30th Nov.

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 30th June & then 31st December of each year.

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 Brahmacahri 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

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.

1. RSSDI Research Retreat 2022
28th & 29th May 2022 in Pune

2. 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 - 30th June 2022
Screening Interview - 7th July 2022
Declaration of Exam Result - 10th July 2022
Last date of payment of course fee - 15th July 2022
Commencement of course - 16th July 2022
Prospectus release date 15th May 2022

Acknowledgement

We are grateful to the following reviewers for reviewing articles for International Journal in Diabetes in Developing Countries for the year 2021.

A AR
A BH
Abbasi E
Abdelnasser Z
Abulmeaty M
Acharya L
Adesiyun, MBBS, FMCPaed, Fespe O
Adisa R
Agarwal K
Agarwal N
Agrawal S
Agrawal A
Ahmadi - Motamayel F
Ahmed M
Ahmed Mphil D
Ajeet J
Ajose AO
Aksoy D
Aktas G
Ali A
Ali S
Ali Khan I
Ali, PhD S
AL-Khateeb A
Al-Lami F
Al-Sayyed PhD, H
Alvani SR
Arora R
Arora V
Arpaci D
Arslan BÇ
Arumalla V
Arun P
Asar G
Asirvatham AJ
Aslam M
Asmah PhD H
Ayar Y
Aydın, PhD A
Ayyagari U
Ayyagari M
Azimi H
Babalola, MD O
Baid L
Bajaj S
Balasubramanian, PhD R
Balbaa M
Balogun, MBBS W
Bansal MD R
Baquer NZ
Barakat B
Barman H
Barman M
Baruah MP
Barvadheesh C
Batra L
Behera, MD KK
Bekele BB
Bhadada SK
Bhale D
Bhat S
Bhati P
Bhattacharya S
Bhattacharya C
Bhattacharya G
Bhatti A
Bhavani N
Bhavatharani N.B
Bitencourt P
Bolboaca S
Boriboonhirunsarn D
Borr SS
Brasil F
Chaudhuri A
Chawla M
Chawla R
Chin YH
Chowdhury S
Chutia H
D.S P
Dabadghao P
Dabas, Pediatrician A
Dabla, Biochemistry PK
Dariya S
Das S
Dayal D
Devaraj NK
Dolatkhah N
Dorairaj P
Dutta D
Ezenwaka C
Farishta F
Fawwad A
G Seshadri, MD K
Ganesan M
Gangopadhyay K
Garg S
Gasbjerg LS
George L
Ghosal, MD S
Ghosh A
Ghosh U
Girei, MD B
Godi S
Goel R
Golboni F
Gopal J
Gossain V
Goswami S
Goyal G
Goyal K
Goyal, DM, AIIMS A
Gupta A
Gupta D
Gupta S
Gupta V
H S A
Haleem A
Hasanat MA
Hussain T
Iman Leong bin Abdullah, MBBS, Psych MF
Jaggi S
Jain N
Jaleel A

Jayalakshmi M
Jethwani P
Jha S
Joshi S
Juttada U
K Darwish R
Kale M
Kansra P
Kapoor N
Kar S
Karmakar S
Karoli R
Kesavadev J
Khaled MB
Khan AM
Kini S
Kochar IS
Kotwal N
Kulshreshtha B
Kumar A
Kumar D
Kumpatla S
Kunikullaya KU.
L S
Lahiry S
Lal A
Lee W
Liu L
Maharaj S
Maheshwari S
Maheshwari, MD, FICP,
FIACM, FACP, FACE A
Mahilmaran A
Maisnam I
Majaliwa E
Malek R
Malhotra S
Malik Ra.
Manickam N
Manivel, MD Physiology R
Mannan M.D. R
Mannari J
Maskey R
Mathur N
Mathur Sk
Mathur S
Menon P S N
Mert M
Meseri R
Minakshisundaram S
Mishra B
Mittal V
Mogarekar M
Mohan V
Mongkolsomlit S
Morales-Bozo I
Mukherjee S
Mukherjee, MD D
Nachimuthu S
Naik D
Nair M
Nambiar V
Nayak M
Ni, clinical pharmacist J
Nicoletto B
Oberoi S
Obirikorang C
P R AVK
Pal A
Pandey, genomics A
Pandya H
Panicker S
Pasupulati AK
Patil A
Patni B
Patwardhan, BAMS, MD
Ayurved K
Paul B
Paul S
Paul T
Phatale H
Prabhu S
Prabhu, M
R C
Rai M
Raizada A
Raizada N
Rajsekar S
Ram U
Ramesh J
Ramesh N
Rankawat G
Rao C
Rao R
Rao, PhD PV
Rastogi A
Rathod MD N
RJ S
Rotte A
Roy K
Roy B
Saboo B
Sachan A
Sadashiv, biochemistry S
Sahin M
Sahoo J
Saito Y
Salem M
Saluja M
Samaria A
Saraf A
Saran S
Sarangi R
Selvaraj B
Senthil G
Seshiah V.
Seth S
Shah R
Shah S
Shah V
Shahid SM
Sharma A
Sharma BB
Sharma R
Sharma B
Sharma MD R
Sharma, MD, DM D
Shenoy MD, M
Shrestha D
Siddiqi, PhD HS
Silver B
Singh A
Singh N.K.
Singh R
Singh S.K.
Singh S
Singhal P
Singla M
Sinha B
Sinha S
Sinha A
Sinishaw M
Sonagra A
Srivastava S

Subramanian K
Sudhakar G.
Sudhir P
Sun Q
Surendran S
Swem F
Talla V
Tanabe K
Tarantino G
Thakur, endocrinology PS
Thoomati, M.S S
Thunga, MPharm, PhD G
Tobita H
Upadhyay D
V R
Valliyot B
Venkatesan R
Verma K
Vikram N
Virmani A
Visaria A
Viswanathan V
VP S
Waisundara V
Waly PhD, M
Wang Y
Weinem M
Yadav S
Yang S
Yoshioka K
Yuen K-H
Yurtcu, PhD E
Zeng B

Registered with Registrar of News Papers, India vide no. RNI-43019/85

International Journal of Diabetes in Developing Countries

Volume 42 | Issue 1 | January–March 2022

Springer