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EDITORIAL

Vitamin B_{12} and diabetes risk—myth or reality

S. V. Madhu[1]

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The role of nutritional factors in contributing to diabetes risk has been the subject of research for several years. However, the focus on micronutrients particularly vitamins has been a more recent phenomenon. While most of the research has focused on vitamin D, the interest in vitamin B_{12} as a modulator of diabetes risk has been growing and the link reportedly getting stronger.

The question of whether vitamin B_{12} deficiency is associated with risk of future diabetes is an important question to ask in our country as a large section of our population is pure vegetarian and is thus prone to deficiency of this vitamin. In fact, vitamin B_{12} deficiency is widely prevalent in our country affecting 47% of our population [1]. A recent study from South India in urban individuals reported that the prevalence of absolute and borderline vitamin B_{12} deficiency was over 50% [2]. Vitamin B_{12} levels decreased as the level of glucose intolerance increased and the proportion of those with vitamin deficiency increased with increasing severity of glucose intolerance, more so among males and vegetarians [2].

The association of vitamin B_{12} deficiency with hyperhomocysteinemia [3] and other metabolic abnormalities such as insulin resistance and dyslipidemia [4] has been known for a while. Also, hyperhomocysteinemia has been well recognized as a cardiovascular (CV) risk factor [4]. A recent study from North India in rural subjects [5] found positive correlation of vitamin B_{12} levels and low HDL levels although the possible mediation by homocysteine could not be confirmed. Also, no significant relationship between vitamin B_{12} levels and any of the other lipid parameters and between hyperhomocysteinemia and any of the obesity or lipid parameters was found. Whether vitamin B_{12} could also mediate diabetes risk is not known and is currently being investigated.

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Evidence for a possible role of vitamin B_{12} deficiency in enhancing diabetes risk comes mainly from studies on pregnant women and their offspring and from studies using nutrigenetic approaches.

Studies from western India [6] suggest that low vitamin B_{12} levels in pregnancy are associated with obesity. These have focused our attention on the potential role of maternal undernutrition on maternal and fetal obesity and insulin resistance. The effects of low vitamin B_{12} levels on obesity and insulin resistance in pregnant women have been confirmed in a study from the UK [7]. Another study from the UK [8] has also shown that maternal vitamin B_{12} levels are associated with maternal obesity and risk of gestational diabetes mellitus (GDM). A more recent systematic review in 2019 [3] concluded that vitamin B_{12} deficiency could be associated with increased risk of GDM. The study included over 1810 pregnant women without GDM and 309 with GDM and showed that GDM patients had lower vitamin B_{12} levels than non-GDM subjects and that vitamin B_{12} level predicted GDM (OR 1.81). The results of these studies reinforce the hypothesis that there is a strong association between vitamin B_{12} and GDM risk. However, more studies would be required to clearly establish any causative link between the two.

Emerging evidence also indicates that vitamin B_{12} deficiency in the mother has a profound influence on fetal growth and development, and this fetal programming can result in increased obesity, adiposity, and insulin resistance later in life [6]. Studies in the Pune cohort suggest that maternal undernutrition is closely linked to fetal obesity and insulin resistance. These studies also describe the thin-fat Indian babies where the babies were thin but with higher levels of adiposity compared with babies of European origin. Over two-thirds of the mothers of these babies displayed vitamin B_{12} deficiency. Low B_{12} levels were also associated with a greater likelihood of adiposity and insulin resistance during childhood [9].Vitamin B_{12} is known to critically influence several cellular pathways particularly nucleic acid synthesis and gene methylation that is involved in the expression of genes. Some of these

pathways may be the basis of fetal metabolic programming that predisposes to insulin resistance in the offspring [6].

A study from South India examined the association of 2 FTO gene variants with greater predisposition to obesity in the CURES cohort and found that these were not only associated with a higher risk of obesity but also with a lower vitamin B_{12} concentration [10]. This interesting observation if replicated in larger studies will provide evidence for the hypothesis that vitamin B_{12} influences obesity and NCD risk by its effects on obesity-related genes. GWAS in Indians has shown that plasma B_{12} concentrations are regulated by newly identified population-specific variants in FUT6 gene [11]. A very recent study confirmed that vitamin B_{12} supplementation regulates several diabetes-associated genes such as TCF7L2, FTO, and CREBP/CBP through methylation of MiR21 and could thus epigenetically influence the risk of obesity, insulin resistance, and type 2 diabetes mellitus [12].

The relationship of vitamin B_{12} and metabolic traits was explored in healthy non-pregnant women in Indonesia by adopting a nutrigenetic approach [13]. Genetic risk scores (GRS) were derived using nine vitamin B_{12}–associated single nucleotide polymorphisms (SNPs) (B_{12}-GRS) and nine metabolic SNPs (metabolic-GRS). The B_{12}-GRS and metabolic-GRS had no effect on vitamin B_{12} levels and metabolic traits. However, the study did show a significant effect on HbA1C levels.

The study by Surendran et al. [14] published in the current issue is a similar nutrigenetic study in South Asians conducted in Sri Lanka. The authors constructed a vitamin B_{12}–related genetic risk score (B_{12}-GRS) based on 10 vitamin B_{12} SNPs and a metabolic trait–related genetic risk score (metabolic-GRS) based on 10 metabolic trait–related SNPs and examined the relationship of these risk scores to vitamin B_{12} levels and different metabolic traits in 109 Sinhalese adults. While there was a significant association of B_{12}-GRS to plasma vitamin B_{12} levels, the authors could not demonstrate any significant relationship between genetically mediated vitamin B_{12} levels and any of the metabolic traits. However, they found a significant interaction of B_{12}-GRS and dietary protein intake on waist circumference suggesting that low genetically mediated vitamin B_{12} levels could be associated with central obesity in the presence of lower dietary protein intakes. While these findings are interesting and open up novel possibilities of gene-nutrient interactions, they need to be replicated in larger studies which are appropriately powered before firm conclusions on such interactions can be drawn. Despite its limitations, this study highlights the need for focused research in the important area of gene-dietary nutrient interactions to understand the complexities of diet-related diabetes risk.

Whether low vitamin B_{12} levels could be causally related to enhanced cardiometabolic risk was evaluated by a unique Mendelian randomization (MR) approach [15]. SNPs identified by earlier GWASs to be robustly associated with vitamin B_{12} levels were analyzed by MR for any significant cardiometabolic risk. A causal relation of vitamin B_{12} was suggested with fasting glucose and beta cell function (HOMA beta) but not with any of the other cardiometabolic factors such as obesity, waist to hip ratio, body fat, HOMA-IR, HbA1C, or serum lipids. If replicated in larger samples, these findings could have major implications in understanding the role of vitamin B_{12} in diabetes risk.

Thus, a growing body of evidence points to a significant association of vitamin B_{12} and risk of diabetes. It would appear that besides macronutrients, recent research suggests that vitamins such as vitamin D and now vitamin B_{12} could also be key modulators of this risk. Larger studies looking closely at gene-environment interactions should unravel the mystery of what promises to be an important link between vitamin B_{12} and diabetes risk.

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REVIEW ARTICLE

The use of ABI in screening for diabetes-related lower limb peripheral arterial disease in IDF middle- and low-income countries: a scoping review

Anette Telmo Thompson[1] · Somasundram Pillay[1,2] · Colleen Aldous[1]

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Abstract

Background The burden of disease from diabetic foot ulcers linked to peripheral arterial disease (PAD) is complicated by limited resources in low- to middle-income countries (LMIC). This precludes the widespread use of sophisticated imaging methods. Screening for PAD can include the use of low-cost tests such as the ankle brachial index (ABI). A scoping review was performed to identify what is known about the use of ABI in screening for diabetes-related PAD in LMIC.

Methods LMIC were defined as per the International Diabetes Federation Atlas. The following databases were searched using a comprehensive search term: Cochrane Library, EbscoHost, PubMed, Web of Science, Science Direct, Scopus, and Google Scholar. A total of 400 records were gleaned. The publication abstracts were screened through several rounds to select only those in which ABI was used in the context of screening for PAD to prevent diabetic foot disease.

Results Twenty publications were included, from MLIC countries such as Brazil, Nigeria, Kenya, Uganda, South Africa, India, China, Singapore, Korea, Taiwan, Hong Kong, Indonesia, Thailand, and the Philippines. Ten ancilliary publications were identified.

Conclusions The widespread reporting and large scale use of ABI in PAD screening in Asia are evident, compared to fewer reports from Africa and South America. Many Asian publications report using faster, automated ABI equipment in conjunction with handheld Doppler evaluation of flow signals. Despite concerns regarding the potential for false elevations secondary to vessel calcification and non-compressibility, ABI remains a useful first encounter test in screening for PAD. Asian publications endorse and report the use of ABI firmly within the core clinical examination and consideration of other risk factors for PAD. No publications were found on large-scale use of automated ABI in the South African or greater African context of diabetic foot screening.

Keywords Diabetic foot screening · PAD · Ankle brachial index · Scoping literature review · MLIC

Abbreviations

ABI	Ankle brachial index
ABPI	Ankle brachial pressure index
CT	Computerized tomography
ECQ	Edinburgh Claudication Questionnaire
IDF	International Diabetes Federation
IWGDF	International Working Group on the Diabetic Foot
MRI	Magnetic resonance imaging
NICE	National Institute for Health and Care Excellence (England)
NIDDM	Non-insulin dependent diabetes mellitus (also known as type II diabetes mellitus)
PAD	Peripheral arterial disease
PVD	Peripheral vascular disease
SEMDSA	Society for Endocrinology Metabolism and Diabetes South Africa
SIGN	Scottish Intercollegiate Guidelines Network
TBI	Toe brachial pressure index
WHASA	Wound Healing Association of South Africa

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Background

Peripheral arterial disease (PAD) is a known risk factor for diabetic foot ulceration [1–5]. PAD can be variously described as peripheral vascular disease (PVD) or lower limb (ischemia). In high-income countries such as the UK, the 2018 NICE guidelines update for assessment of PAD-added new recommendations to the 2016 guidelines [3]. In 2018, the use of ABPI for screening (not as a pure diagnostic tool) is retained after reviewing the evidence on test and screening modalities [6]. In low- to middle-income countries, health resources are limited and thus preclude large-scale use of costly MRI or CT modalities. The costs of PAD in diabetes and related lower limb amputations in KwaZulu-Natal, a province of South Africa, encompass not only human suffering but increasing drain on fiscus [7]. Screening for PAD remains an important tool to reduce the burden of social inequality on health.

Clinical signs of diminished blood flow to the lower limbs may be seen as dry, shiny, hairless skin; brittle and rigid nails; elevated pallor; and dependent rubor due to the loss of the veno-arteriolar reflex and loss of skin temperature sensation. Pain may or may not be present. It must be noted that in the African or Indian populations, elevation pallor and dependent rubor may be obscured by skin pigmentation. Vascular examination of all peripheral pulses consists of palpation of pedal pulses as well as the brachial, radial, femoral, popliteal, dorsalis pedis, and posterior tibial arteries. According to the Wound Healing Association of South Africa (WHASA), lower limb clinical vascular examination should include an examination of the skin, surface temperature, pedal pulses, capillary refill time, ABI, and a grading based on the examination. [4] In a small 2019 study from the University of Malta which compared different screening modalities in the detection of PAD in primary care settings, pulse palpation, wave-form analysis, ankle brachial pressure index, absolute toe pressure, toe brachial pressure index, and transcutaneous oxygen pressure were compared. The highest percentage of participants who had PAD was for the Doppler Waveform (93.0%). This was followed by TBPI (72.0%), ABPI (57.0%), ATP (35.0%), TCPO (30.0%), and pulse palpation (23.0%). The study demonstrated the existence of inconsistencies between the six different modalities used and advocated not only awareness among clinicians but the need for more studies with a view to provide evidence as to which screening modality yields the most valid results [8] .

As patients with diabetes often present atypically, and may have non palpable pedal pulses, handheld Doppler evaluation of flow signals provides the next level of non-invasive clinical assessment.

Quality of Doppler signals can be graded as:

- Triphasic: normal arterial flow; usually associated with a palpable pulse
- Biphasic: mild to moderate PAD
- Monophasic or absent: severe PAD
- Bounding biphasic or monophasic pulses indicate stenotic vessels.

The ABI is a test conducted using a sphygmomanometer with appropriate cuffs for the ankle and the brachial (arm) pulses. The index is a calculated ratio of the systolic blood pressure at the ankle versus that in the upper arm. The use of a handheld Doppler instrument greatly facilitates the location of atypical pulses. Automated ABI machines incorporate both ankle and brachial cuffs such that measurement of both lower limbs is conducted at the same time. Interpretation of ABI is shown in Table 1.

Aim

This scoping review set out to find what has been published on the usage of the ankle brachial pressure index (ABPI) test in screening for diabetes-related lower limb peripheral arterial disease in middle- and low-income countries as defined by the World Health Organization.

Methods

Scoping reviews differ from literature reviews and systematic reviews. Recently scoping reviews have emerged as an alternative method to synthesize literature on a given topic. Scoping reviews generate descriptive narratives that review the sources of available evidence. The work of Arksey and O'Malley [10] and that of Levac et al. [11] outlined six stages of a scoping study or scoping review. The present review followed the methods outlined for the first five of those stages.

The research team was established by approaching fellow academics with expertise in the area to be scoped (stage 1). The first team meeting decided on a pertinent research question "What is the usage of ABPI in screening for diabetes-related lower limb peripheral arterial disease in resource limited settings?" (stage 2). Whereas TBPI, ATP, palpation, and TcPO2 have been documented as alternative screening methods [8], our study chose to only search for ABPI.

Criteria for inclusion or exclusion of studies (stage 3): a search was conducted online for key word synonyms in order to anticipate different terms that might be used in the literature. For example, "resource limited settings" could be expressed as "third world" or "developing world" or "MLIC" middle- to low-income countries." Boolean operators were used to include all the synonyms in the first search term to prepare as comprehensive a sweep of the literature as possible.

To establish which countries were resource-limited, the team used the IDF classification of limited resource countries

Table 1 Ankle brachial index interpretation [9]

ABI value	Interpretation
> 1.3	False elevation; heavy vessel calcification
0.9–1.29	Normal range
0.5–0.89	Peripheral artery disease, associated with intermittent claudication
< 0.5	Critical limb ischemia; associated with ulceration and rest pain

as found in the IDF World Atlas [12]. By using the IDF website's interactive filter for economic development, an atlas for each of low-income countries and middle-income countries was generated (Figs. 1 and 2).

Search term and data sources

The following search term was developed to list specific country or continent names and applied to the Cochrane Library, EbscoHost, PubMed, Web of Science, Science Direct, Scopus and Google Scholar databases: ("diabetes mellitus") AND ("peripheral arterial disease") AND ("ankle brachial index") AND (Africa OR "South America" OR Brazil OR Mexico OR Russia OR India OR China OR Philippines).

It must be noted that the search term for Web of Science was identical but needed insertion of a TS Field tag to denote topic. (TS = (diabet* AND "peripheral arterial disease" AND ("ankle brachial index") AND ("Africa" OR "South America" OR Brazil OR Mexico OR Russia OR India OR China OR Philippines)).

The search across the Cochrane database yielded one result [13].

Study selection, data collection, and interpretation (stage 4): the search across the remaining databases identified 400 records. Since PAD in diabetes mellitus places patients at significant risk for cardiovascular, ocular, and renal damage in addition to limb loss, these records included references in which the focus was not strictly that of screening for PAD in the lower limbs. Once duplications, indices, and abstract lists for conferences were deleted ($n = 161$), abstracts were screened by the team to eliminate records with obvious focus on PAD in areas other than the lower limb (Fig. 3).

Full texts of the 24 core papers were requested from the university library services. On receipt of these, it was found that two abstracts related to the same paper as one database had

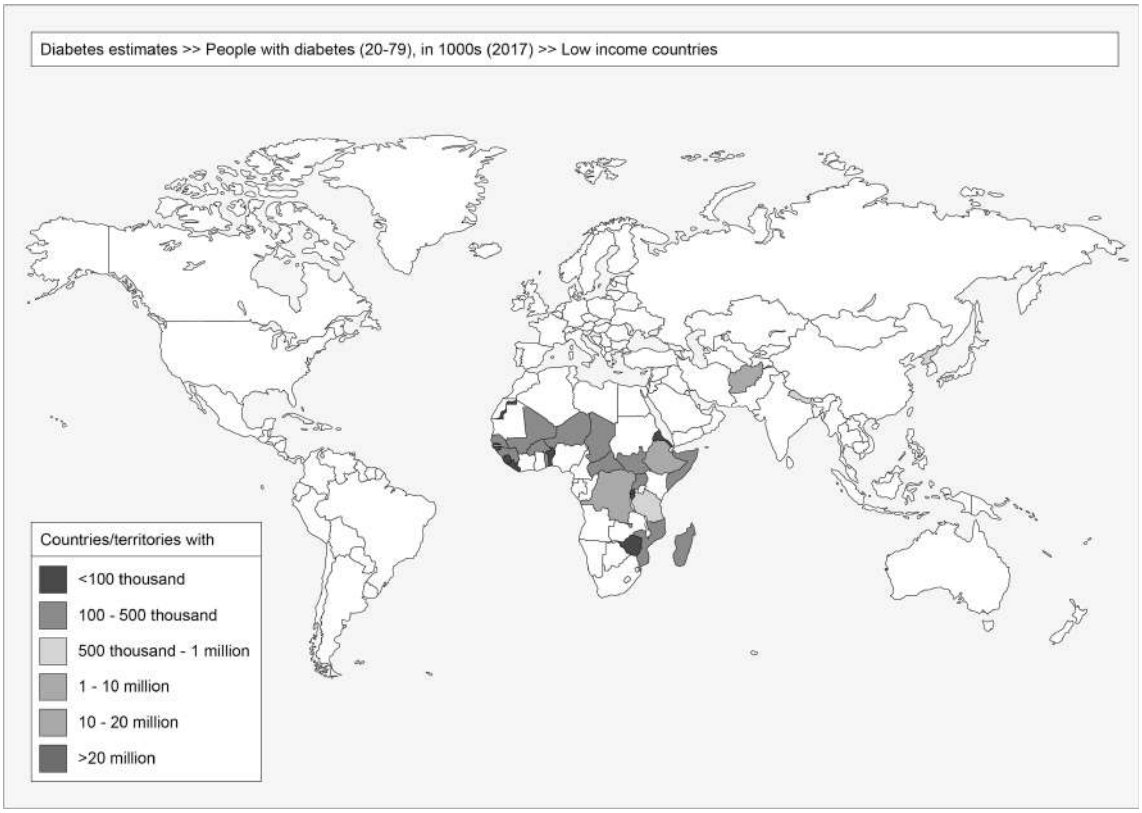

Fig. 1 IDF estimates of diabetes prevalence in low income countries (Africa, Madagascar, Afghanistan, Nepal, and North Korea) [12] (Reprinted with permission)

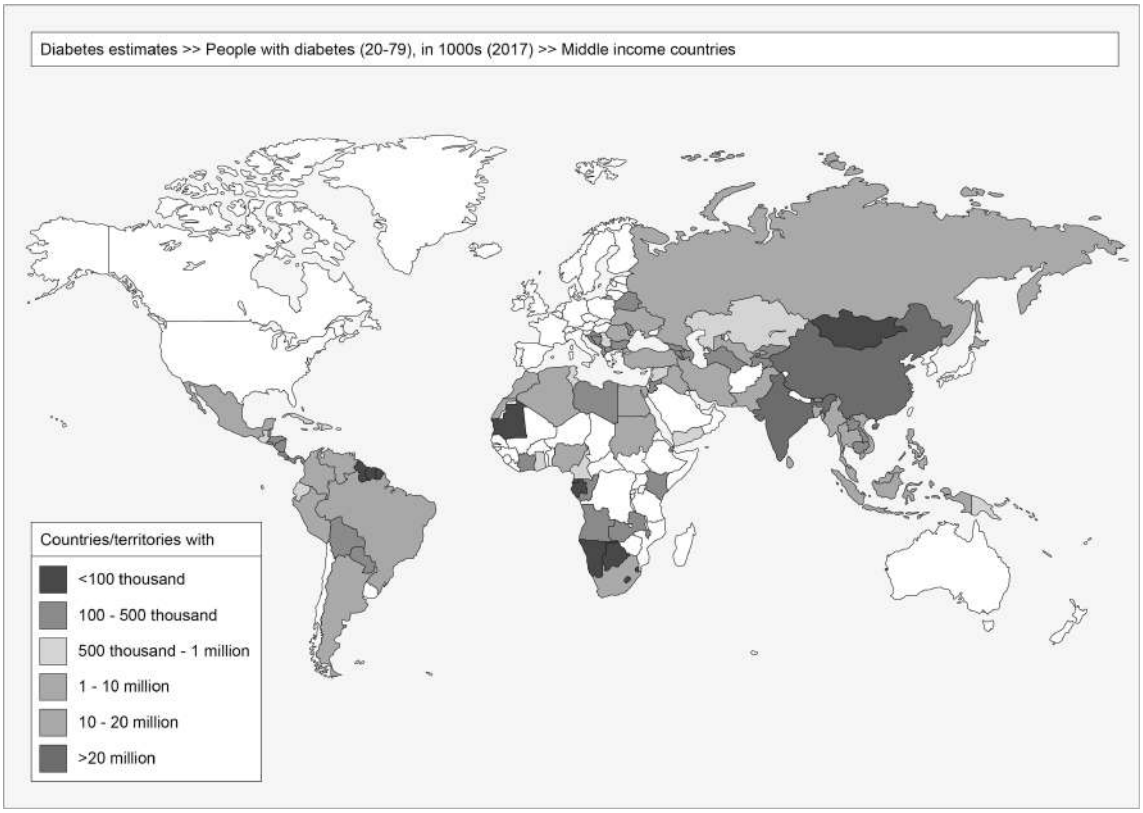

Fig. 2 IDF estimates of diabetes prevalence in middle-income countries (Mexico, South America, Africa, India, Russia, China, the Philippines) [12] (Reprinted with permission)

referenced the Chinese author's first name as his surname. Three papers were found to have English abstracts but foreign language texts (Mexican Spanish and African French) and were, regretfully, discarded since none of the team members had sufficient fluency in these languages. The single Cochrane review, together with the remaining 20 core papers, was then examined.

The Cochrane review included a single Spanish prospective study that compared the manual Doppler method of obtaining an ABI with the automated oscillometric method [13] and thus was not included in this review as Spain is classified as a high-income country by the IDF. Furthermore, the Cochrane review excluded screening studies in which patients were asymptomatic (did not present with exertional leg pain) and in which angiography or duplex ultrasonography was excluded. Of note, however, the review discussion acknowledged that systematic review of studies using ABI in alternative patient groups such as high-risk patients without leg pain and patients with atypical leg pain is required. It also endorsed that primary studies that include patients not previously diagnosed with PAD and asymptomatic patients with comorbidities such as diabetes mellitus are also required.

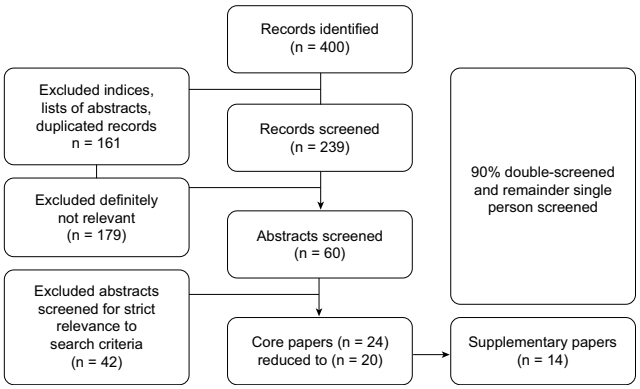

Fig. 3 Flow diagram showing process of eliminating duplications and irrelevant records from the total number of records identified from abstracts (study identification and selection)

Data synthesis

Extracted data were synthesized descriptively with studies grouped by commonly reported themes. Simple summation was used in MS Excel to provide quantitative data (stage 5).

Results

As per the Arksey and O'Malley framework for conducting a scoping review, "a thematic construction is used to provide an overview of the breadth of the literature but not a synthesis" [11]. Results are thus presented as a numerical analysis of the extent and nature of studies

using tables or charts, together with common thematic groupings and/or analysis.

Countries identified

The 20 core papers emanated from countries using ABI in their screening process to detect peripheral vascular disease in patients with diabetes. They included Brazil, Nigeria, Kenya, Uganda, South Africa, India, China, Singapore, Korea, Taiwan, Hong Kong, Indonesia, Thailand, and the Philippines.

Number of patients screened

The total number of patients reported by the 20 core papers [14–33] was 35,340. Within these papers, published in this century with one exception [23], their analyses of results present common statements regarding risk factors for PAD, correlations to hypertension, age, duration of diabetes, systolic blood pressure, dyslipidaemia, gender, smoking, HbA1c levels, ethnic differences, as well as endorsement for the use of ABPI as a reasonable screening tool, noting its simplicity and low cost of examination.

Nature of clinical operators

Among the core papers, the ABI test was variously carried out by vascular surgeons [14], medical doctors or interns [17–19, 22–31, 33], experienced technician under supervision of a physician [15], trained examiner [16], trained physician [32], and trained personnel [21] .

With one exception [14], all papers excluded other types of diabetes mellitus and reported only on non-insulin dependent diabetes mellitus (NIDDM) participants.

Types of ABI equipment

While most studies in this review used conventional sphygmomanometer cuffs together with a handheld 8 MHz Doppler ultrasound probe, others used automated ABI measurement devices [26, 29, 32]. Automated ABI measurement was used on 17,906 (50.7%) of the 35,340 patients reported in the records selected by this scoping review.

Two studies of case control design, 18 cross sectional studies

Two papers [15, 31] studied patients with NIDDM and control groups without diabetes. The paper by Binu et al. in South India [15] excluded symptomatic, cardiovascular and smokers from the diabetic group and excluded hypertensive, smokers, coronary artery disease, and metabolic syndrome from the control group. They found an asymptomatic (no exertional leg pains) PAD prevalence of 24% in the diabetic group compared to 4% in the non-diabetic group. The other case control paper reported on a larger study carried out at the National Diabetes Management and Research Centre in Ghana in which the ECQ assessment was used to differentiate the participants into those with no exertional leg pains, atypical exertional pains, pain at rest, and "classical" intermittent claudication. They found that 18.6% of participants with diabetes had an ABI less than 0.9, compared to 8.1% of those without diabetes.

Age and gender data

Although increased age was stated as an expected risk factor, a number of studies in India, China, and Singapore found that male patients displayed PAD at a younger age than females [12, 18–20]. Some studies found females to be more at risk than males [11, 14, 16, 17, 20, 22, 23, 28] while others found males to be more at risk than females [15, 21, 26, 29] and a number of studies found gender not to be significant [12, 13, 27, 30].

Two papers were gender specific: Rheeder et al. [27] examined only female South African patients while Sarkar et al. [28] examined only male patients in Eastern India. Both studies administered the WHO questionnaire developed by Rose et al. [34] to test for exertional symptoms and to grade intermittent claudication where present. Both studies were too small to draw inferences on their larger diabetic population. The paper by Rheeder et al. was aimed at finding concordance between ABI and toe brachial index (TBI) and correlations to pedal pulse palpation and radiographic evidence of medial artery calcification [27]. Unfortunately, although it was population group-specific (black South Africans), it did not report on the clinical data collected.

Unusual reported risk factors for PAD

Among the expected risk factors for PAD documented by the 20 core papers (see Table 2) were some noteworthy data. In the Kenyan study [24], foot deformities were found to be a risk factor for PAD while in the Ugandan study, glibenclamide a sulfonylurea drug was found to be associated with a higher incidence of peripheral arterial disease [25]. No distinction was made as to whether only glibenclamide was associated with an increased risk of PAD or whether all sulfonylureas or other medications were implicated.

Body mass index

Obesity screening yielded unexpected associations in that heavier BMI was only found to be a risk factor in a few papers [15, 31, 33] while others found that leaner patients (low BMI) were at risk [18, 19, 26, 29] and some found BMI to have no significance [16, 21, 23–25, 30, 32].

Table 2 Summary of data in 20 core studies screened (N/S denotes not significant, shaded blocks indicate data not collected/reported). Bullets indicate data collected/reported

Authors	Countries	Number of participants	NIDDM duration	Age group	Gender	HBP	Systolic BP	Lipidaemia	HbA1C	BMI	Diff in Pop Groups	Smoking	Neuropathy
Andrade et al [14]	Brazil	236	longer	older	Female	•	•						
Binu et al [15]	India	100		M Y	N/S	•		•		heavier			
Do N Sales et al [16]	Brazil	73	longer	older	N/S	•	•			N/S		N/S	
Eshcol et al [17]	India	2493	longer	older	Female>	•	•	•	N/S			•	
Ghosh et al [18]	India	84	shorter	N/S	Male >	•	•	•		Leaner		male	
Guan et al [19]	China	1397	longer	older	Female	•	•	N/S	•	Leaner		•	
Khurana et al [20]	India	200	longer	Older	Female			•	•				
Narayanan et al [21]	Singapore	521	longer	Older	Male Y	•		•	N/S	N/S	•	•	•
Li et al [22]	China	3924	M ≠ F	Older	Male Y	N/S		•	•	•		•	•
Mohan et al [23]	India	4941	Longer	M Y	Female>	•	•	•	•	N/S		N/S	
Mugambi et al [24]	Kenya	218	Longer	Older	Male>	•	•			N/S		N/S	•
Okello et al [25]	Uganda	229	N/S	Older	Female>	•	N/S	N/S	N/S	N/S		N/S	
Rhee et al [26]	Korea, Indonesia, Thailand, Taiwan, China, Hong Kong	6625	longer	Older	Female>	•		•	•	Leaner	•	•	
Rheeder et al [27]	S Africa (*gender specific)	85	longer		Female*						•		
Sarkar et al [28]	India (*gender specific)	31	longer	Older	Male*		•						
Sosale et al [29]	India	600	shorter	older	Male	•		•	N/S	leaner			
Umuerri et al [33]	Nigeria	380	longer	older	N/S	•		N/S		heavier		N/S	
Wang et al [30]	China	2010	Longer	older	N/S	•		N/S		N/S	•	•	•
Yeboah et al [31]	Ghana	485		older	Female	•				heavier		N/S	
Zhang et al [32]	China	10681		older	Male	•		•	•	N/S		•	•

Ethnic and/or population differences

Ethnic and population prevalence differences were noted in the large PAD-Search study across six countries in Asia [26] as well as between the three population groups in Singapore [21]. All the African studies only measured the black population group [24, 25, 27, 31, 33] and thus did not report on prevalence in other population groups.

Discussion

Macrovascular diabetes-related complications like PAD and resulting lower limb amputations place a strain on the economies of developing countries. One of the major goals in addressing the burden of lower limb complications in diabetes should be the detection of PAD by simple non-invasive means. Risk stratification by means of ABI is one such option allowing for prioritization of resources to high-risk populations in order to have the greatest impact.

It can be difficult to provide in-depth preventative screening for every patient owing to resource constraints in low-and middle-income countries. Automated ABI measurement devices have been used for large PAD screening studies since they offer cost reductions in terms of improved accuracy, time taken to test, and human resources.

Screening for PAD in Africa using ABI remains a conundrum. Our review showed that, although some countries in Africa have reported using ABI in screening for lower limb PAD, there has been no report of large-scale implementation of ABI in the diabetic population to screen for lower limb PAD in the context of diabetic foot disease in South Africa. In contrast, the low- and middle-income countries in Asia and South America already actively include ABI in their clinical screening of patients with diabetes.

Limitations of ABI testing for diagnosis of PAD include concerns that have been raised in connection with ABI results above 1.3 as these are considered to be indicative of stenotic vessels. It has been argued that, because of vessel stenosis, normal readings could represent low readings elevated by stenosis. This would only apply in the case of using an ABI test in isolation and no publication reported this. The use of exercise modifiers and the background data from a complete physical examination which must include identification of known risk factors is as much a part of the search for PAD as the ABI screening test.

It is hoped that the advent of low-cost automated ABI used in conjunction with handheld Doppler equipment will allow more low-resource health systems, particularly those in Africa, to close the gap in large scale screening for PAD so as to prevent the costly sequelae of diabetic foot disease.

Authors' contributions ATT was the project lead in the scoping review. CA provided background research on scoping review methodology. ATT, SP, and CA participated in the iterative record selection process. Each author read a set number of abstracts and contributed to the final selection of included records.

ATT wrote the first draft of the article and prepared the tables and figures. SP and CA participated in revising the article through several iterations providing critical intellectual content. All the authors have read and approved the final manuscript.

Compliance with ethical standards

Ethics approval was obtained from the Biomedical Research Ethics Committee, University of KwaZulu-Natal number BE264/17. This article does not contain any studies with human participants performed by any of the authors.

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

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REVIEW ARTICLE

Association of RETN gene polymorphism at +299 G>A with type 2 diabetes mellitus: a meta-analysis

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Abstract

Introduction Resistin (RETN) protein plays an important role in the regulation of energy, glucose, and lipid homeostasis and maintenance of fasting blood glucose level by modulating hepatic insulin action. Though RETN gene polymorphism at single nucleotide polymorphism (SNP) rs3745367 (+299 G>A) and SNP rs3745368 (+62G>A) has been indicated to be relevant to type 2 diabetes mellitus (T2D), results are contradictory due to incomplete and inconsistent studies.

Material and methods Meta-analysis was conducted to evaluate the relationship between RETN gene polymorphism at SNP rs3745367 and rs3745368 with risk of T2D. A present study included 5276 subjects for polymorphism for SNP rs3745367 and 3617 subjects for SNP rs3745368 reported in 16 different studies available at Pubmed, EMBASE, and Google Scholar from January 2001 to May 2018. Inclusion and exclusion criteria were followed as per international norms. Publication bias was assessed using a funnel plot. The Review Manager 5.3 software was used for statistical analysis. Polymorphism was evaluated using the odds ratio (OR) corresponding to 95% confidence interval (CI). Heterogeneity among the studies was calculated using Q test. I^2 was calculated to assess the variation caused by heterogeneity. Association of RETN polymorphism and T2D was analyzed using three genetic models viz. allelic, recessive, and dominant.

Results and Conclusion Significant association was observed between RETN gene polymorphism at SNP rs3745367 and T2D. The pooled odds ratio (OR) was 1.45 (95% CI 1.10–1.92) with a p value of 0.009 under an allelic genetic model, whereas no significant association was found between RETN gene polymorphism at SNP rs3745368 and T2D. The pooled OR was 1.10 (95% CI, 0.66–1.83) with a p value of 0.71 under an allelic genetic model.

Keywords Allelic genetic model · Gene polymorphism · Meta-analysis · Resistin · Single nucleotide polymorphism · Type 2 diabetes mellitus

Introduction

Diabetes is a group of metabolic disorders characterized by hyperglycemia due to defect in insulin secretion, insulin action, or both. T2D is a multifactorial disease that occurs due to combined action of environmental and genetic factors [1]. Heritability of T2D ranges from 20 to 80% as evidenced by study of various populations in different countries and twin-based studies [2]. The risk of developing T2D is 40% in individuals having either parent with T2D and 70% in individuals having both affected parents [3]. Diabetic patients are increasing at alarming rate. According to the International Diabetes Federation (IDF) Diabetes Atlas, there are 451 million people with type 2 diabetes worldwide and this is expected to increase 693 million by 2045 [4].

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Resistin, also known as adipose tissue–specific secretory factor (ADSF) is a cysteine-rich adipose-derived peptide hormone. In humans, it is encoded by the RETN gene [5] located on chromosome no. 19p13.3. Resistin prepeptide in humans is comprised of 108 amino acid residues. Resistin in mouse and rat is 114 amino acid residues long and is highly expressed in mouse adipose tissue [6]. In humans, the resistin is expressed at higher level in pre-adipocytes than in mature adipocytes but expression pattern is not totally consistent [7]. It is also expressed in human peripheral blood monocytes [8]. Resistin increases the production of low-density lipoprotein (LDL) in human hepatocytes but degrades LDL receptors in liver. As a result, liver is unable to clear LDL cholesterol from the body. Thus, resistin accelerates the accumulation of LDL in arteries, thereby increasing the risk of heart disease [9]. Resistin also plays role in the regulation of energy, glucose, and lipid homeostasis [10] and maintenance of fasting blood glucose level by modulating hepatic insulin action [11, 12]. Genetic variants in RETN gene have been examined by many groups, and it is estimated that up to 70% of variation in circulating resistin levels could be explained by genetic factors [13]. In some studies, resistin was reportedly upregulated in insulin resistance, T2D, and cardiovascular diseases (CVD) [14].

Nevertheless, some association studies have been conducted in different ethnicities to evaluate the potential risk of variants in RETN gene in developing T2D. But their results are contradictory. This might be due to differences in ethnic groups. To date, no systematic study has been conducted to evaluate RETN gene polymorphisms and risk of developing T2D. Thus, meta-analysis was conducted to evaluate association of two genetic variants of RETN gene rs3745367 and rs3745368 and susceptibility to T2D. Eleven studies for SNP rs3745367 and five studies for SNP rs3745368 have been examined by suitable statistical software and are being reported in the present study.

Materials and methods

Literature search strategy and inclusion criteria

Relevant research papers published from January 2001 to May 2018 were retrieved from three electronic databases

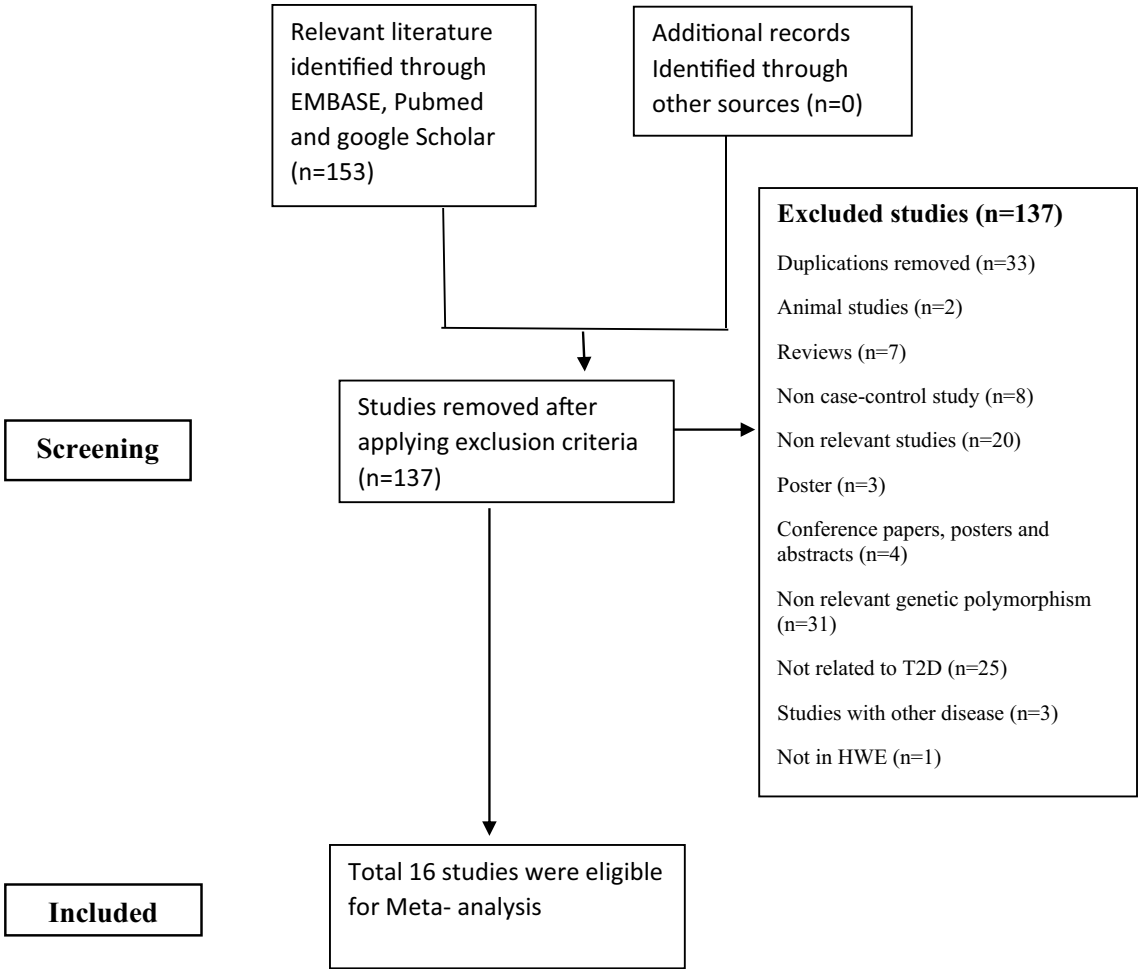


Fig. 1 A PRISMA flow diagram of inclusion criteria in meta-analysis study

Table 1 General characteristics of included studies for rs3745367

Author and year	Country	T2D criteria	Genotyping method	Study design	Sample size (T2D/control)	T2D (GG/GA/AA)	Control (GG/GA/AA)
Ma (2002) [15]	Italy	WHO	PCR-allelic specific Assay	Case–control	312/303	174/114/24	169/120/14
Osawa (2002) [16]	Japan	ADA	PCR sequencing	Case–control	99/99	40/46/13	35/47/14
Conneely (2004) [17]	Finland	OGTT	Mass spectrometry	Case–control	781/409	439/293/49	236/149/24
Kunnari (2005) [18]	Finland	NA	PCR-RFLP	Case–control	258/494	156/84/16	282/185/27
Suriyaprom (2009) [19]	Thailand	ADA	PCR-RFLP	Case–control	95/105	33/46/16	58/40/7
Lau[1] (2011) [20]	India	ADA	TaqMan genotyping	Case–control	146/60	63/69/14	18/37/5
Lau[2] (2011) [20]	Malaysia	ADA	TaqMan genotyping	Case–control	144/75	63/56/25	30/34/11
Lau[3] (2011) [20]	China	ADA	TaqMan genotyping	Case–control	137/73	56/63/18	33/30/10
Zhang (2013) [21]	China	WHO	PCR-RFLP	Case–control	738/279	280/353/105	23/123/33
Khalil (2014) [22]	Egypt	NA	PCR-RFLP	Case–control	60/45	8/29/23	19/20/6
Al-hilali (2015) [23]	Iraq	NA	PCR-RFLP	Case–control	50/25	3/31/16	11/12/2
Kaur (2016) [24]	India	ADA	PCR-RFLP	Case–control	30/30	13/13/4	21/8/1
Vardali (2017) [25]	Turkey	NA	Allelic discrimination assay	Case–control	217/212	42/101/74	122/75/15

[1, 2, 3] Lau conducted study in 3 different populations as mentioned

namely, Pubmed, EMBASE, and Google Scholar using the keywords "Resistin," "RETN," "gene polymorphism," "type 2 diabetes mellitus," "T2D," and "SNP." References were also evaluated from the retrieved papers. Inclusion criteria for the study included the following: (i) case–control studies evaluating relationship of polymorphism of RETN gene with T2D; (ii) studies containing original data; (iii) studies with genotypic or allelic frequencies; (iv) clear definition of T2D; (v) if similar article published more than once, the nearest and integrated article was selected. Review articles, conference abstracts, animal and in vitro studies, studies based on linkage considerations, and cohort studies were excluded from the study. Inclusion criteria for meta-analysis is shown in a PRISMA flow diagram (Fig. 1).

Data extraction

Research papers containing animal studies, non-case–control studies, non-relevant studies, data presented in conferences as posters, conference papers and abstracts, review articles, and papers having duplication were excluded from the study. General characteristics included different parameters viz. surname of first author, year of publication, country of subjects, diagnosis criteria of T2D, genotyping method, study design, sample size, and genotypic data or allelic frequencies (Tables 1 and 2). A total of 16 research papers were included in the present study. Eleven studies involving 3067 cases and 2209 controls were evaluated to study the association of rs3745367 polymorphism with T2D and 5 studies involving 2058 cases and 1559 controls were evaluated to assess the association of rs3745368 polymorphism with T2D.

Statistical analysis

The Review Manager 5.3 software was used to perform the statistical analysis. The association between RETN gene polymorphisms for SNPs rs3745367 and rs3745368 was evaluated using the odds ratio (OR) corresponding to 95% confidence interval (CI). Heterogeneity among the studies was calculated using Q test. I^2 was calculated to assess the variation caused by heterogeneity. Pooled OR was assessed using different effect models according to heterogeneity among the studies. A random effect model was employed when a significant heterogeneity ($I^2 > 50\%$)

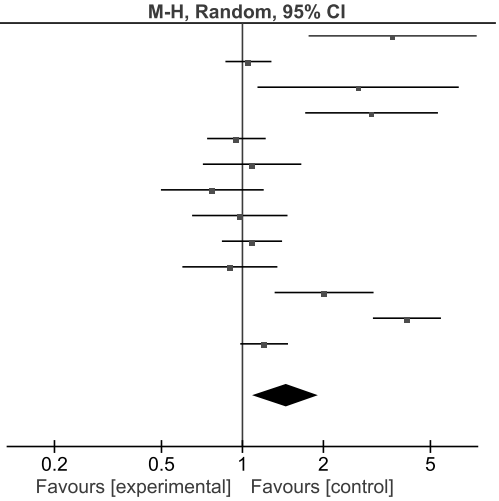
Table 2 General characteristics of included studies for rs3745368

Author and year	country	T2D criteria	Genotyping method	Study design	Sample size (T2D/control)	T2D (GG/GA/AA)	Control (GG/GA/AA)
Tan (2003) [26]	Taiwan	WHO	PCR-RFLP	Case–control	1102/743	920/176/6	558/178/7
Berthold (2005) [27]	Germany	NA	PCR-RFLP	Case–control	384/434	360/23/1	400/34/0
Jiang (2014) [28]	China	WHO	Sequenom iplex platform	Case–control	352/161	256/87/9	122/35/4
Tellez (2016) [29]	Mexico	IDF	PCR-RFLP	Case–control	45/46	39/5/1	41/4/1
Thammakun (2017) [30]	Thailand	ADA	PCR-RFLP	Case–control	175/175	88/71/16	116/53/6

(a)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
AL-HILALI 2015	63	100	16	50	5.8%	3.62 [1.76, 7.43]
CONNEELY 2004	391	1562	197	818	8.9%	1.05 [0.86, 1.28]
KAUR 2016	21	60	10	60	5.0%	2.69 [1.14, 6.37]
KHALIL 2014	75	120	32	90	6.8%	3.02 [1.71, 5.33]
KUNNARI 2005	120	516	239	988	8.7%	0.95 [0.74, 1.22]
LAU (CHINESE) 2011	99	274	50	146	7.7%	1.09 [0.71, 1.66]
LAU (INDIAN) 2011	97	292	47	120	7.6%	0.77 [0.50, 1.20]
LAU (MALAY) 2011	106	288	56	150	7.8%	0.98 [0.65, 1.47]
MA 2002	162	624	148	606	8.7%	1.09 [0.84, 1.40]
OSAWA 2002	72	198	77	198	7.8%	0.90 [0.60, 1.35]
SURIYAPROM 2009	78	190	54	210	7.7%	2.01 [1.32, 3.07]
VARDARLI 2017	249	434	105	424	8.5%	4.09 [3.06, 5.47]
ZHANG 2013	563	1476	189	558	8.9%	1.20 [0.98, 1.48]
Total (95% CI)		**6134**		**4418**	**100.0%**	**1.45 [1.10, 1.92]**
Total events	2096		1220			

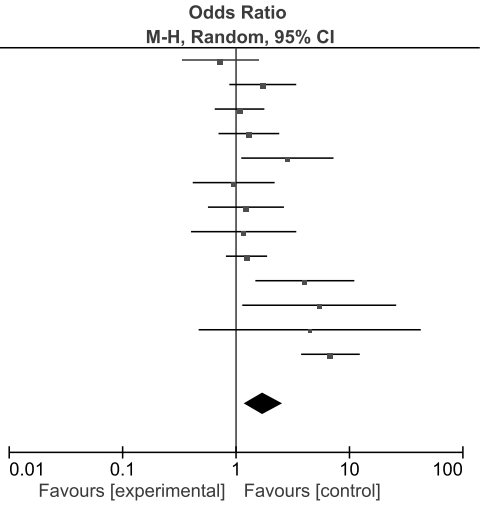
Heterogeneity: Tau² = 0.22; Chi² = 106.52, df = 12 (P < 0.00001); I² = 89%
Test for overall effect: Z = 2.59 (P = 0.009)



(b)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
OSAWA 2002	13	99	17	99	8.2%	0.73 [0.33, 1.60]	2002
MA 2002	24	312	14	303	8.9%	1.72 [0.87, 3.39]	2002
CONNEELY 2004	49	781	24	409	10.1%	1.07 [0.65, 1.78]	2004
KUNNARI 2005	18	258	27	494	9.3%	1.30 [0.70, 2.40]	2005
SURIYAPROM 2009	16	95	7	105	7.2%	2.84 [1.11, 7.23]	2009
LAU (CHINESE) 2011	18	137	10	73	7.9%	0.95 [0.42, 2.19]	2010
LAU (MALAY) 2011	25	144	11	75	8.3%	1.22 [0.57, 2.64]	2010
LAU (INDIAN) 2011	14	146	5	60	6.4%	1.17 [0.40, 3.40]	2010
ZHANG 2013	105	738	33	279	10.7%	1.24 [0.81, 1.88]	2013
KHALIL 2014	23	60	6	45	6.8%	4.04 [1.48, 11.04]	2014
AL-HILALI 2015	16	50	2	25	4.2%	5.41 [1.13, 25.81]	2015
KAUR 2016	4	30	1	30	2.5%	4.46 [0.47, 42.51]	2016
VARDARLI 2017	74	217	15	212	9.5%	6.80 [3.75, 12.33]	2017
Total (95% CI)		**3067**		**2209**	**100.0%**	**1.75 [1.18, 2.60]**	
Total events	399		172				

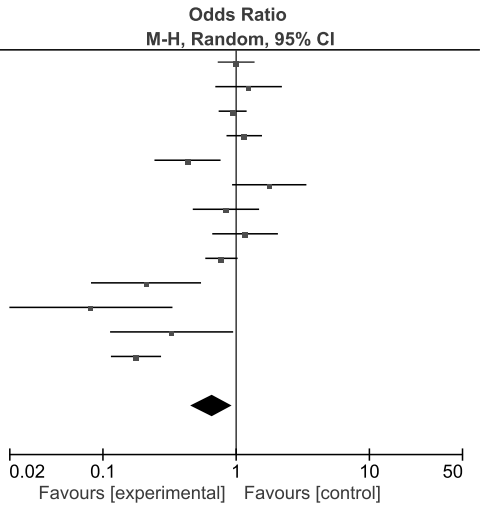
Heterogeneity: Tau² = 0.34; Chi² = 41.33, df = 12 (P < 0.0001); I² = 71%
Test for overall effect: Z = 2.77 (P = 0.006)



(c)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
MA 2002	174	312	169	303	9.2%	1.00 [0.73, 1.37]	2002
OSAWA 2002	40	99	35	99	7.9%	1.24 [0.70, 2.20]	2002
CONNEELY 2004	439	781	236	409	9.5%	0.94 [0.74, 1.20]	2004
KUNNARI 2005	156	258	282	494	9.3%	1.15 [0.85, 1.56]	2005
SURIYAPROM 2009	33	95	58	105	7.9%	0.43 [0.24, 0.76]	2009
LAU (INDIAN) 2011	63	146	18	60	7.5%	1.77 [0.93, 3.37]	2010
LAU (CHINESE) 2011	56	137	33	73	7.9%	0.84 [0.47, 1.49]	2010
LAU (MALAY) 2011	63	144	30	75	7.9%	1.17 [0.66, 2.06]	2010
ZHANG 2013	280	738	123	279	9.4%	0.78 [0.59, 1.03]	2013
KHALIL 2014	8	60	19	45	5.8%	0.21 [0.08, 0.54]	2014
AL-HILALI 2015	3	50	11	25	3.8%	0.08 [0.02, 0.33]	2015
KAUR 2016	13	30	21	30	5.2%	0.33 [0.11, 0.95]	2016
VARDARLI 2017	42	217	122	212	8.7%	0.18 [0.11, 0.27]	2017
Total (95% CI)		**3067**		**2209**	**100.0%**	**0.66 [0.46, 0.93]**	
Total events	1370		1157				

Heterogeneity: Tau² = 0.33; Chi² = 90.42, df = 12 (P < 0.00001); I² = 87%
Test for overall effect: Z = 2.33 (P = 0.02)



◀ **Fig. 2** Forest plot of relationship between RETN gene polymorphism of SNP rs3745367 and the risk of T2D among different population using allelic genetic model (**a**), under recessive genetic model, (**b**) and under dominant genetic model (**c**). Black diamond denotes the pooled OR. Blue square represents the weight of study. Horizontal line shows 95% confidence interval (CI)

was detected among studies; otherwise, a fixed effect model was applied. Publication bias was assessed using funnel plot. Egger's linear regression test and Begg–Mazumdar's test were used to assess the asymmetry in funnel plot using Comprehensive Meta-Analysis version 3 (CMA) software.

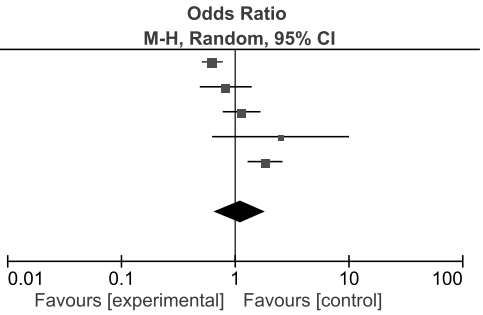
Meta-analysis results

To evaluate the association between RETN gene polymorphism of SNP rs3745367 and T2D risk, three genetic models viz. allelic genetic model (A vs G), recessive genetic model (AG + GG vs AA), and dominant genetic model (GG vs AG + AA) were used. Random and fixed effects models were applied according to significant heterogeneity. RETN gene polymorphism of SNP rs3745367 was found to be significantly associated with risk of T2D in all three genetic models. A comparison of A vs. G under allelic model produced an OR of 1.45 (95% CI 1.10–1.92) which was statistically significant with a p value of 0.009 as shown by forest plot in Fig. 2a.

(a)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
TAN 2003	188	2204	192	1486	24.9%	0.63 [0.51, 0.78]	2003
BERTHOLD 2005	25	768	34	868	20.4%	0.83 [0.49, 1.40]	2005
JIANG 2014	105	704	43	322	22.7%	1.14 [0.78, 1.67]	2014
TELLEZ 2016	7	90	3	92	8.9%	2.50 [0.63, 10.00]	2016
THAMMAKUN 2017	103	350	65	350	23.1%	1.83 [1.28, 2.61]	2017
Total (95% CI)		**4116**		**3118**	**100.0%**	**1.10 [0.66, 1.83]**	
Total events	428		337				

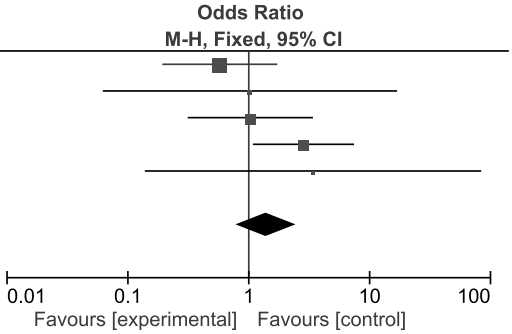
Heterogeneity: Tau² = 0.26; Chi² = 29.95, df = 4 (P < 0.00001); I² = 87%
Test for overall effect: Z = 0.37 (P = 0.71)



(b)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
TAN 2003	6	1102	7	743	40.5%	0.58 [0.19, 1.72]
TELLEZ 2016	1	45	1	46	4.7%	1.02 [0.06, 16.87]
JIANG 2014	9	352	4	161	26.0%	1.03 [0.31, 3.39]
THAMMAKUN 2017	16	175	6	175	26.5%	2.83 [1.08, 7.42]
BERTHOLD 2005	1	384	0	434	2.3%	3.40 [0.14, 83.68]
Total (95% CI)		**2058**		**1559**	**100.0%**	**1.38 [0.78, 2.44]**
Total events	33		18			

Heterogeneity: Chi² = 5.18, df = 4 (P = 0.27); I² = 23%
Test for overall effect: Z = 1.10 (P = 0.27)



(c)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
TAN 2003	920	1102	558	743	24.9%	1.68 [1.33, 2.11]	2003
BERTHOLD 2005	360	384	400	434	20.4%	1.27 [0.74, 2.19]	2005
JIANG 2014	256	352	122	161	22.2%	0.85 [0.55, 1.31]	2014
TELLEZ 2016	39	45	41	46	10.3%	0.79 [0.22, 2.81]	2016
THAMMAKUN 2017	88	175	116	175	22.2%	0.51 [0.33, 0.79]	2017
Total (95% CI)		**2058**		**1559**	**100.0%**	**0.97 [0.58, 1.64]**	
Total events	1663		1237				

Heterogeneity: Tau² = 0.27; Chi² = 25.80, df = 4 (P < 0.0001); I² = 84%
Test for overall effect: Z = 0.11 (P = 0.91)

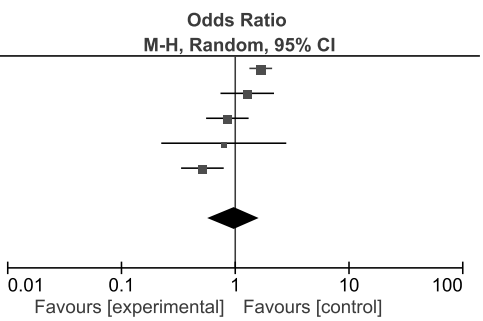


Fig. 3 Forest plot of relationship between RETN gene polymorphism of SNP rs3745368 and the risk of T2D among different populations using allelic genetic model (**a**), recessive genetic model (**b**), and dominant genetic model (**c**). Black diamond denotes the pooled OR. Blue square represents the weight of study. Horizontal line shows 95% confidence interval (CI)

Table 3 Results of sensitivity analysis of the relationship between RETN gene polymorphism of SNP rs3745367 and T2D risk for allelic model

Study omitted	Number of studies	OR (95% CI)	*p* value	I^2 (%)	$p_{Heterogeneity}$	Effect model
Ma (2002)	12	1.50 (1.10–2.05)	0.01	89	< 0.000*	Random
Osawa (2002)	12	1.51 (1.12–2.04)	0.006	89	< 0.000*	Random
Conneely (2004)	12	1.51 (1.09–2.07)	0.01	89	< 0.000*	Random
Kunnari (2005)	12	1.52 (1.12–2.06)	0.008	89	< 0.000*	Random
Suriyaprom (2009)	12	1.41 (1.05–1.90)	0.02	89	< 0.000*	Random
Lau (Indian) (2011)	12	1.53 (1.14–2.05)	0.005	89	< 0.000*	Random
Lau (Malai) (2011)	12	1.50 (1.11–2.03)	0.008	89	< 0.000*	Random
Lau (Chinese) (2011)	12	1.48 (1.09–2.0)	0.01	90	< 0.000*	Random
Zhang (2013)	12	1.49 (1.08–2.06)	0.02	90	< 0.000*	Random
Kahlil (2014)	12	1.37 (1.03–1.83)	0.03	89	< 0.000*	Random
Al-hilali (2015)	12	1.36 (1.02–1.81)	0.04	89	< 0.000*	Random
Kaur (2016)	12	1.41 (1.05–1.88)	0.02	89	< 0.000*	Random
Vardali (2017)	12	1.26 (1.03–1.53)	0.02	72	< 0.000*	Random

*Statistically significant ($p < 0.05$)

Using recessive and dominant genetic models respectively, comparison of AG + GG vs. AA and GG vs. AG + AA gave ORs of 1.75 (CI 1.18–2.60) and 0.66 (0.46–0.93) with *p* value of 0.006 and 0.02 respectively which is again statistically significant (Fig. 2b, c).

Association was also studied between RETN gene polymorphism of SNP rs3745368 and risk of T2D using allelic, recessive, and dominant genetic models. Comparison of A vs. G (Fig. 3a), AG + GG vs. AA (Fig. 3b), and GG vs. AG + AA (Fig. 3c) gave ORs of 1.10 (CI 0.66–1.83), 1.38 (CI 0.78–2.44), and 0.97 (CI 0.58–1.64) with *p* value of 0.71, 0.27, and 0.91 respectively, which were statistically insignificant.

Heterogeneity testing

For RETN gene polymorphism of SNP rs3745367, significant heterogeneity was detected under three genetic models, i.e., A vs. G, AG + GG vs. AA, and GG vs. AG + AA, such that the I^2 values were 89%, 71%, and 87% respectively (Fig. 2a–c). Therefore, a random effect model was applied to estimate pooled OR of SNP. For the polymorphism of SNP rs3745368 under three genetic models, i.e., A vs. G, AG + GG vs. AA, and GG vs. AG + AA, the I^2 values were 87%, 23%, and 84% respectively (Fig. 3a–c). Therefore, a random effect model was applied to allelic and dominant genetic models while fixed effect model was applied to recessive genetic model to estimate the pooled OR of SNP as the I^2 value was less than 50%.

Sensitivity analysis

In order to evaluate the influence of each study on the outcome, sensitivity analysis was performed by omitting each single study included in the meta-analysis one at a time. For polymorphism of SNP rs3745367, the pooled ORs ranged from 1.26 (95% CI 1.03–1.53) to 1.53 (95% CI 1.14–2.05) under an allelic model, which did not change from a pooled OR of 1.45 (95% CI 1.10–1.92) under the same genetic model in total sample (Table 3).

For polymorphism of SNP rs3745368, the pooled ORs ranged from 0.81 (95% CI 0.57–1.61) to 1.30 (95% CI 0.86–1.97) under an allelic model, which did not change from pooled OR 1.10 (95% CI 0.66–1.83) under the same genetic model in total sample (Table 4).

Table 4 Results of sensitivity analysis of the relationship between RETN gene polymorphism of SNP rs3745368 and T2D risk for allelic model

Studies omitted	Number of studies	OR (95% CI)	*p* value	I^2 (%)	$p_{Heterogeneity}$	Effect model
Tan (2003)	4	1.30 (0.86–1.97)	0.21	61	0.05	Random
Berthold (2005)	4	1.20 (0.64–2.25)	0.57	90	< 0.000*	Random
Jiang (2014)	4	1.11 (0.57–2.17)	0.76	89	< 0.000*	Random
Tellez (2015)	4	1.02 (0.60–1.72)	0.95	89	< 0.000*	Random
Thammakun (2017)	4	0.81 (0.57–1.16)	0.25	59	0.06	Random

*Statistically significant ($p < 0.05$)

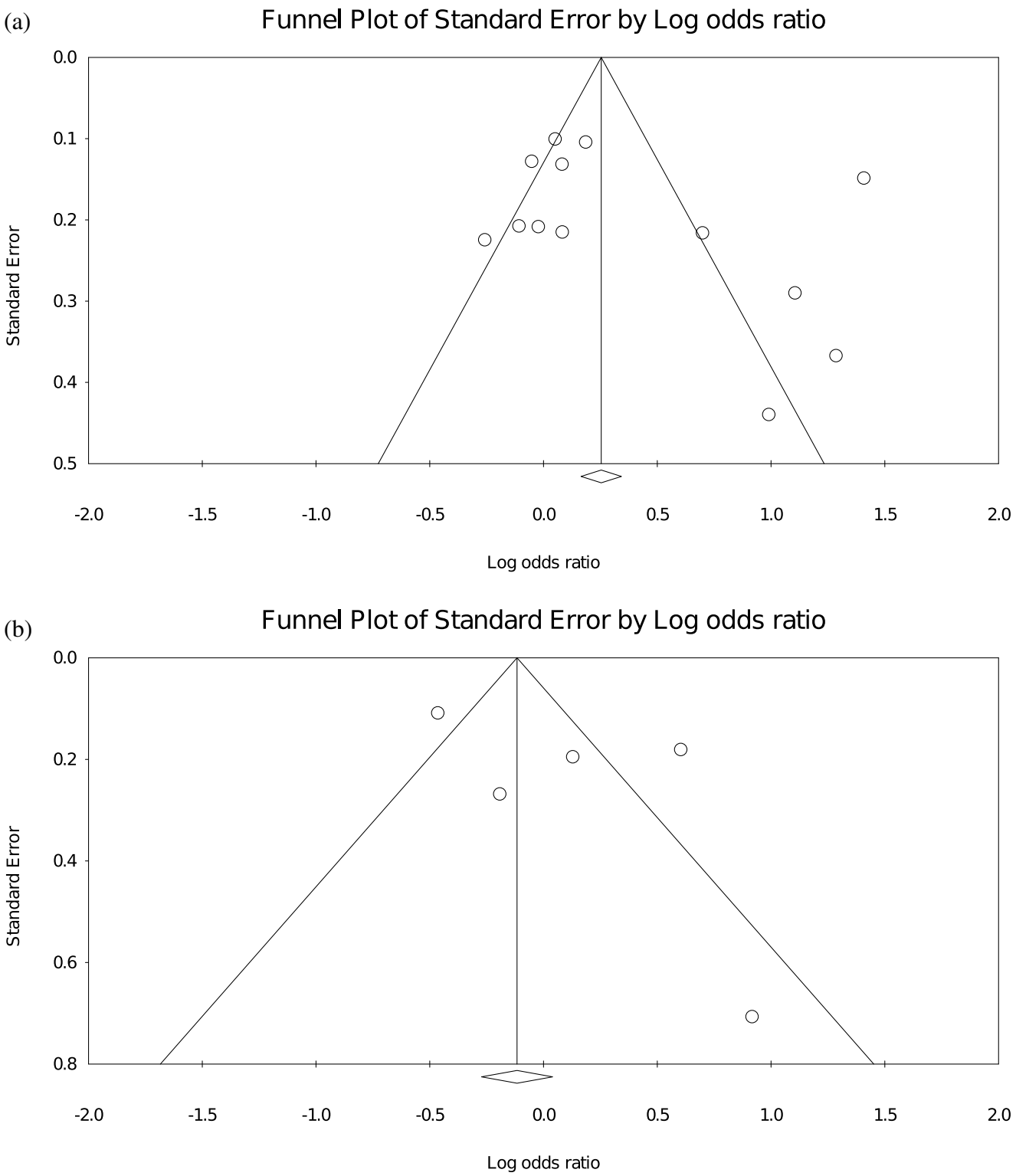


Fig. 4 Funnel plot for studies of the association of RETN gene polymorphism of SNP rs3745367 (**a**) and rs3745368 (**b**) under an allelic model

Publication bias

Publication bias was assessed using a funnel plot. No visual publication bias was detected in the funnel plot (Fig. 4a, b). To check the asymmetry of funnel plot, Begg's and Mazumdar's adjusted rank correlation test and Egger's linear regression test were performed. No significant publication bias was observed in this meta-analysis for polymorphism of SNP rs3745367. Begg's and Mazumdar's p values of 0.127, 0.160, and 0.200

and Egger's p values of 0.270, 0.360, and 0.168 were obtained for the allelic genetic model (A vs. G), recessive genetic model (AG + GG vs. AA), and dominant genetic model (GG vs. AA + AG) respectively.

For RETN gene G>A polymorphism of SNP rs3745368, no significant publication bias was observed in the meta-analysis. Begg's and Mazumdar's p value of 0.806 for all the three genetic models and Egger's p values of 0.306, 0.985, and 0.314 were observed for an allelic, recessive, and dominant genetic models respectively.

Discussion

Studies among different ethnicities revealed inconsistency that might have low statistical significance because of small sample size. A study of Thai population showed significantly higher resistin concentrations in GA/AA genotypes than GG genotype and resistin gene polymorphism at position +299 (G>A) was more frequent in T2D patients than in the control group [24]. In Iraqi population, polymorphism of TNF-α and RETN rs3745367 in development of insulin resistance in T2D patients was evaluated [16]. Contrarily, no significant association between RETN gene polymorphism of SNP rs3745367 and risk of T2D was found in Japanese [15] and Italian subjects [19]. Some authors also reported increased serum resistin levels in T2D subjects [22, 31]. RETN gene polymorphism of SNP rs3745367 was found to be a predisposing factor of T2D development in Egyptian patients [23]. However, RETN gene polymorphism rs1423096 is more significantly associated with T2D than rs3745367 [26]. Serum resistin concentrations as well as HOMA-IR were remarkably more in mutant genotype (GA/AA) as compared with wild type (GG) in German population [27]. In contrast, there was no difference in GG and GA/AA genotypes in Han Chinese patients thus neglecting the risk of T2D incidence [28]. Due to incomplete and conflicting findings of relationship between RETN gene polymorphism of SNP rs3745367 and T2D risk, the research results remained debatable.

Therefore, meta-analysis was conducted to obtain effective result from the available evidences with respect to association between RETN variants and T2D risk. Meta-analysis carried out using all the three genetic models viz. allelic, recessive, and dominant genetic model involving 5276 subjects of different ethnicities revealed that RETN gene polymorphism of SNP rs3745367 was associated with T2D risk with p values of 0.009, 0.006, and 0.002 respectively. Thus, it was concluded that the presence of AA genotype and A allele predicted the probability of developing insulin resistance in T2D, whereas GG genotype and G allele served as a protective factor for disease.

None of studied genetic model revealed any significant association for RETN gene polymorphism of SNP rs3745368 with T2D. The p values for meta-analysis study conducted on 3617 subjects and calculated by allelic, recessive, and dominant genetic models were 0.71, 0.27, and 0.91 respectively. RETN gene polymorphism of SNP rs3745368 was an independent risk factor in developing T2D in Taiwanese [29]. No significant association of RETN gene polymorphism of SNP 3745368 with T2D risk was found in German [30] and Han Chinese population [17]. A meta-analysis study in Mexican population for RETN gene polymorphism of SNP rs3745368 and rs1862513 was not associated with a risk of developing obesity and T2D [18]. However, RETN gene polymorphism at position rs3745368 probably increased the susceptibility to T2D in Thais [20].

Omission of a single study did not have significant change in observed pooled effect estimates for RETN gene polymorphism of SNP rs3745367 or SNP rs3745368 thereby confirming the authenticity and reliability of conducted meta-analysis. Even no significant publication bias was observed in the present study, and all the available relevant research papers were included in this study.

With available reports, the current meta-analysis found a significant association between RETN gene polymorphism at +299 G>A and T2D risk.

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Compliance with ethical standards

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

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ORIGINAL ARTICLE

A genetic approach to examine the relationship between vitamin B_{12} status and metabolic traits in a South Asian population

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Abstract

Background Observational studies in South Asian populations have suggested an association between vitamin B_{12} status and metabolic traits; however, the findings have been inconclusive. Hence, the aim of the present study was to use a genetic approach to explore the relationship between metabolic traits and vitamin B_{12} status in a Sri Lankan population and to investigate whether these relationships were modified by dietary intake.

Methods A total of 109 Sinhalese adults (61 men and 48 women aged 25–50 years) from Colombo City underwent anthropometric and biochemical measurements, dietary intake analysis, and genetic tests. Genetic risk scores (GRS) based on 10 metabolic single nucleotide polymorphisms (SNPs) (metabolic-GRS) and 10 vitamin B_{12} SNPs (B12-GRS) were constructed.

Results The B12-GRS was significantly associated with serum vitamin B_{12} ($p = 0.008$) but not with metabolic traits ($p > 0.05$), whereas the metabolic-GRS had no effect on metabolic traits ($p > 0.05$) and vitamin B_{12} concentrations ($p > 0.05$). An interaction was observed between B12-GRS and protein energy intake (%) on waist circumference ($p = 0.002$). Interactions were also seen between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio ($p = 0.015$).

Conclusion Our findings suggest that a genetically lowered vitamin B_{12} concentration may have an impact on central obesity in the presence of a dietary influence; however, our study failed to provide evidence for an impact of metabolic-GRS on lowering B_{12} concentrations. Given that our study has a small sample size, further large studies are required to confirm our findings.

Keywords SNP · Body mass index · Obesity · Metabolic traits · Vitamin B_{12} pathway · Sinhalese · Sri Lanka · Nutrigenetics

Abbreviations

SNPs	Single nucleotide polymorphisms
MTHFR	Methylenetetrahydrofolate reductase
CPS1	Carbamoyl-phosphate synthase 1
CUBN	Cubulin
CD320	CD320 molecule

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TCN2	Transcobalamin 2
CLYBL	Citrate lyase beta like
FUT2	Fucosyltransferase 2
TCN1	Transcobalamin 1
FUT6	Fucosyltransferase 6
MUT	Methylmalonyl-CoA mutase
CAP10	Calpain 10
KCNJ11	Potassium voltage-gated channel subfamily J member 11
TCF7L2	Transcription factor 7-like 2
FTO	Fat mass and obesity-associated
MC4R	Melanocortin 4 receptor
BMI	Body mass index
SD	Standard deviations
WC	Waist circumference
WHR	Waist-to-hip ratio

Introduction

In recent years, the incidence of obesity in Sri Lanka has increased markedly [1]. The prevalence of being overweight or obese in Sri Lankan adults is 34.4% (25.2% and 9.2% in 2005 and 2006, respectively), with an upward trend being observed [1, 2]. Obesity increases the risk for certain health conditions, such as insulin resistance, diabetes mellitus, and hypertension [3]. South Asians have been observed to exhibit increased visceral fat and waist circumference (WC), hyperinsulinemia, and insulin resistance; this has been termed the "South Asian phenotype" [4]. Despite a known genetic contribution, the increase in obesity has been largely associated with changes in lifestyle habits [5, 6]. It is imperative that modifiable risk factors for obesity and associated metabolic problems are identified, especially if they can be easily addressed.

Vitamin B_{12} is a micronutrient that has been identified as a modifiable risk factor associated with the progression of metabolic disorders. In humans, vitamin B_{12} acts as an essential coenzyme involved in DNA synthesis and cellular energy production [7]. Subclinical deficiency of vitamin B_{12} has been linked to higher levels of homocysteine; this may have important consequences in the progression of chronic diseases, by inducing oxidative stress and inflammation [8]. Vitamin B_{12} deficiency has also been linked to many other complications including an increased risk of obesity [9–11], diabetes [12–14], and cardiovascular disease [15]. Currently, one study has investigated the effect of genetically instrumented vitamin B_{12} concentrations on body mass index (BMI) in individuals with European ancestry; however, there were no associations between the vitamin B_{12} genetic risk score (GRS) and BMI [16].

Genetic studies have implicated several gene loci in the predisposition to vitamin B_{12} deficiency, but no study has yet been carried out in the Sri Lankan population [17]. The mechanisms by which obesity and its comorbidities are related to vitamin B_{12} deficiency are poorly understood. Hence, we conducted a gene-based approach to explore the relationship between metabolic traits and vitamin B_{12} status in a Sinhalese cohort and investigated whether these relationships were modified by dietary intake in the Genetics Of Obesity and Diabetes (GOOD) study.

Study participants

The GOOD study is a cross-sectional study that was conducted in the city of Colombo, Sri Lanka, between April and August 2017. Healthy adults between the ages of 25 and 50 years were enrolled into the study. Exclusion criteria were having a previous history of type 2 diabetes, cardiovascular disease, or hypertension, having a BMI of more than 40 kg/m^2 or being classed morbidly obese by a physician, being blood related to other participants in the study, having any communicable disease, being pregnant or lactating, taking dietary or vitamin supplements, and taking medications that affect lipid metabolism or hypertension (Fig. 1).

Anthropometric measures

Body weight was measured to the nearest 100 g using an electronic scale (Seca 815, Seca GmbH. Co. kg, Germany) and height was measured to the nearest millimeter using a stadiometer (Seca 217, Seca GmbH. Co. kg, Germany). The BMI calculation was based on the body weight (kg) divided by the square of body height (m). Waist circumference and hip circumference were measured using a metal tape (Lufkin W606PM®, Parsippany, NJ, USA). Body fat percentage was estimated using a handheld bioelectrical impedance analysis technique (Omron Body Fat Monitor BF306, Omron, Milton Keynes, UK).

Biochemical analysis

Blood samples (10 ml) were collected by a trained phlebotomist in the morning, after a 12-h overnight fast. Fasting serum insulin and vitamin B_{12} levels were determined using the chemiluminescent microparticle immunoassay method on an Architect i1000 analyzer (Abbott Laboratories, IL, USA). Fasting plasma glucose concentrations were measured using the glucose hexokinase method using the Beckman Coulter AU5800 analyzer (Beckman Coulter®, California, USA). Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography using the BioRad D10 HPLC analyzer (BioRad, Hercules, CA, USA).

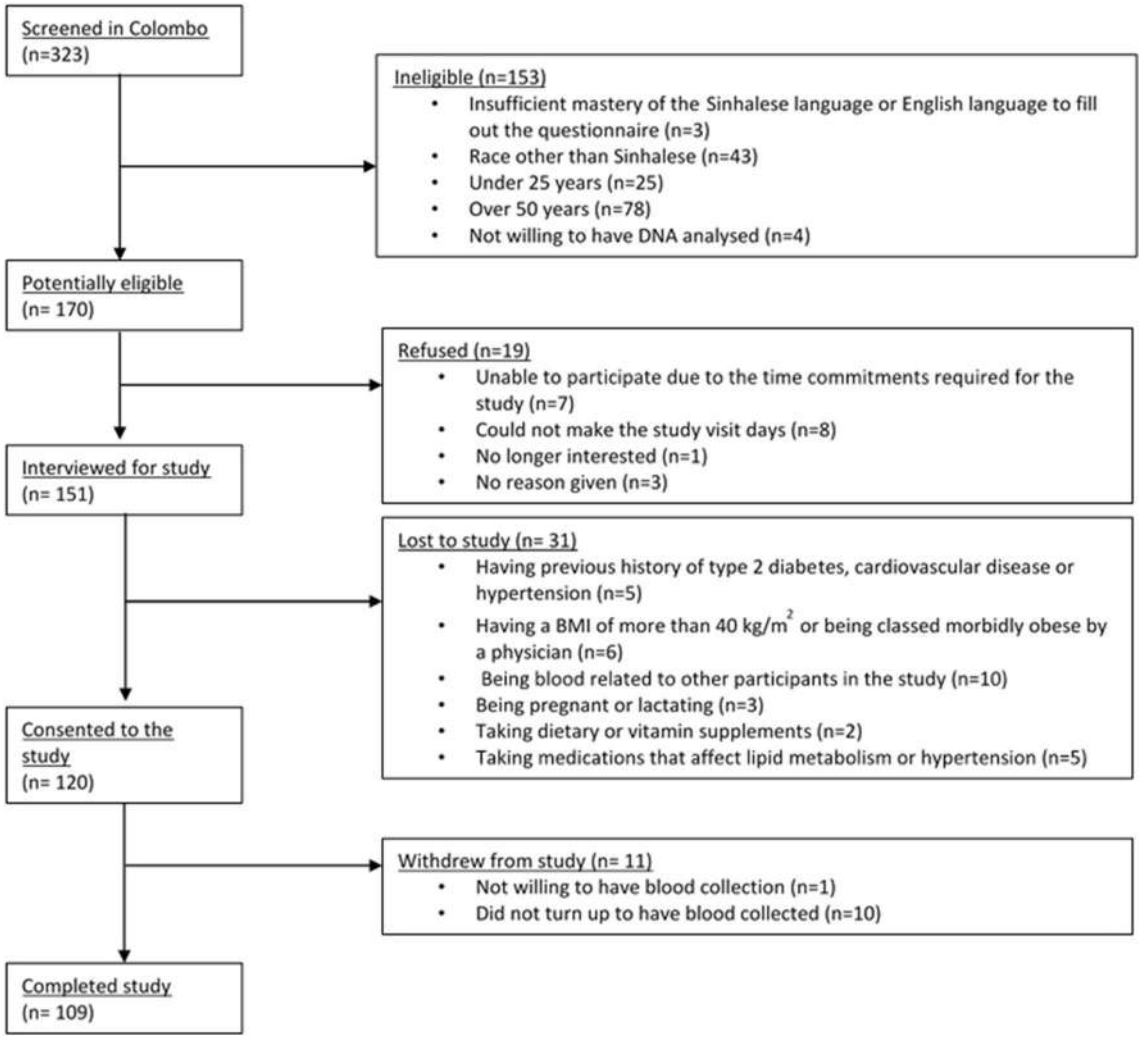

Fig. 1 Flow chart of the subject recruitment process

Dietary intake analysis

Dietary intakes were assessed using a previously validated and published [18] interviewer-administered food frequency questionnaire (FFQ) containing 85 food items. In brief, participants were asked to estimate the usual frequency (number of times per day, week, or month/never) and the portion sizes of various food items. The recorded data was analyzed with the NutriSurvey 2007 database (EBISpro, Germany) to estimate energy as well as macro- and micronutrient consumption [19].

"The Global Physical Activity Questionnaire" (GPAQ), developed by the World Health Organization (WHO), was used to measure physical activity [20]. Individuals were classified as vigorously active, when they both exercised and engaged in demanding work activities, and moderately active, when the participants either exercised or carried out heavy physical work. The remaining study participants were classified into the sedentary group.

SNP selection and genotyping

We selected 10 metabolic disease-related single nucleotide polymorphisms (SNPs) (associated with obesity and diabetes) (fat mass and obesity-associated [*FTO*], rs9939609 and rs8050136; melanocortin 4 receptor [*MC4R*], rs17782313 and rs2229616; transcription factor 7-like 2 [*TCF7L2*], rs12255372 and rs7903146; potassium voltage-gated channel subfamily J member 11 [*KCNJ11*], rs5219; calpain 10 [*CAPN10*], rs3792267, rs2975760, and rs5030952) for our analysis based on previously published candidate gene association and genome-wide association (GWA) studies for metabolic disease-related traits [21–29].

The 10 vitamin B_{12}-related SNPs (methylenetetrahydrofolate reductase [*MTHFR*], rs1801133; carbamoyl-phosphate

synthase 1 [*CPS1*], rs1047891; cubulin [*CUBN*], rs1801222; CD320 molecule [*CD320*], rs2336573; transcobalamin 2 [*TCN2*], rs1131603; citrate lyase beta like [*CLYBL*], rs41281112; fucosyltransferase 2 [*FUT2*], rs602662; transcobalamin 1 [*TCN1*], rs34324219; fucosyltransferase 6 [*FUT6*], rs778805 and methylmalonyl-CoA mutase [*MUT*], rs1141321) were chosen on the basis of the recent review article by Surendran et al. [17].

Blood samples for the measurement of DNA were transported in dry ice to the UK. Genomic DNA was extracted from a 5-ml whole blood sample from each participant and genotyping was performed at LGC Genomics (http://www.lgcgroup.com/services/genotyping), which employs the competitive allele-specific PCR-KASP® assay.

The Hardy-Weinberg equilibrium (HWE) *p* values were computed for the following 20 SNPs. The SNP *FUT2* rs602662 and calpain 10 (*CAP10*) rs3792267 deviated from the HWE; however, these SNPs were not excluded from analysis. The *FUT2* SNP rs602662 previously departed from HWE in a GWA study conducted in India; the authors ruled out that the deviation was not due to a genotyping error and still used this SNP for analysis in their study [30]. In addition, the KASP™ genotyping technology used in our study has been independently assessed to be over 99.8% accurate. Validation of the KASP™ genotyping was conducted at LGC genomics, where the genotyping results were assessed by two project managers separately to confirm that the data was accurate, and this ruled out genotyping artifacts as possible reasons for deviation from HWE. The reasons for deviation from HWE could be due to population or racial grouping substructure (subgrouping), non-random mating, linkage disequilibrium (incomplete mixing of different ancestral population), or chance findings [31].

Statistical analysis

The SPSS statistical package (version 22; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Allele frequencies were estimated by gene counting (Table 1). The normality of variable distribution was verified by the Shapiro-Wilk test, and data not normally distributed were log transformed prior to analysis. We performed an independent *t* test to compare the means of the quantitative variables between men and women. Comparison of the means between the two groups was analyzed by the chi-square test for categorical outcomes.

A schematic representation of the study design is presented in Fig. 2. The unweighted, risk allele GRS method was calculated for each participant as the sum of risk allele counts across each SNP which predicted vitamin B_{12} status or metabolic disease risk. The B12-GRS was generated from the SNPs in

Table 1 Genotype distribution of vitamin B_{12}-related SNPs and metabolic disease-related SNPs

Gene	rs number	Major allele	Minor allele	Common Homozygotes (%)	Heterozygotes (%)	Rare Homozygotes (%)	Minor allele frequency	HWE *p* value
MTHFR	rs1801133	C	T	89 (81.7)	19 (17.4)	1 (0.9)	0.100	0.990
CPS1	rs1047891	C	A	56 (51.9)	44 (40.7)	8 (7.4)	0.278	0.873
CUBN	rs1801222	C	T	78 (72.2)	29 (26.9)	1 (0.9)	0.144	0.338
CD320	rs2336573	C	T	99 (90.8)	10 (9.2)	0 (0)	0.046	0.616
TCN2	rs1131603	T	C	107 (98.2)	2 (1.8)	0 (0)	0.009	0.923
CLYBL	rs41281112	C	T	105 (96.3)	4 (3.7)	0 (0)	0.018	0.845
FUT2	rs602662	G	A	60 (55.6)	30 (27.8)	18 (16.7)	0.306	**0.000**
TCN1	rs34324219	C	A	107 (98.2)	2 (1.8)	0 (0)	0.009	0.923
FUT6	rs778805	C	T	29 (26.6)	53 (48.6)	27 (24.8)	0.491	0.776
MUT	rs1141321	G	A	28 (25.7)	60 (55.0)	21 (19.3)	0.470	0.271
CAPN10	rs3792267	G	A	79 (72.5)	24 (22.0)	6 (5.5)	0.165	**0.035**
CAPN10	rs2975760	T	C	66 (60.6)	38 (34.9)	5 (4.6)	0.220	0.874
CAPN10	rs5030952	C	T	101 (92.7)	8 (7.3)	0 (0)	0.037	0.691
KCNJ11	rs5219	C	T	49 (45.0)	45 (41.3)	15 (13.8)	0.344	0.373
TCF7L2	rs12255372	G	T	57 (52.3)	45 (41.3)	7 (6.4)	0.271	0.633
TCF7L2	rs7903146	C	T	45 (41.3)	54 (49.5)	10 (9.2)	0.340	0.274
FTO	rs9939609	T	A	48 (44.0)	47 (43.1)	14 (12.8)	0.344	0.641
MCR	rs17782313	T	C	48 (44.0)	50 (45.9)	11 (10.1)	0.330	0.700
FTO	rs8050136	C	A	48 (44.0)	47 (43.1)	14 (12.8)	0.340	0.641
MC4R	rs2229616	G	A	99 (91.7)	9 (8.3)	0 (0)	0.042	0.651

MAF minor allele frequency, *HWE* Hardy-Weinberg equilibrium, χ^2 chi-squared value

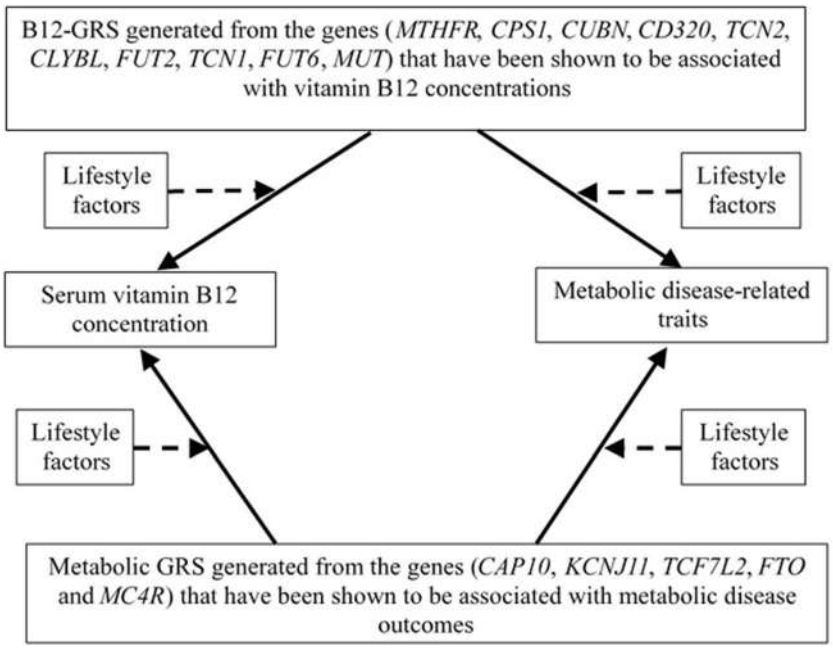

Fig. 2 Diagram representing the study design. The diagram shows four possible associations and four possible interactions. One-sided arrows with unbroken lines represent genetic associations and one-sided arrows with broken lines represent interactions between a lifestyle factor and GRS on serum vitamin B_{12}/metabolic traits. We tested the association between the metabolic-GRS and vitamin B_{12} concentrations and metabolic disease-related traits. We then tested the associations between the B12-GRS and vitamin B_{12} status and metabolic disease-related traits. Lastly, we tested whether these genetic associations were modified by lifestyle factors (macronutrient intake and physical activity levels)

the genes *MTHFR*, *CPS1*, *CUBN*, *CD320*, *TCN2*, *CLYBL*, *FUT2*, *TCN1*, *FUT6*, and *MUT*, which have been shown to be associated with vitamin B_{12} concentrations. Furthermore, another unweighted GRS was created using allele markers previously reported to be associated with metabolic disease traits. The metabolic-GRS was generated from the SNPs in the genes *CAP10*, *KCNJ11*, *TCF7L2*, *FTO*, and *MC4R*. A value of 0.1 or 2 was assigned to each SNP, which denotes the number of risk alleles on that SNP. These values were then calculated by adding the number of risk alleles across each SNP. The average number of risk alleles per person for the B12-GRS was 8.69 (SD = 1.70), which ranged from 5 to 15. The sample was stratified, by the median, into a "low genetic risk group," for those with a GRS ≤ 9 risk alleles ($n = 79$), and into a "high genetic risk group," for those with a GRS ≥ 10 risk alleles ($n = 30$). For the metabolic-GRS, the average number of risk alleles per person was 7.00 (SD = 2.28), which ranged from 1 to 13. The sample was stratified, into a "low genetic risk group," for those with a GRS ≤ 8 risk alleles ($n = 88$), and into a "high genetic risk group," for those with a GRS ≥ 9 risk alleles ($n = 21$). Linear regression was used to examine the association of the two GRS scores with the biochemical and anthropometric outcomes (glucose, insulin, HbAC1, vitamin B_{12}, body fat %, BMI, WC, and waist-to-hip ratio (WHR)). The interaction between the two GRS scores and dietary factors on biochemical and anthropometric outcomes was determined by including interaction terms (GRS × diet) in the regression model. Models were adjusted for age, sex, BMI, and total energy intake, wherever appropriate.

Correction for multiple testing was applied using Bonferroni correction [adjustment p value for association analysis was < 0.00313 [2 GRS × 8 biochemical and anthropometric outcomes (Fasting blood glucose, fasting insulin, glycated hemoglobin, vitamin B_{12}, fat %, BMI, WC, and WHR) = 16 test)] and for interaction < 0.00078 [2 GRS × 8 biochemical and anthropometric × 4 lifestyle factors (dietary carbohydrate energy %, dietary protein energy %, dietary fat energy %, and physical activity levels)) = 64]. Given that there are no studies on GRS and no previously reported effect sizes for the South Asians, we were unable to perform a power calculation.

Results

Characteristics of the participants

In this study, 109 participants (mean age, 38.34 ± 6.92 years; BMI, 24.58 ± 4.12 kg/m^2) were included. Table 2 illustrates the main characteristics of the study participants stratified according to sex. No significant difference between men and women was observed in the levels of fasting glucose, insulin, HbAC1, and plasma vitamin B_{12} ($p > 0.05$).

Table 2 Anthropometric and biochemical characteristics of men and women participants ($n = 109$; men 61, women 48)

	Total (n = 109) Mean ± SD	Men ($n = 61$) Mean ± SD	Women ($n = 48$) Mean ± SD	p value*
Age (years)	38.24 ± 6.92	37.34 ± 6.97	39.38 ± 6.77	0.129
Height (cm)	164.97 ± 9.15	170.95 ± 6.18	157.36 ± 6.16	< 0.0001
Weight (kg)	67.07 ± 13.05	71.76 ± 11.81	61.11 ± 12.17	< 0.0001
BMI (kg/m^2)	24.58 ± 4.12	24.51 ± 3.52	24.68 ± 4.80	0.844
Waist circumference (cm)	83.73 ± 17.97	89.83 ± 14.04	75.99 ± 19.52	< 0.0001
Hip circumference (cm)	91.16 ± 17.78	92.27 ± 13.83	89.75 ± 21.87	0.488
WHR	0.92 ± 0.11	0.98 ± 0.08	0.85 ± 0.11	< 0.0001
Fat (%)	27.25 ± 7.37	23.52 ± 5.12	32.00 ± 7.08	< 0.0001
Obesity cases[a]	40.37%	37.70%	43.75%	0.523
Fasting blood glucose (mg/dL)	85.64 ± 12.64	87.41 ± 15.41	83.40 ± 7.40	0.100
Fasting blood insulin (pmol/L)	68.55 ± 49.97	71.77 ± 59.12	64.46 ± 35.28	0.451
Fasting blood HbA1C (mmol/mol)	35.62 ± 5.91	35.20 ± 5.99	36.16 ± 5.84	0.402
Fasting blood B12 (pmol/L)	380.65 ± 132.83	389.80 ± 135.00	369.02 ± 130.52	0.420
Physical activity levels (low %/moderate%/high%)	72.5/19.3/8.3	70.5/19.7/9.8	75.0/18.8/6.3	0.777
Total energy (kcal/day)	2097.92 ± 456.01	2173.68 ± 427.82	2001.65 ± 476.72	0.050
Protein (energy %)	11.29 ± 2.31	11.25 ± 2.41	11.33 ± 2.20	0.853
Fat (energy %)	21.87 ± 5.31	21.64 ± 5.22	22.16 ± 5.45	0.613
Carbohydrate (energy %)	69.62 ± 8.80	69.89 ± 10.29	69.28 ± 6.52	0.721
Dietary fiber (g)	16.78 ± 8.18	17.24 ± 8.46	16.20 ± 7.85	0.513
Polyunsaturated fatty acids (g)	3.32 ± 1.69	3.36 ± 1.66	3.27 ± 1.75	0.779

Data presented as mean ± SD

BMI body mass index, *SD* standard deviations, *WHR* waist-to-hip ratio

*$p < 0.05$, statistically significant differences in mean values between men/women, unadjusted

[b] Obesity cases refer to the percentage of individuals with a BMI of over 25

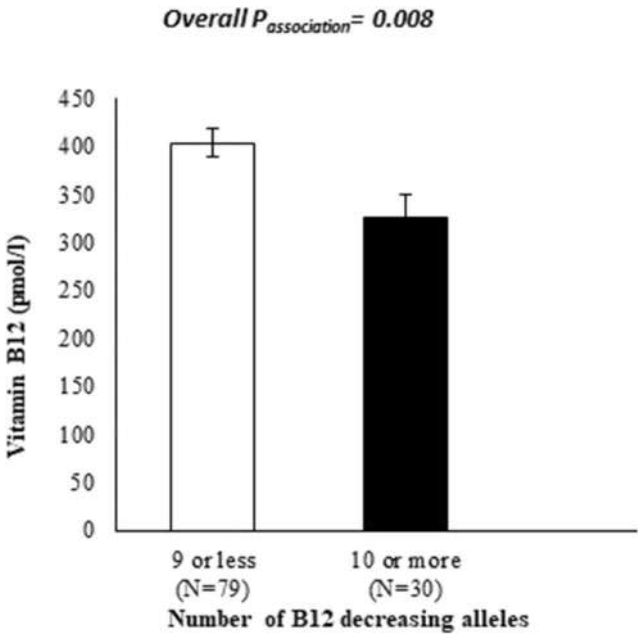

Fig. 3 Association between the B12-GRS and serum vitamin B_{12} levels. Vitamin B_{12} decreasing alleles ranged from 5 to 15. Individuals with ≤ 9 or ≥ 10 alleles were grouped to obtain a reasonable number of individuals in each group

Association between B12-GRS and obesity GRS with biochemical and anthropometric measurements

A significant association between B12-GRS and serum vitamin B_{12} was observed ($p = 0.008$) (Supplementary Table 1 and Fig. 3). However, this finding was not significant after correction for multiple testing. No associations between the B12-GRS and metabolic traits ($p > 0.05$) were observed (Supplementary Table 1). Furthermore, no associations between the metabolic-GRS and vitamin B_{12} or metabolic traits ($p > 0.05$) were observed (Supplementary Table 2).

Interaction between the B12-GRS and dietary factors on biochemical and anthropometric measurements

An interaction was found between the B12-GRS and protein energy (%) on log transformed WC ($p = 0.002$). However, further stratification of participants based on their consumption of low, medium, and high dietary protein (energy %) did not show statistically significant associations between the GRS and the outcome in any of the tertiles, which could account for the small sample size (Supplementary Table 3).

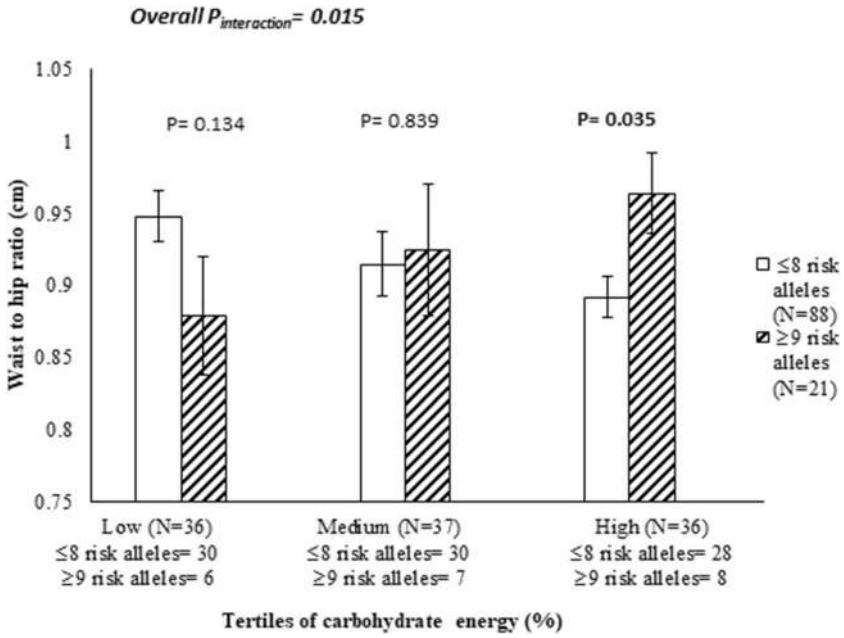

Fig. 4 Interaction between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio (cm) ($P_{\text{interaction}} = 0.015$). Among those who consumed a high carbohydrate diet, individuals who carried nine or more risk alleles had significantly higher levels of waist-to-hip ratios compared to individuals carrying eight or less risk alleles ($p = 0.035$)

Interaction between the metabolic-GRS and dietary factors on biochemical and anthropometric measurements

We observed a significant interaction between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio ($P_{\text{interaction}} = 0.015$) (Fig. 4 and Table 3). Individuals who carried eight or less risk alleles for metabolic disease had 7.47% lower WHR measurements (cm) in the highest tertile of carbohydrate energy intake (%) (mean ± SD = 78.00 ± 7.90%) compared to those with nine or more risk alleles ($p = 0.035$) (Table 3).

Interactions were also seen between the metabolic-GRS and carbohydrate energy (%) on log fasting insulin concentrations ($p = 0.011$) and log WC ($p = 0.031$) and the metabolic-GRS and protein energy (%) on log fasting insulin levels ($p = 0.032$) and log WC ($p = 0.011$) (Table 3 and Supplementary Table 3).

Interaction between the B12-GRS and physical activity on biochemical and anthropometric measurements

No statistically significant interactions were observed between the two GRSs (vitamin B_{12} and metabolic) and physical activity on biochemical and anthropometric measurements (Table 3 and Supplementary Table 3). After correction for multiple testing, none of these gene-diet and gene-physical activity interactions remained statistically significant.

Discussion

To our knowledge, this is the first study to use a genetic approach to explore the relationship between metabolic traits and vitamin B_{12} status in a South Asian population. Our study confirmed the strength of the association between B12-GRS and B_{12} concentrations and demonstrated the impact of genetically instrumented B_{12} concentrations on waist circumference, an indicator of central obesity, through the influence of dietary protein intake. Furthermore, our study has also showed a significant effect of metabolic-GRS on waist-to-hip ratio through the influence of high carbohydrate intake. Given that the total daily intake of protein is low and carbohydrate is high in Sri Lankan adults [32], our findings, if replicated in future studies, might carry significant public health implications in terms of revising the food-based dietary guidelines which could prevent central obesity and the associated CVD-related outcomes.

In this study, we constructed a GRS consisting of ten vitamin B_{12} decreasing SNPs in genes involved in vitamin B_{12} metabolism [17]. The B12-GRS was associated with vitamin B_{12} levels, suggesting that it would be an ideal instrument for vitamin B_{12} status. Given the lack of association between the B12-GRS and metabolic disease traits in our study, we were unable to provide evidence for linear decreases in vitamin B_{12} concentrations having substantive effects on metabolic disease traits. However, we found a significant interaction between the B12-GRS and protein energy (%) on log WC. Interestingly, individuals who carried nine or less alleles had lower WC when consuming a high protein diet compared to those consuming a low protein diet. Although no statistically significant differences in WC were observed between the

Table 3 Interaction between the B12-GRS and lifestyle factors on anthropometric measurements

Interaction between the GRS and lifestyle factors on log waist circumference (cm)			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between B12-GRS and physical activity levels
0.002 ± 0.004 (0.727)	0.037 ± 0.011 **(0.002)**	− 0.003 ± 0.003 (0.344)	− 0.051 + 0.037 (0.173)
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.007 ± 0.006 (0.212)	− 0.024 ± 0.009 **(0.011)**	0.007 ± 0.003 **(0.031)**	0.020 ± 0.044 (0.654)
Interaction between the GRS and dietary factors on waist-to-hip ratio			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between-B12 GRS and physical activity levels
0.002 ± 0.004 (0.660)	0.013 ± 0.010 (0.196)	− 0.003 ± 0.002 (0.241)	0.018 ± 0.032 (0.584)
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.009 ± 0.005 (0.079)	− 0.012 ± 0.008 (0.158)	0.007 ± 0.003 **(0.015)**	0.038 ± 0.039 (0.323)
Interaction between the GRS and lifestyle factors on log BMI			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between B12-GRS and physical activity levels
− 0.002 ± 0.003 (0.539[a])	0.009 ± 0.008 (0.259[a])	− 0.001 ± 0.002 (0.762[a])	0.015 ± 0.023 (0.513[a])
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.004 ± 0.004 (0.245[a])	− 0.004 ± 0.006 (0.480[a])	0.002 ± 0.002 (0.322[a])	− 0.005 ± 0.028 (0.851[a])

Values are beta coefficients ± standard errors. *P* values are inserted in brackets. *P* values were obtained by using a general linear model adjusted for age, sex and BMI

Values in bold represent statistical significance ($p < 0.05$)

[a] *P* values were obtained by using a general linear model adjusted for age and sex

alleles of the B12-GRS, the impact of the B12-GRS on WC was observed only under the influence of a high protein diet. Further investigations are required to confirm this finding to determine the clinical significance and potential applications as part of weight management interventions.

At present, carbohydrates constitute the majority of the energy intake among South Asian countries such as Sri Lanka (~ 71.2%) [32]; in contrast, the consumption of carbohydrates is lower in Western countries (~ 45%) [33]. Furthermore, high carbohydrate intake has been associated with an increased risk of diabetes in a South Indian population [34] and an increase in WC among premenopausal (20–45 years) Sri Lankan women [35]. In the present study, we found a significant interaction between the metabolic-GRS and carbohydrate energy percentage on waist-to-hip ratio, where the individuals carrying more than nine risk alleles had a higher waist-to-hip ratio among those in the highest tertile of carbohydrate energy percentage. There are no previous reports of the risk variants used in our GRS, but Goni et al. [36] found that carbohydrates (total and complex) interacted with a GRS of 16 obesity/lipid metabolism polymorphisms to modify the effect on body fat mass in 711 individuals of Caucasian ancestry. In our study, we only observed interactions of the metabolic-GRS on WC and waist-to-hip ratio, which suggests that effects are likely to be on central obesity as opposed to common obesity.

South Asians have a higher risk of developing obesity-related non-communicable diseases relative to white Caucasians despite lower BMI levels; this has been termed the "South Asian phenotype." The distinctive features of this phenotype include a higher WC, abdominal adiposity combined with insulin resistance, and a greater predisposition to diabetes [4]. The role of vitamin B_{12} in promoting this adverse phenotype has been suggested by Yajnik et al., who demonstrated that offspring born to mothers with a low vitamin B_{12} and high folate status had a greater risk of developing insulin resistance during childhood [12]. According to Yajnik et al., vitamin B_{12} deficiency prevents the generation of tetrahydrofolate from 5-methyltetrahydrofolate in the one-carbon metabolism cycle; as a result, homocysteine levels accumulate leading to altered lean tissue deposition and reduced protein synthesis [12]. Furthermore, vitamin B_{12} is involved in the conversion of methylmalonyl-CoA to succinyl-CoA by the enzyme methylmalonyl-CoA mutase (adenosyl-B12 as a cofactor). Subsequently, vitamin B_{12} deficiency results in elevated methylmalonyl-CoA, inhibiting the mitochondrial enzyme carnitine palmitoyltransferase, which may promote lipogenesis and insulin resistance [12, 37].

No studies to date have investigated interactions between the two GRSs and physical activity on metabolic traits and B_{12} concentrations in Asian Sri Lankans. Although 60% of Sri Lankan adults are reported to be highly physically active [38], no significant interactions were found between the two GRSs and physical activity on metabolic traits, which could be due to a small sample size and measurement bias associated with self-reported physical activity questionnaire. The strengths of our study include the use of a validated food frequency questionnaire [18] to measure macronutrient intake, the comprehensive measurements of lifestyle factors, and the use of GRSs which increased the statistical power of our study [39]. Nevertheless, some limitations need to be acknowledged. The first limitation concerns the relatively small sample size of the study; however, we were still able to identify significant gene-diet interactions. Furthermore, we used Bonferroni correction to correct for multiple testing and this can often lead to larger power, specifically where studies have a small sample size and a small number of disease-associated markers. This is also true for when studies have a large allele frequency difference due to a small sample size [40]. Secondly, information about the type of oil used for frying, the estimation of different dietary fat components (monounsaturated or saturated fatty acids), and vitamin B_{12} intake was not collected. This could have limited our in-depth analysis of interactions of specific macronutrients and vitamins with the two GRSs. Furthermore, the study was limited to Sinhalese adults in Colombo, and the conclusions may not be applicable to other ethnic groups in Sri Lanka. Finally, none of the genetic associations or gene-lifestyle interactions were statistically significant after correction for multiple testing; however, given that this is the first study using a genetic approach to establish a relationship between vitamin B_{12} status and metabolic disease outcomes in South Asians, we have taken into consideration the significant findings; hence, further large studies are required to replicate our findings.

In summary, our study suggests that a genetically lowered vitamin B_{12} concentration may have an impact on central obesity in the presence of a dietary influence; however, our study failed to show an impact of the metabolic-GRS on lowering B_{12} concentrations through a dietary influence. Our study also showed a significant effect of the metabolic-GRS on waist-to-hip ratio, another indicator of central obesity, through the influence of a high carbohydrate intake. However, after correction for multiple testing, none of these findings were statistically significant. Hence, further replication studies are highly warranted on large samples to confirm or refute our findings.

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Author contributions SS and KSV drafted the manuscript; SS performed the statistical analysis; and SS, VKS, DJA, and RL were responsible for the study conception. KW provided guidance to the research; SS and RL conducted data and sample collection; SS, RJ, and SA were involved in the dietary data analysis; SS and SaS were involved in the physical activity data analysis; KSV designed the gene-diet interaction study; and SS, VKS, JAL, and RJ critically reviewed the manuscript. All authors contributed to and approved the final version of the manuscript.

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Data availability Data from this project will not be shared because additional results from the study are yet to be published.

Compliance with ethical standards

Ethics approval and consent to participate This study was approved by the Ethical Review Committee of the University of Colombo (EC-17-107) and the University of Reading Research Ethics Committee (17/25). All participants signed informed consent prior to their participation.

Consent for publication Not applicable.

Competing interests All authors declare that there is no conflict of interest associated with their contribution to this manuscript.

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ORIGINAL ARTICLE

The Efficacy and Safety of an Insulin Infusion Protocol in a Medical Intensive Care Unit at a Tertiary Care Hospital: a Prospective Study

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Abstract

Aims To evaluate the efficacy and safety of a modified insulin infusion protocol, targeting a blood glucose level of 140–180 mg/dL, in a medical intensive care unit at a tertiary care university hospital.

Methods We compared glycemic control parameters before and after using the KCMH insulin infusion protocol in a MICU (pre- and post-protocol groups). In the pre-protocol group, hyperglycemia was managed by conventional care. In the post-protocol group, hyperglycemia was managed according to the KCMH protocol. Study outcomes were a proportion of patients achieving a glycemic target of 140–180 mg/dL within 8 h after initiation of an insulin infusion, various glycemic control parameters, and clinical outcomes.

Result Twenty-eight patients were in the pre-protocol group, and 27 were in the post-protocol group. The proportion of patients achieving a glycemic target within 8 h after initiation of an insulin infusion was significantly higher in the post-protocol group compared to the pre-protocol group (70.4% vs. 21.4%, $p < 0.001$). Patients in the post-protocol group achieved a blood glucose target faster than the pre-protocol group (7.4 ± 4.1 h vs. 12.5 ± 7.5 h, $p = 0.004$). Mean blood glucose levels during insulin infusion were significantly lower in the post-protocol group compared to the pre-protocol group (170.9 ± 15.3 mg/dL vs. 205.6 ± 46.7 mg/dL, $p = 0.001$). The glycemic variability indices were also better in the post-protocol group.

Conclusion Implementation of a KCMH insulin infusion protocol in a medical ICU resulted in better glycemic control than conventional care without excess risk of hypoglycemia.

Trial registration The study was approved by the Institutional Review Board and Thai Clinical Trial Registry (clinicaltrials.in.th: TCTR20161105001).

Keywords Hyperglycemia · Critical care · Insulin infusion protocol

Abbreviations

HPA	Hypothalamic–pituitary–adrenal axis
MICU	Medical Intensive Care Unit
KCMH	King Chulalongkorn Memorial Hospital
APACHE II	Acute Physiology and Chronic Health Evaluation II
SOFA	Sequential Organ Failure Assessment
ARDS	Acute respiratory distress syndrome
TPN	Total parenteral nutrition
POCT	Point of Care Glucose Test

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Introduction

Hyperglycemia is common in critically ill patients. The incidence is 32–46% among patients admitted to an intensive care unit [1, 2]. Hyperglycemia during critical illness occurs as a result of an increase in hepatic glucose production, augmented lipolysis, and enhanced insulin resistance from an activation of the hypothalamic–pituitary–adrenal (HPA) axis, a sympatho-adrenal system and pro-inflammatory cytokines [3]. Severe hyperglycemia can cause many deleterious effects such as osmotic

diuresis and dehydration, endothelial dysfunction, and generation of free oxygen radicals which further cause tissue damage [4]. Stress hyperglycemia is known to be associated with an increased risk of morbidity and mortality [5–7]. Randomized trials and a meta-analysis have demonstrated that treatment of stress hyperglycemia with a glycemic target of 140–180 mg/dL is associated with lower mortality [8, 9].

Since critically ill patients are clinically diverse and highly complex, safe and effective strategies for optimal blood glucose control can be challenging. Conventional care using sliding scale insulin therapy or physician-based insulin dose adjustment may not be adequate for glycemic control in a critical care setting. A number of recent guidelines have therefore recommended in-hospital management of diabetes using an insulin protocol [10–13]. The purpose of this study was to evaluate the efficacy and safety of a modified insulin infusion protocol in a medical intensive care unit (ICU) in a tertiary care university hospital.

Materials and Methods

A modified insulin infusion protocol was implemented in two medical ICUs at King Chulalongkorn Memorial Hospital (KCMH), a 1300-bed tertiary care teaching hospital in Bangkok, Thailand, in August of 2016. Each medical ICU has eight beds with a nurse-to-patient ratio of 1:1. We compared clinical data of patients admitted to these medical ICUs before the insulin protocol was implemented from April to July of 2016 (a pre-protocol group) and after the insulin protocol was implemented from August to November of 2016 (a post-protocol group). In the pre-protocol group, hyperglycemia in the MICUs was managed by conventional care, i.e., insulin infusion was initiated and titrated according to physicians' judgment. In the post-protocol group, hyperglycemia in the medical ICUs was managed using the KCMH insulin infusion protocol. This protocol was adapted from a modified Yale insulin infusion protocol [14] to target a blood glucose level of 140–180 mg/dL to be in accordance with current guidelines [11–13, 15]. In addition, the insulin initiation rate was adjusted according to both body weight and initial glucose level. The detail of the KCMH insulin infusion protocol is included in supplement 3 (Example of KCMH insulin infusion protocol). Before implementation of the KCMH protocol, all medical ICU nursing staff and physicians were trained to be familiar with the use for 2 weeks.

Subjects

In both the pre-protocol and the post-protocol groups, we included patients age > 18 years who were admitted to the medical ICUs with capillary blood glucose level ≥ 180 mg/dL. To be included in the analysis, patients had to receive intravenous insulin infusion therapy for more than 24 h. Patients with type 1 diabetes, pancreatic diabetes mellitus, diabetic ketoacidosis, and hyperglycemic or hyperosmolar coma were excluded. In the post-protocol group, patients who had a protocol violation, defined as incorrect titration of an insulin infusion rate more than 20%, were also excluded from the analysis. A written informed consent was obtained by either the patient or a family member. The study was approved by the Institutional Review Board and Thai Clinical Trial Registry (clinicaltrials.in.th: TCTR20161105001).

Measurement

Capillary blood glucose levels were measured by glucose dehydrogenase PQQ modified by site-directed mutagenesis (Mutant Q-GHD) enzyme (Roche Accu-Chek Performa II). The diagnostic performance was within an acceptable range of ISO15197. An inter-assay variability between each strip lot ranged between 3.1–4.4% at blood glucose levels of 30–400 mg/dL.

Outcomes

A primary outcome was a proportion of patients who achieved a glycemic target of 140–180 mg/dL within 8 h after initiation of an intravenous insulin infusion. Any measured capillary plasma glucose value that fell between 140 and 180 mg/dL during the first 8 h was classified as "a target reached." Secondary outcomes were mean blood glucose levels during the entire period of insulin infusion, time to reach a glucose target, glycemic variability (measured by coefficient of variation [CV] and standard deviation [SD]), percentage of time in a glucose target range during the entire period of insulin infusion, incidence of hypoglycemia, insulin dose, frequency of glucose monitoring, and length of ICU stay.

Hypoglycemia was defined as plasma glucose level less than 70 mg/dL, and severe hypoglycemia was defined as plasma glucose level less than 40 mg/dL.

We also collected demographic data including age, gender, reason for ICU admission, comorbidities, and severity of the illness defined by the APACHE II score and the SOFA score. In this study, the severity scores were recorded as an initial score, a peak score, and a mean score. The scores were collected on a daily basis during the ICU admission, and the highest score recorded was used as a peak score. A mean score was an average of all the scores recorded. Glycemic control parameters including point-of-care testing (POCT) for glucose at insulin initiation and at various time points were also recorded.

Statistical analysis

From our pilot study, consisting of 12 patients, the proportion of patients who achieved a glycemic target within 8 h in the pre-protocol group was 42%. We estimated that use of the insulin protocol would result in a 2-fold increase in the proportion of patients achieving a glycemic target within 8 h. As a

result, a sample size of 25 in each group would yield at least 90% power to detect a difference in the primary outcome using a chi-square test with a 2-sided statistical significance level of 0.05.

Data are displayed as mean ± SD or percentage as appropriate. An independent sample *t* test and chi-square were used to compare between the pre- and post-protocol parameters. As the initial POCT values at insulin initiation in the pre-protocol group were higher than those in the post-protocol group which might affect the primary outcome, we used multiple regression analysis to adjust for covariates, including preexisting steroid use, total parenteral nutrition administration, POCT values at POCT initiation, and use of insulin protocol. Hazard ratio was calculated using Cox proportional hazards model. *p* values of less than 0.05 were considered statistically significant. All statistical analysis was conducted by SPSS version 22.

Results

During April 1 to November 30, 2016, a total of 526 patients admitted to the two medical ICUs were assessed for eligibility. Upon admission, only 147 patients had hyperglycemia defined as a capillary blood glucose value ≥ 180 mg/dL. Of those, 73 patients were in the pre-protocol group and 74 patients were in the post-protocol group. Twenty-eight patients in the pre-protocol group and 27 patients in the post-protocol group who had received intravenous insulin infusion for more than 24 h and did not have a protocol violation were included in the final analysis (Fig. 1). Clinical characteristics of both groups were mostly comparable except that the pre-protocol group had significantly higher pre-infusion blood glucose levels, and the post-protocol group had a higher prevalence of liver diseases (Table 1). Although the APACHE II score and the SOFA score were comparable between the two groups, both the peak APACHE II score and the peak SOFA score were significantly higher in the post-protocol group (Table 1).

For the primary outcome, 70.4% of patients in the post-protocol group achieved a glycemic target of 140–180 mg/dL within 8 h after insulin infusion compared to 21.4% in the pre-protocol group ($p < 0.001$, Table 2). Patients in the post-protocol group also achieved a blood glucose target faster than those in the pre-protocol group (Table 2). In the pre-protocol group, 24 patients (86%) achieved a glucose target of 140–180 mg/dL, whereas 4 patients (14%) never reached this goal, so we did the analysis of time to target in only 24 patients. During insulin infusion, the percentage of blood glucose values that stayed within the target range was significantly greater and the mean glucose levels during insulin infusion were also significantly lower in the post-protocol group compared to those in the pre-protocol group (Table 2 and Fig. 2).

In addition, patients in the post-protocol group had significantly lower glycemic variability as reflected by significantly lower SD and CV compared to those in the pre-protocol group (Table 2). The duration of insulin infusion was significantly

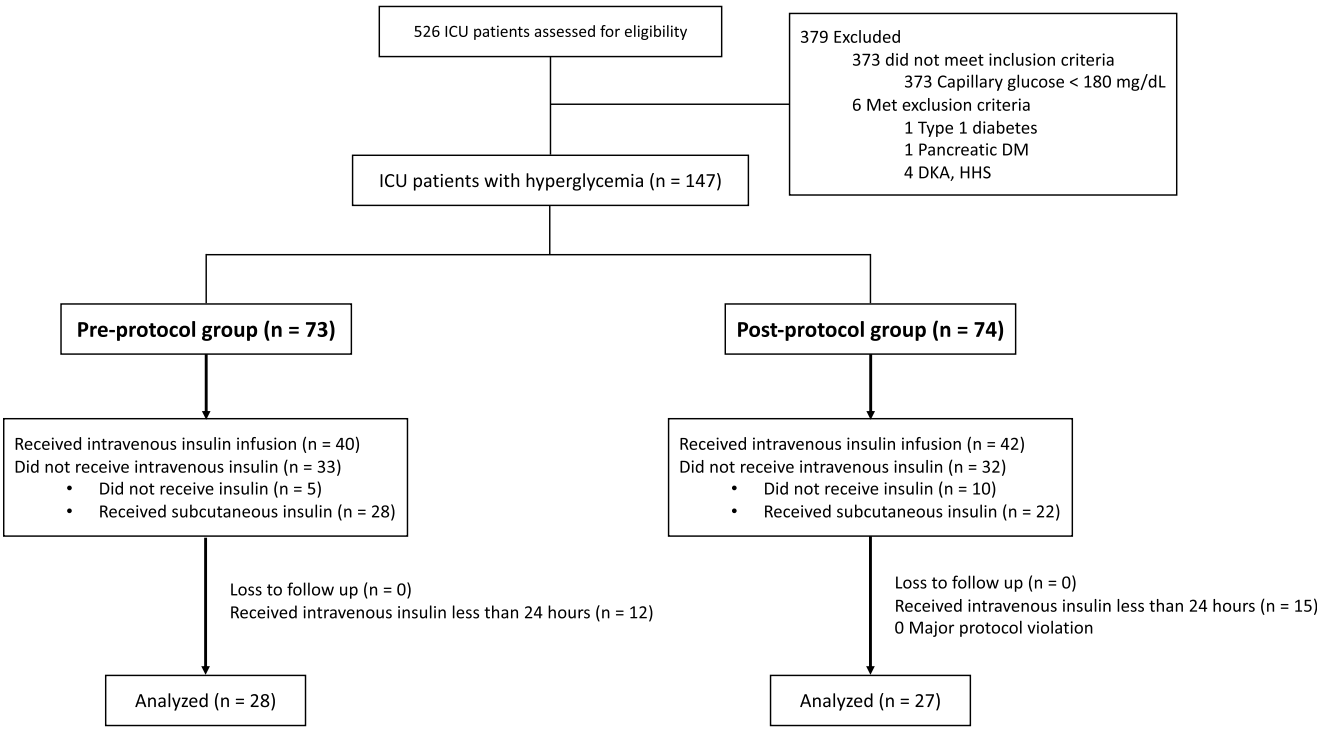

Fig. 1 Patient flow through the insulin protocol study

Table 1 Baseline characteristics

	Pre-protocol group ($n = 28$)	Post-protocol group ($n = 27$)	p value
Female—n (%)	13 (46)	17 (63)	0.22
Age—years[a]	64 ± 15	63 ± 17	0.29
Comorbidities—n (%)			
Diabetes mellitus	20 (71)	19 (70)	0.93
Hypertension	16 (57)	18 (67)	0.47
Dyslipidemia	16 (57)	16 (59)	0.87
Cancer	7 (25)	8 (30)	0.70
Acute or chronic kidney disease	10 (36)	12 (44)	0.51
Liver diseases	1 (4)	6 (21)	0.04
Reasons for ICU admission—n (%)			
Septic shock	11 (39)	9 (32)	0.65
Cardiac arrest	4 (14)	2 (7)	0.41
ARDS/respiratory failure	7 (25)	7 (26)	0.94
Treatment—n (%)			
Steroid administration	12 (43)	11 (41)	0.87
TPN	3 (11)	4 (15)	0.61
Severity of illness			
APACHE II[a]	21 ± 8	24 ± 8	0.32
Peak APACHE II	23 (19–28)	28 (24–34)	0.009*
SOFA[b]	8 (3–12)	10 (7–12)	0.10
Peak SOFA	14 (9–16)	16 (12–17)	0.05
Pre insulin infusion POCT[a] —mg/dL	299 ± 98	220 ± 44	< 0.001

ARDS acute respiratory distress syndrome, *TPN* total parenteral nutrition, *APACHE II* Acute Physiology and Chronic Health Evaluation II, *SOFA* Sequential Organ Assessment Score

*Statistically significant

[a] Mean, SD

[b] Median, IQR

longer in patients in the post-protocol group, but the median dose of insulin was marginally higher than those in the pre-protocol group (Table 2). Not surprisingly, the frequency of POCT in the post-protocol group was significantly higher than that in the pre-protocol group. Multivariate analysis showed that protocol implementation was the only factor associated with successful glycemic control (adjusted odds ratio 12.75, 95% CI 2.74–59.26, $p = 0.001$) (Supplement Table 2). POCT values at insulin initiation were not associated with achievement of a glycemic target within 8 h.

The incidence of hypoglycemia was not significantly different between the two groups and there was no severe hypoglycemia in both groups. Patients in the post-protocol group stayed in the ICU longer than those in the pre-protocol group, but the difference was not statistically significant (Table 2). However, the mortality rate in the post-protocol group was significantly higher than that in the pre-protocol group (hazard ratio 2.36, 95% CI = 1.01–4.90, $p = 0.027$). Analysis of the primary and secondary outcomes including the mortality rate in patients with known diabetes mellitus showed similar results (Supplement Table 1).

Because of a significantly higher peak APACHE II score and a higher peak SOFA score in the post-protocol group (Table 1), further adjustment with a peak APACHE II score was performed. An adjusted hazard ratio for mortality was 1.23 (95% CI = 0.45–3.35, p 0.687), which was no longer statistically significant after adjustment (Supplement Fig. 1). In the subgroup of patients with known diabetes mellitus, however, an adjusted hazard ratio for mortality was 1.1 (95% CI = 1.02–1.19, $p = 0.015$, Supplement Fig. 2).

Discussion

In our study, implementation of the KCMH insulin infusion protocol in medical ICUs resulted in a significantly greater proportion of patients achieving a glycemic target within 8 h

Table 2 Clinical outcomes between the two groups

	Pre-protocol group ($n = 28$)	Post-protocol group ($n = 27$)	p value
Primary outcome			
Glycemic target achieved within 8 h, n (%)	6 (21.4)	19 (70.4)	< 0.001*
Secondary outcome			
Time to glycemic target[b] (hours)	10 (9–23)[c]	6 (5–10)	0.001*
Percent of glucose values in target range (%)	30 ± 16 [c]	45 ± 21	0.004*
Mean plasma glucose[a] (mg/dL)	206 ± 47	171 ± 15	0.001*
SD[b] (mg/dL)	63 (49–79)	43 (19–71)	0.002*
CV[a] (%)	34 ± 12	27 ± 8	0.001*
Duration of insulin infusion[b] (hrs)	30 (26–46)	66 (31–116)	0.002*
Total insulin dose per patient (units)	74 (29–110)	96 (44–177)	0.069
Frequency of POCT per patient (times)	13 (10–20)	34 (17–46)	< 0.001*
Hypoglycemia, times (%)			
Non-severe hypoglycemia (< 70 mg/dL)	3 (0.7)	4 (0.4)	0.648
Severe hypoglycemia (< 40 mg/dL)	0 (0)	0 (0)	
ICU length of stay (days)[b]	5 (3–13)	8 (5–19)	0.067
Mortality, n (%)	11 (39.3)	19 (70.4)	0.021*

SD standard deviation, *CV* coefficient of variation, *POCT* point of care glucose test

*Statistically significant

[a] Mean, SD

[b] Median, IQR

[c] $N = 24$

than the conventional care. Various secondary outcomes, including mean blood glucose levels, time to a glycemic target, and glycemic variability, also indicated better glycemic control by the use of the insulin protocol compared to the conventional care. We observed no difference in hypoglycemic events and no cases of severe hypoglycemia.

The Yale insulin infusion protocol was initially developed in 2004, and the glycemic target in the original protocol was between 100 and 139 mg/dL [14]. The protocol has shown good efficacy in glycemic control with the median time to target of 9 h. Sixty-six percent of blood glucose values recorded during the total period of insulin infusion were within a clinically desirable range (100–139 mg/dL) with a low (0.3%) rate of hypoglycemia [15].

In 2011, the Yale insulin infusion protocol was modified to be in accordance with updated guidelines on glycemic target. The modified protocol with a glycemic target of 120–160 mg/dL has shown similar efficacy to the original protocol. The median time to target was 7 h, and the average blood glucose value during the insulin infusion period was 156 ± 23 mg/dL whereas hypoglycemia was infrequent [14, 16–19]. Implementation of the modified Yale insulin infusion protocol in other settings, such as in a cardiothoracic ICU or in a hospital with limited resources, has also shown similar efficacy and safety [20].

In our study, the KCMH insulin infusion protocol was adapted from the modified Yale insulin infusion protocol. In brief, the insulin initiation rate was adjusted according to body weight and initial glucose level. In addition, a glycemic target was adjusted to 140–180 mg/dL according to the current guidelines [11–13, 21], and the protocol for management of hypoglycemia was also added. Our study has shown that implementation of the KCMH insulin infusion protocol resulted in similar efficacy and safety as those from previous studies with the modified Yale insulin infusion protocol [14]. Using our protocol, the majority of patients (74%) achieved a glucose target of 140–180 mg/dL within 8 h and the median time to target was 6 h. Moreover, the use of our protocol resulted in time in target range of 45%, which was similar to a previous result from anothor study [22].

Glycemic variability is known to be associated with increased oxidative stress [23, 24] and probably an increase in mortality [25–27]. Commonly used glycemic variability indices are SD and CV, both of which have been associated with mortality [28, 29]. Critically, ill patients who had SD level of more than 20 mg/dL had a higher mortality rate (24%) compared to those with SD less than 20 mg/dL (2.5%) [30]. Another study has demonstrated a significant difference in SD levels between survivors and non-survivors in a critical care setting. The survivors had SD of 30 ± 23 mg/dL whereas non-survivors had SD of 41 ± 29 mg/dL ($p = 0.001$) [27, 31]. Similarly, time in an acceptable glucose target has been shown to be associated with mortality. Maintaining plasma glucose level of 140–180 mg/dL for more than 50% of total plasma glucose values measured was independently associated with

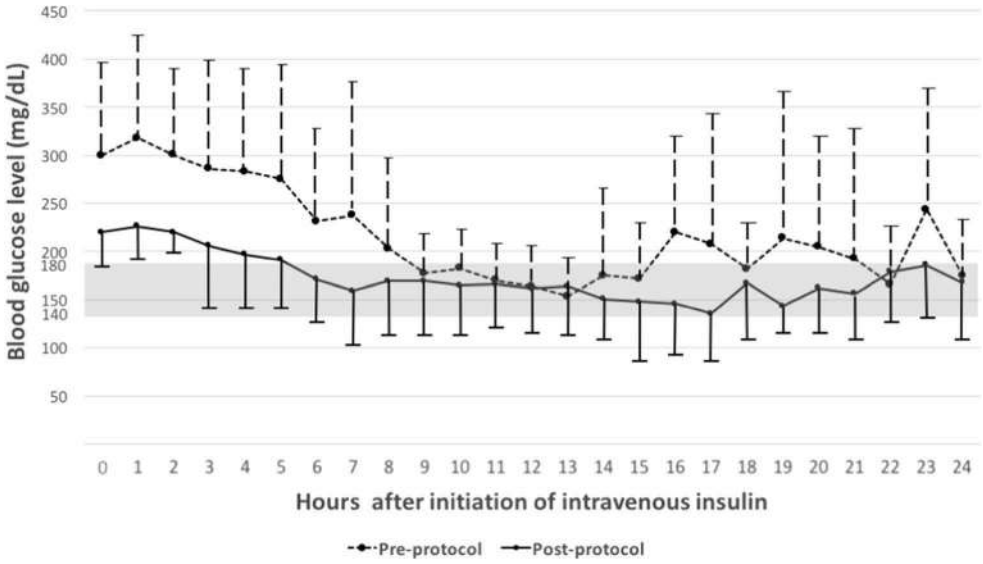

Fig. 2 Mean plasma glucose values in the pre-protocol group (dash line) and the post-protocol group (solid line) during the first 24 h of insulin infusion. The gray box indicates plasma glucose level of 140–180 mg/dL, the target range for glucose control. Data are expressed as mean and SD

an increased rate of survival regardless of glucose control strategy [22].

Given significantly lower time to reach target blood glucose, lower SD and CV values and a significantly higher percentage of time in target range in the post-protocol group, the patients in this group could benefit from less exposure to glucotoxicity and were less susceptible for the complications of hyperglycemia. A lower mortality in this group might have been expected. Although the reduction in mortality rate was not demonstrated in our study, similar to many of the studies conducted in critical care settings, enrolled patients are oftentimes diverse and have multiple factors that could possibly confound the mortality rate. Unexpectedly, a higher mortality rate was found in the post-protocol group, since the mortality rate could be influenced by a variety of other factors besides glycemic control. Further analysis was performed to explain this finding. At baseline, there were no significant differences between the two groups, except for a higher prevalence of liver diseases in the post-protocol group. However, the majority of patients with liver diseases survived, so the underlying liver disease could not explain the excess mortality rate in the post-protocol group. We further analyzed the severity of illness and found that baseline APACHE II and baseline SOFA scores were not significantly different between the two groups. Nonetheless, detailed analysis has revealed that patients in the post-protocol group had a significantly higher peak APACHE II score than that of the pre-protocol group. Similarly, the peak SOFA score in the pre-protocol group was also higher but the difference was not statistically significant ($p = 0.05$ in Table 1). After adjustment with the peak APACHE II score, the difference in mortality rate was no longer statistically significant, suggesting that the higher peak APACHE II score could have explained, at least in part, the higher mortality rate in the post-protocol group.

There are certain limitations in our study. Firstly, we implemented the protocol only in the medical ICUs; thus, the result of this study may not be generalized to other settings. Secondly, the use of the insulin protocol is both time- and resource-consuming despite superiority in glycemic control parameters. The duration of insulin infusion was longer in the post-protocol group, but the total insulin dose was not significantly different between the two groups. The frequency of POCT was significantly greater in the post-protocol group. Based on the cost of POCT at our hospital, this would add an average of an additional cost of 14 US dollars per patient using the protocol. The average duration of ICU length of stay was 3 days longer in the post-protocol group. However, we believe that the insulin infusion should not be the reason for a prolonged ICU stay since the transition from intravenous to subcutaneous insulin administration could be initiated anytime once the patients were clinically stable. In this regard, further studies regarding the cost-effectiveness of protocol implementation are needed. Lastly, we did not design the study as a randomized-controlled trial since the treatment protocol could not have been blinded and protocol contamination between the two groups might have been encountered. Nonetheless, our study did reflect the efficacy of the protocol in real-life clinical practice.

Conclusions

Implementation of the KCMH insulin infusion protocol, targeting blood glucose values of 140–180 mg/dL, in medical

ICUs resulted in better glycemic control than conventional care without excess risk of hypoglycemia.

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Compliance with ethical standards

The study was approved by the Institutional Review Board and Thai Clinical Trial Registry (clinicaltrials.in.th: TCTR20161105001).

Conflicts of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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ORIGINAL ARTICLE

The association between gallstone disease (GSD) and the incidence of prediabetes and type 2 diabetes mellitus (type 2 DM): a prospective cohort study

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Abstract

Objectives The present study aimed to investigate the role of GSD in different phases of type 2 DM development in the Jinchang cohort study.

Methods A total of 28,760 eligible participants were divided into two mutually exclusive subgroups of normoglycemia (n = 23,725) and prediabetes (n = 5035) at baseline. These subgroups were followed up for incident prediabetes (n = 4512) and incident type 2 diabetes mellitus (n = 754), respectively. Cox proportional hazard models were used to determine hazard ratios (HRs) for incident prediabetes among normoglycemic individuals and incident type 2 diabetes mellitus among prediabetic individuals.

Results The mean duration of follow-up was 2.18 years for incident prediabetes and 2.30 years for incident type 2 diabetes mellitus. GSD is significantly associated with incident type 2 diabetes mellitus among prediabetic individuals (HR = 1.292; 95% CI, 1.071–1.560), but not with incident prediabetes among normoglycemic individuals (HR = 0.999; 95% CI, 0.849–1.079). When gallstone and cholecystectomy were studied separately, only cholecystectomy was found to be significantly associated with incident type 2 diabetes mellitus among individuals with prediabetes (HR = 1.703; 95% CI, 1.299–2.233) and the association seems to be stronger in women (HR = 1.929; 95% CI, 1.242–2.994) than in men (HR = 1.555; 95% CI, 1.077–2.247).

Conclusion Our findings suggest that GSD is more closely related to the late-phase mechanisms in the development of type 2 DM than early-phase mechanisms.

Keywords Gallstone · Cholecystectomy · Prediabetes · Type 2 diabetes mellitus · Cohort study

Highlight

- This is the first paper to investigate the role of GSD in different phases of type 2 DM development, and we found that GSD is more closely related to the late-phase mechanisms in the development of type 2 DM than early-phase mechanisms.
- Both gallstone and cholecystectomy are not associated with the incidence of prediabetes among normoglycemic individuals. In prediabetic individuals, cholecystectomy instead of gallstone is related to a high risk of type 2 DM.
- Previous studies show that GSD can lead to a higher risk of type 2 DM only in women. However, the relationship between cholecystectomy and type 2 DM among prediabetic individuals was found both in men and women when gallstone and cholecystectomy were studied separately in our study.

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Introduction

Gallstone is a common digestive system disease and related to a high prevalence of cardiovascular disease [1], digestive system cancers, and some other diseases [2, 3]. Cholecystectomy is considered the gold standard treatment of symptomatic gallstones [4]. It was also considered that gallbladder (GB) removal is a relatively harmless procedure [5, 6], but more and more researches indicate that cholecystectomy is not a completely safe way. There is emerging evidence showing that cholecystectomy itself may lead to an excessive risk for the metabolic syndrome [7, 8], which is a risk factor of gallstone diseases.

With the development of social economy, the number of diabetics around the world is growing sharply and become one of the biggest public health problems of the twenty-first century. Prediabetes defined as blood glucose concentrations higher than normal, but lower than diabetes thresholds, is a high-risk state for diabetes development [9], but this concept

has always been controversial. The objection is that diagnosed people with higher blood glucose than normal as having prediabetes brings more problems than benefits in terms of prevention and treatment, and the fact is also that individuals with prediabetes received less treatment than participants with diagnosed diabetes [10]. Although there is a dispute, several epidemiologic studies have reported that changes in lifestyle and eating habits can reduce the incidence of diabetes in prediabetic individuals [9, 11, 12].The risk factors of diabetes have been researched by varieties of studies. However, studies about the risk factors in different stages of type2 DM development are limited, and a cohort study indicates that the same factor may play different roles in different stages of type 2 DM development [13].

Several prospective cohort studies have found a relevance between diabetes and GSD, and the association between cholecystectomy and type 2 DM seems to be stronger [14, 15]; however, the mechanisms are still unclear about this correlation. This study aimed to investigate the role of GSD in different stages of type 2 DM development to provide clues for the study of the mechanism.

Material and methods

Study population

This is a cohort study using data from the baseline and the first round of follow-up of the Jinchang cohort study which consists of all current and retired workers of the Jinchuan Nonferrous Metals Corporation (JNMC), a mining and metals company that has employed about 50,000 people in Jinchuan. Details of the Jinchang cohort study had been described in previous studies [16, 17]. Briefly, we began the baseline survey in June 2011 and ended in December 2013 and the first round of follow-up was finished in November 2015, and a total of 33,355 participants were followed up. For the present study, we excluded 2212 participants who had type 2 DM at baseline, those with missing data on ultrasonic ($n = 1900$) and fasting serum glucose ($n = 79$) at baseline were also removed. Finally, 28,760 eligible participants were enrolled in this study.

Definition of type 2 diabetes mellitus and prediabetes

According to the American Diabetes Association standards [18], type 2 DM was defined as having a fasting plasma glucose level ≥ 7.0 mmol/L or receiving anti-diabetic medication with "type 2 DM" as the indication. Prediabetes was defined as a fasting plasma glucose (FPG) between 5.6 and 6.9 mmol/L in workers without prior diabetes diagnosis.

Covariates

LOGIQ P5 ultrasonic system (GE ultrasound company in Korea) was used to examine GSD, which was defined as either having gallstones or having had a cholecystectomy. The collection of other confounding factors includes epidemiological survey and physical and biochemical examinations. Age, sex, education, smoking (smoker, non-smoker), alcohol consumption (drinker, non-drinker), and family history of diabetes were obtained through epidemiological surveys conducted by specially trained postgraduates. Physical and biochemical examinations were performed by clinicians at the tertiary Jinchuan Workers' Hospital, and body mass index (BMI), waist circumference (WC, measured to the nearest 0.5 cm), hypertension(defined as having a systolic blood pressure > 140 mmHg and (or) diastolic blood pressure > 90 mmHg or receiving blood pressure–lowering medication), serum HDL, serum LDL, uric acid (UA), systolic blood pressure (SBP), diastolic blood pressure (DBP), serum total cholesterol (TC), and triglycerides (TG) were considered in our study. Standing height (measured to the nearest 0.1 cm), weight (measured to the nearest 0.1 kg), WC, and blood pressure (BP) were measured in the morning. BP was measured twice in sitting position from the right arm, and then the two values were averaged. The blood sample was collected in the fasting state in the morning and the biochemistry analysis for HDL, LDL, UA, TG, and TC was performed by chemistry automatic analyzer (Hitachi 7600-020, Kyoto, Japan).

Statistical analysis

Participants were divided into normoglycemia group and prediabetes group based on the fasting plasma glucose (FPG) levels at baseline, and all the analyses were performed separately within the two groups. Continuous variables and categorical variables were expressed as means ± SD (standard deviation) and percentages. The baseline characteristics of participants between GSD and no GSD groups were compared using the Student's t test and the chi-square test. Incidence density of prediabetes and diabetes was expressed as the number of cases divided by person-years.

Cox proportion hazards regression was performed to analyze the association between GSD and risk of incident prediabetes or incident type 2 DM, and we estimated the adjusted hazard ratio (HR) with 95% confidence intervals (CI) comparing GSD-group with the non-GSD group. The Cox proportion models were initially adjusted for age and sex (model 1) and then for BMI, WC, education, smoking status, drinking status, hypertension, and family history of diabetes (model 2). In model 3, we additionally adjusted for the TC, TG, DBP, SBP, UA, LDL, and HDL besides the factors in model 2.

Statistical analysis was performed using SPSS statistical software (version 20; Chicago, USA). All reported p values

were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

A total of 28,760 subjects were enrolled in this study, including the subgroups of normoglycemia ($n = 23,725$) and prediabetes ($n = 5035$) at baseline. Compared with the population without GSD, those with GSD are elder, low-level educated, have higher BMI, WC, blood pressure, and lower HDL. In normoglycemic individuals, the levels of TC, TG, and LDL are higher in the populations with GSD than those without. In the participants with prediabetes, of whom with GSD is more likely to have family history of diabetes and higher level of UA. Specific information is shown in Table 1.

After 11,586 person-years of follow-up, 4512 participants with normoglycemia at baseline developed prediabetes (incidence rate 85 per 1000 person-years), including 3041 men (incidence rate 98.7 per 1000 person-years) and 1471 women (incidence rate 70.4 per 1000 person-years). The results of Cox regression analysis showed that both gallstone and cholecystectomy were not associated with the incidence of prediabetes among normoglycemic individuals; specific information is shown in Table 2. A total of 754 individuals with prediabetes at baseline developed type 2 DM over 11,586 person-years of follow-up (incidence rate 65.1 per 1000 person-years), including 583 men (incidence rate 68.9 per 1000 person-years) and 171 women (incidence rate 54.7 per 1000 person-years). The relationship between GSD and type 2 DM among prediabetic individuals is shown in Table 3. We found a significant association between GSD and incident type 2 DM among individuals with prediabetes in model 1 which adjusted only for age and sex (HR = 1.498; 95% CI, 1.251–1.1.795), The association was moderately attenuated but still exists in the multivariable-adjusted model (HR = 1.292; 95% CI, 1.071–1.560). When men and women were studied separately, we found that GSD was significantly associated with the incidence of type 2 DM in women (HR = 1.476; 95% CI, 1.035–2.106) instead of men (HR = 1.228; 95% CI, 0.976–1.544) after multivariable adjustment. Further studies have revealed that only cholecystectomy can lead to an excessive risk of type 2 DM, and the association seems to be stronger in women (HR = 1.929; 95% CI, 1.242–2.994) than in men (HR = 1.555; 95% CI, 1.077–2.247). The association between gallstone and the incidence of type 2 DM had only been found in model 1 in total populations, and this association was not observed after further adjustment in model 2 and model 3.

Table 1 Baseline characteristics of study population

Characteristics	Normoglycemia		*p* value	Prediabetes		*p* value
	With GSD	Without GSD		With GSD	Without GSD	
No. of participants	2060	21,665		692	4343	
Sex, % male	1037 (50.3)	13,241 (61.1)	< 0.05	420 (60.7)	3259 (75.0)	< 0.05
Age (year)	49.5 (11.7)	42.9 (11.5)	< 0.05	55.3 (11.3)	50.1 (12.2)	< 0.05
Middle school and above (%)	1157 (56.2)	15,273 (70.5)	< 0.05	294 (42.5)	2388 (55)	< 0.05
BMI (kg/m^2)	24.2 (3.2)	23 (3.1)	< 0.05	25.4 (3.3)	24.395 (3.1)	< 0.05
WC (cm)	84.4 (9.7)	83.0 (10.0)	< 0.05	87.8 (10.1)	86.7 (9.6)	< 0.05
Smoking (%)	40.8	47.5	< 0.05	46.4	57.2	< 0.05
Alcohol consumption (%)	23.8	25.5	> 0.05	26.2	37.4	< 0.05
Hypertension (%)	31	20.4	< 0.05	50.1	40.7	< 0.05
Family history of diabetes (%)	15.7	14.3	> 0.05	14	17.1	< 0.05
Serum HDL (mmol/L)	1.3 (0.3)	1.4 (0.3)	< 0.05	1.3 (0.3)	1.3 (0.4)	> 0.05
Serum LDL (mmol/L)	3.1 (0.7)	3.0 (0.7)	< 0.05	3.2 (0.8)	3.3 (0.8)	> 0.05
Uric acid (μmol/L)	323.7 (80.3)	323.2 (80.3)	> 0.05	343.8 (84.8)	351.9 (83.5)	< 0.05
Systolic Blood pressure (mmHg)	124.4 (19.4)	119.5 (18.1)	< 0.05	134.3 (19.6)	130.7 (20.2)	< 0.05
Diastolic Blood pressure (mmHg)	79.7 (12.6)	77.0 (11.7)	< 0.05	84.2 (12.7)	82.9 (12.8)	< 0.05
Serum Total cholesterol (TC)	4.7 (0.9)	4.6 (0.9)	< 0.05	4.9 (0.9)	4.9 (0.9)	> 0.05
Triglycerides (mmol/L)(TG)	2.0 (1.5)	1.8 (1.4)	< 0.05	2.4 (1.74)	2.3 (1.8)	> 0.05

Variables are presented as mean (standard deviation) or percentage

Chi-square test and *t* test were used to compare the difference between GSD and non-GSD group

Table 2 The association between GSD and incidence of prediabetes in normoglycemic individuals

	Person-years	Cases	Hazard ratio (95% CI)		
			Model 1	Model 2	Model 3
Total					
Without GSD	46,970	3994	1.000	1.000	1.000
With GSD	4741	518	1.091 (0.994–1.198)	0.996 (0.905–1.095)	0.999 (0.906–1.102)
Gallstone	2891	321	1.095 (0.976–1.228)	0.979 (0.871–1.100)	0.957 (0.849–1.079)
Cholecystectomy	1850	197	1.084 (0.938–1.253)	0.977 (0.839–1.136)	0.989 (0.854–1.144)
Male					
Without GSD	28,421	2736	1.000	1.000	1.000
With GSD	2389	305	1.091 (0.967–1.230)	1.008 (0.892–1.140)	0.995 (0.878–1.128)
Gallstone	1625	209	1.078 (0.935–1.243)	0.984 (0.852–1.138)	0.955 (0.823–1.109)
Cholecystectomy	764	96	1.105 (0.900–1.357)	1.049 (0.852–1.290)	1.080 (0.876–1.332)
Female					
Without GSD	18,550	1258	1.000	1.000	1.000
With GSD	2351	213	1.033 (0.891–1.197)	0.929 (0.799–1.080)	0.931 (0.797–1.088)
Gallstone	1266	112	1.100 (0.906–1.337)	0.959 (0.787–1.169)	0.936 (0.764–1.147)
Cholecystectomy	1085	101	0.965 (0.786–1.184)	0.922 (0.734–1.144)	0.922 (0.743–1.144)

Model 1: adjusted for age and sex

Model 2: model 1 + BMI, WC, education, smoking, alcohol consumption, hypertension, and family history of diabetes

Model 3: model 2 + TC, TG, DBP, SBP, UA, LDL, HDL

The survival curves show that participants with GSD have lower overall survival rate, compared with those without GSD among prediabetic individuals (p value = 0.000) (Fig. 1). However, in individuals with normal blood glucose, there was no significant difference in overall survival rate between the populations with and without GSD (p value = 0.226) (Fig. 2).

Table 3 The association between GSD and incidence of type 2 diabetes in prediabetic individuals

	Person-years	Cases	Hazard ratio (95%CI)		
			Model 1	Model 2	Model 3
Total					
Without GSD	9939	601	1.000	1.000	1.000
With GSD	1647	153	1.498 (1.251–1.795)	1.342 (1.113–1.517)	1.292 (1.071–1.560)
Gallstone	1045	90	1.316 (1.052–1.646)	1.148 (0.911–1.446)	1.105 (0.875–1.396)
Cholecystectomy	602	63	1.898 (1.458–2.471)	1.757 (1.341–2.302)	1.703 (1.299–2.233)
Male					
Without GSD	7448	486	1.000	1.000	1.000
With GSD	1012	97	1.351 (1.084–1.685)	1.260 (1.004–1.581)	1.228 (0.976–1.544)
Gallstone	692	65	1.277 (0.984–1.659)	1.129 (0.862–1.480)	1.092 (0.831–1.436)
Cholecystectomy	320	32	1.516 (1.058–2.172)	1.585 (1.100–2.284)	1.555 (1.077–2.247)
Female					
Without GSD	2490	115	1.000	1.000	1.000
With GSD	635	56	1.765 (1.270–2.454)	1.535 (1.088–2.166)	1.476 (1.035–2.106)
Gallstone	353	25	1.383 (0.893–2.140)	1.129 (0.719–1.773)	1.104 (0.690–1.754)
Cholecystectomy	282	31	2.362 (1.556–3.587)	2.060 (1.333–3.183)	1.929 (1.242–2.994)

Model 1: adjusted for age and sex

Model 2: model 1 + BMI, WC, education, smoking, alcohol consumption, hypertension, and family history of diabetes

Model 3: model 2 + TC, TG, DBP, SBP, UA, LDL, HDL

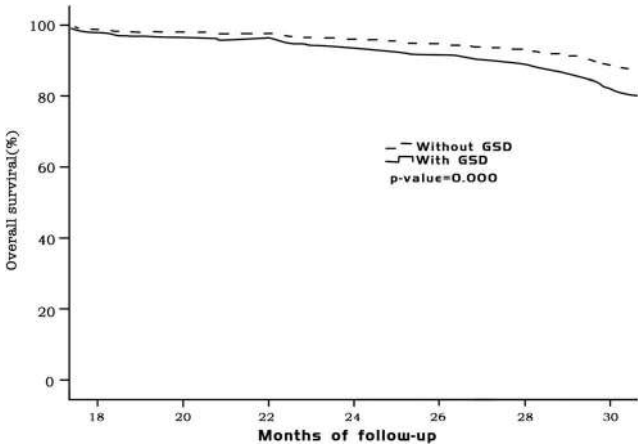

Fig. 1 Overall survival rate in prediabetic individuals

Discussion

To our knowledge, this is the first prospective cohort study to investigate the role of GSD in different phases of type 2 DM development. We found that both gallstone and cholecystectomy were not associated with the high risk of prediabetes among normoglycemic individuals after adjusting for the potential confounding factors. Cholecystectomy was significantly associated with the development of type 2 DM among prediabetic individuals, but the relationship between gallstone and the development of type 2 DM had not been found after multivariable adjustment.

In a large prospective cohort study of 0.5 million adults in China [15], the prevalence of GSD was 5.7%, which is lower than 9.6% of our study populations. This difference may be explained by the different measurements of GSD. For the presence of GSD, their study was self-reported and participants with asymptomatic GSD may be classified into non-GSD group. However, GSD in our study was measured with ultrasonic system which can identify each individual with GSD as much as possible. In another cohort study in Korea [14], the prevalence of GSD at baseline was 2.1%, which is lower than the prevalence in our study. Since the average age in the Korean cohort study is lower than ours, this difference may be explained by age and ethnicity. Both studies in China and Korea had found the association between GSD and type 2 DM in women, which was consistent with our study.

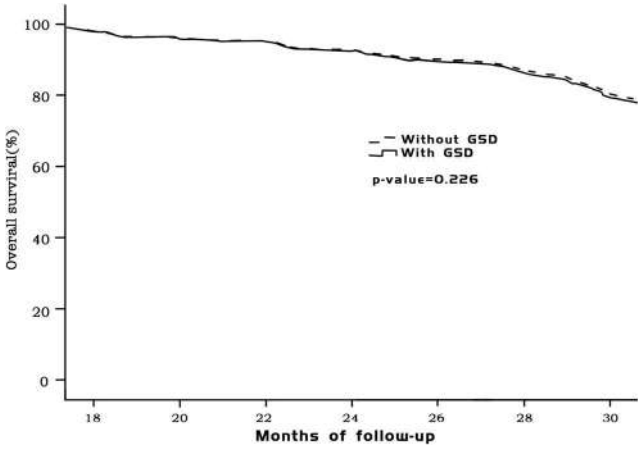

Fig. 2 Overall survival rate in individuals with normal blood glucose

The relevance between gallstone and type 2 DM was first revealed in a large-scale Europe-wide prospective cohort study [19]. Further study in Korea [14] indicated that the presence of gallstones is related to a high risk of type 2 DM only in women but not in men. However, gallstone was not associated with an increased risk of prediabetes and type 2 DM among individuals with normoglycemia and prediabetes in our study. But our results do not contradict theirs for the previous studies only marked the baseline population as no diabetes. However, our study population at baseline was divided into individuals with normoglycemia and prediabetes, and all analyses were performed separately. Lv et al. [15] found a stronger association between GSD and diabetes among the patients who had a longer history of GSD; meanwhile, participants' median follow-up time in our study is relatively short. These could also partly explain the results why we did not find a relationship between gallstone and diabetes.

The relationship between cholecystectomy and type 2 DM was studied only in the Korean population [14] before ours and a significant association was found only in women (HR = 2.00; 95% CI, 1.003–3.97). But the present study found a relationship between cholecystectomy and type 2 DM both in men and women among individuals with prediabetes, even though the estimated hazard ratio was higher in women (HR = 1.929; 95% CI, 1.242–2.994) than in men (HR = 1.555; 95% CI, 1.077–2.247). Female sex hormones can adversely influence hepatic bile secretion and gallbladder function, and progestins can reduce bile salt secretion and impair gallbladder emptying [20]. Therefore, this gender difference may be explained by sex hormone differences. Although this weaker association in men was found only in individuals with prediabetes, our results suggest that cholecystectomy can also be a risk factor of type 2 DM for men. Further studies addressing sex difference are needed.

Gallstone always occurs in persons with obesity, low serum levels of HDL and LDL cholesterol [21], and metabolic syndrome [22, 23], all of which are risk factors of type 2 DM. Although our study did not find a higher incidence of prediabetes and type 2 DM among participants with gallstone, we estimated that gallstone could be regarded as an intermediate variability to predict type 2 DM. When the gallbladder is removed, these problems are likely to worsen. Ali et al. [24] found a significant increase in BMI after cholecystectomy. Cholecystectomized patients had higher levels of plasma TGs, total cholesterol, and LDL-cholesterol, but lower levels of HDL cholesterol than controls in a Mexican population [25]. Recent evidences [7, 8, 26] pointed out that cholecystectomy may be a risk factor of metabolic syndrome. However, in the present study, the association between cholecystectomy

and type 2 DM was attenuated but still exists after adjusting for WC, BMI, TG, BP, LDL, and HDL, suggesting that these factors might only explain this association partly. More studies are needed to elucidate the mechanisms by which GSD might cause type 2 DM.

The results of Shen et al. [27] suggest that cholecystectomy can increase the risk of metabolic syndrome significantly while gallstones cannot, which is similar to our findings. The major function of the GB is to concentrate and store bile, and the GB will be emptied 70–80% after a meal to balance the metabolic stress caused by periodic food intake [28]. After cholecystectomy, the hepatic bile will be continuously secreted into the duodenal lumen and raise the risk of metabolic disorders. In addition, GB itself has a metabolic function, which is related to glucose metabolism [8]. Therefore, these discoveries could help to explain the closer relationship between cholecystectomy and type 2 DM.

There are several limitations in our study. First, participants in our study were followed up for a relatively short time, and this may weaken the relationship between GSD and type 2 DM, as the effects of GSD may not be fully apparent in a short period of time. Second, some diabetics may be distinguished as the non-diabetic because only fasting plasma glucose was used as an indicator to identify the type 2 DM in our study so that the association between GSD and type 2 DM may be attenuated. Third, there are many kinds of gallstones, but there was not a distinction in our study. In addition, the causes of cholecystectomy are unclear and which may not be gallstones.

Our study found that cholecystectomy is significantly associated with a high risk of type 2 DM among prediabetic individuals. The association between cholecystectomy and incident prediabetes among normoglycemic individuals has not been found. This is a new discovery guiding us to pay more attention to the role of GSD in the late phase of type 2 DM development. The present results also suggest that cholecystectomy is not a completely harmless procedure on overall metabolic regulation; therefore, careful consideration should be given before cholecystectomy. Although we did not find a higher incidence of prediabetes and type 2 DM among participants with gallstones in our study, gallstones and type 2 DM have been found to share many important risk factors. Therefore, patients with gallstones should pay more attention to the risk of diabetes in early detection and prevention.

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Compliance with ethical standards

Ethical approval This article was approved by the Ethics Committee of Lanzhou University.

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

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ORIGINAL ARTICLE

Association of *Helicobacter pylori* infection with metabolic and inflammatory profile in type 2 diabetes mellitus

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Abstract

Objective To evaluate the effect of *Helicobacter pylori* (*H. pylori*) infection upon metabolic and inflammatory parameters in type 2 diabetes mellitus (T2DM).

Methods A total of 72 patients with T2DM were included in the study. These patients were divided into two groups as *H. pylori* infection positive or negative. For each patient, the following data were collected: age, gender, duration of diabetics, anti-diabetic treatment, the body mass index (BMI), and laboratory parameters (lipid profile, GLU, HbA1c, HCY, HsCRP, ghrelin, leptin, leukocyte, and platelet counts).

Results Totally 47 patients (65.28%) were *H. pylori* positive and 25 patients (34.72%) were *H. pylori* negative. Diabetic patients infected by *H. pylori* showed significantly increased Lpa (297.83 ± 299.51 vs 154.24 ± 83.63, $p < 0.05$), higher HbA1c (9.21 ± 2.15 vs 8.00 ± 1.77, $p < 0.05$), and decreased leptin (4.59 ± 7.55 vs 9.82 ± 10.76, $p < 0.05$) than non-infected patients. Additionally, 72.2% of the patients with HbA1c > 7% were found to be *H. pylori* positive and 44.4% of the patients with HbA1c ≤ 7% were *H. pylori* positive. The levels of other parameters were not significantly different between two groups ($p > 0.05$), although CRP levels determined by high-sensitivity assay showed mild and variable increases in *H. pylori* infection.

Conclusion *H. pylori* affects glycemic control in T2DM and might promote the development of diabetes.

Keywords *Helicobacter pylori* · Type 2 diabetes mellitus · Metabolic and inflammatory profile

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped pathogenic bacterium which can establish a persistent infection within human gastric mucosa, and affects approximately up to half of the world population [1]. This problem is particularly notable in developing countries, and the prevalence rate in China may be as high as 54.7% [2]. The effects of this organism may not only be confined to the gastrointestinal tract but also be associated with extra-intestinal ailments such as its role in diabetes and increased insulin resistance [3].

Diabetes mellitus is a group of metabolic diseases characterized by high levels of blood sugar (glucose). Type 2 diabetes mellitus (T2DM) is increasingly common and is responsible for the death of an estimated 3.8 million adults across the world [4]. Although major risk factors (e.g., lifestyle, genetic background, socioeconomic factors) for T2DM have been identified, they provide only partial explanations.

The relationship of *H. pylori* and DM was first explored in 1989 by Simon et al. [5]. But previous studies provided inconsistent conclusions concerning the association between various clinical manifestation of diabetes and *H. pylori* infection [6–9]. Recent study suggests the role of inflammation in the pathogenesis of T2DM, which is an important process induced by *H. pylori* infection [10]. It has been shown that *H. pylori* infection may increase insulin resistance through inducing chronic inflammation and affecting the levels of

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some insulin-regulating gastrointestinal hormones. This research sought to investigate the possible relationship between *H. pylori* infection and T2DM patients as well as the potential mediators concerning this correlation.

Patients and methods

The study was a hospital-based, analytic observational study and performed through a cross-sectional method. T2DM patients aged 18–65 and recruited from the Second Affiliated Hospital of Bengbu Medical College were included into the study. Our research protocol was approved by ethical committee of Bengbu Medical College, and a written informed consent was signed by each participants according to national and institutional guidelines. Exclusion criteria were as follows: (1) patients over 65 years; (2) women patients who were currently pregnant or breastfeeding; (3) patients with a history of gastrointestinal tract surgery; (4) patients who were currently using antisecretory drugs (proton-pump inhibitor or H2 receptor blockers); (5) patients who had undergone or were currently undergoing *H. pylori* eradication therapy; (6) patients who were obliged to continuous use of antibiotics for various reasons.

We compiled a brief checklist covering demographic data and clinical parameters, including age, gender, duration of diabetics, and anti-diabetic treatment. The body mass index (BMI) is a statistical measure based on a person's weight and height (weight/height^2 = kg/m^2). We also checked the laboratory parameters. GLU (glucose oxidase method); TC (CHOD-PAP substrate method); TG (GPO-PAP enzymatic method); HDL-C (selective inhibition method); LPa (latex-enhanced immunoturbidimetry); Apo A, Apo B, and HsCRP (turbidimetric immunoassay); and HCY (enzyme method) were measured on Beckman Coulter UniCel DxC 800 Synchron. LDL-C was calculated by the Friedewald formula. HbA1c was determined by ion-exchange HPLC method (HLC-723G8 automatic glycosylated hemoglobin analyzer). Commercially available human enzyme-linked immunosorbent assay (ELISA) for the determination of plasma ghrelin and leptin (eBioscience Inc., USA) was performed according to manufacturers' instructions. The leukocyte and platelet counts were determined by flow cytometry, and Mindray BC-5800 automatic blood cell analyzer was used for the detection.

In all patients, the diagnosis of *H. pylori* infection was determined by ELISA for anti-*H. pylori* IgG, with a reported sensitivity and specificity of 96% [11]. Measurement of specific anti-*H. pylori* IgG reveals an immune response that represents either a current infection or a previous exposure, since IgG disappears only several months after eradication of the microorganism. Then, we divided the subjects into two groups according to *H. pylori* infection as *H. pylori*–positive patients and *H. pylori*–negative patients. The association between *H. pylori* infection and demographic factors, and biochemical and anthropometric indicators was investigated in all patients.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) 20.0 Package program was used for the analysis of data. The data were presented as mean ± SD. Adjustment to normality was checked through the Kolmogorov-Smirnov test. Quantitative variables with normal distribution were compared by *H. pylori* status using independent sample *t* test or the Mann-Whitney *U* test as appropriate. Qualitative parameters were analyzed with the chi-square test. Two-tailed *p* values of below 0.05 were considered significant.

Results

A total of 72 T2DM patients with a mean age of 54 (± 7) were enrolled in this study, including 44 males and 28 females. The presence of *H. pylori* infection was diagnosed in 47 (65.28%) of 72 diabetic patients. In male patients, the prevalence of *H. pylori* infection was 68.18% while it was 60.71% in females. The demographic and laboratory characteristics of the study population, divided into *H. pylori*–positive and *H. pylori*–negative groups, are displayed in Tables 1 and 2.

Patients infected with *H. pylori* and non-infected with *H. pylori* were not significantly different in terms of gender, age, BMI, duration of diabetics, and anti-diabetic treatment. The level of GLU, TC, TG, HDL-C, Apo A, Apo B, HsCRP, HCY, leukocyte, and platelet counts were not significantly different between *H. pylori*–positive and *H. pylori*–negative groups ($p > 0.05$), although CRP levels determined by high-sensitivity assay showed mild and variable increases in *H. pylori* infection. Diabetic patients infected by *H. pylori* showed significantly increased serum Lpa (297.83 ± 299.51 vs 154.24 ± 83.63, $p < 0.05$) and decreased leptin (4.59 ± 7.55 vs 9.82 ± 10.76, $p < 0.05$) than non-infected patients (Table 1). HbA1c, the major fraction of glycated hemoglobin, is most valuable glucose monitoring index for patients with diabetes [12]. A significant relationship between glycemic control and the presence of *H. pylori* was detected. Patients with *H. pylori* infection had higher HbA1c level (9.21 ± 2.15 vs 8.00 ± 1.77, $p < 0.05$). Additionally, 55.6% of the patients with HbA1c ≤ 7% were *H. pylori* negative and 72.2% of the patients with HbA1c > 7% were found to be *H. pylori* positive (Table 2), indicating that *H. pylori*–infected group had worse glycemic control.

Table 1 Characteristics of study subjects with respect to the *H. pylori* infection

Parameters	*H. pylori* positive	*H. pylori* negative	*p* value
Patients (*n*)	47	25	–
Age (year)	55.21 ± 7.44	53.04 ± 7.14	–
Male/female (*n*)	30/17	14/11	–
TC (mmol/L)	5.07 ± 1.63	2.30 ± 2.42	> 0.05
TG (mmol/L)	2.30 ± 2.42	2.39 ± 2.06	> 0.05
HDL (mmol/L)	1.24 ± 0.30	1.18 ± 0.39	> 0.05
LDL (mmol/L)	2.78 ± 1.30	2.55 ± 0.94	> 0.05
APOA (g/L)	1.05 ± 0.31	1.00 ± 0.35	> 0.05
APOB (g/L)	1.03 ± 0.46	0.97 ± 0.25	> 0.05
GLU (mmol/L)	10.99 ± 4.38	9.88 ± 3.22	> 0.05
LP-a (mg/L)*	297.83 ± 299.51	154.24 ± 83.63	0.022
HCY (μmol/L)	7.30 ± 5.44	10.60 ± 20.53	> 0.05
hsCRP (mg/L)	11.30 ± 16.67	7.08 ± 12.75	> 0.05
HbA1c (%)*	9.25 ± 2.10	8.00 ± 1.77	0.014
Leukocyte (10^9/L)	6.95 ± 3.49	7.94 ± 4.14	> 0.05
Platelet counts (10^9/L)	177.77 ± 64.85	195.92 ± 80.06	> 0.05
Ghrelin (pg/mL)	80.21 ± 166.89	10.79 ± 20.22	> 0.05
Leptin (pg/mL)*	4.59 ± 7.55	9.82 ± 10.76	0.037
Body mass index (kg/m^2)	28.4 ± 1.8	27.6 ± 1.6	> 0.05
Anti-diabetic treatment (oral anti-diabetics/insulin)	25/22	10/15	> 0.05
Duration of diabetics	2.9 ± 0.7	2.8 ± 0.6	> 0.05

*Significant parameters

Discussion

It is well known that diabetic patients have an increased risk of suffering chronic infections because of cellular and humoral immune deficiency. It has been reported that the prevalence of *H. pylori* infection varies between 30 and 80% in diabetic patients [13–16]. In this recent study of our institute, the prevalence of *H. pylori* infection in diabetics was found to be 65.28% and this rate was concordant with results observed in different related studies.

H. *pylori* plays a major pathogenic role in a wide array of gastric disorders, including simple gastritis, peptic ulcers, and gastric malignancies. During the last two decades, the associations among *H. pylori* and several extragastric manifestations, such as iron deficiency anemia, cardiovascular disease, as well as diabetes mellitus (DM), and other metabolic syndromes [17–20], strongly emerged in literature. The relationship between *H. pylori* and DM was first explored in 1989. From then on, it has been suggested that infection with *H. pylori* is potentially linked to DM in many aspects [21–24]. However, the question of whether *H. pylori* infection is associated with poorer glycemic control in patients with DM remains controversial.

Table 2 The relationship between HbA1c in diabetic patients and *H. pylori* positivity

HbA1c (%)	Total T2DM *n*	*H. pylori* positive *n* (%)	*H. pylori* negative *n* (%)	*p* value
≤ 7	18	8 (44.4%)	10 (55.6%)	< 0.05
> 7	54	39 (72.2%)	15 (27.8%)	

This study showed a positive association between *H. pylori* status and HbA1c levels, a valid and sensitive biomarker for long-term glycemic control among a group of middle-aged to elderly Chinese subjects with T2DM. Patients with *H. pylori* infection had significantly elevated HbA1c level. Additionally, compared with low HbA1c (≤ 7%), the patients with HbA1c > 7% showed a significantly higher *H. pylori*–infection rate (72.2% vs 44.4%), indicating that *H. pylori*–infected group had worse glycemic control.

The mechanisms linking *H. pylori* to glycemic control in T2DM are complicated. It is well known that insulin resistance and abnormal insulin secretion are the main pathogenic factors in T2DM. *H. pylori* might affect pathophysiological process of insulin resistance through subclinical chronic inflammation, by which the bacterium influences glycemic control in diabetics [25]. An alternative hypothesis is that gastrointestinal conditions resulting from *H. pylori* infection could delay gastric emptying and consequently causes poor glucose control. Furthermore, *H. pylori*–

induced gastritis can potentially affect the secretion of gastric-related hormones, which are secreted from gastric mucosa and are involved in energy homeostasis, modulating insulin sensitivity and glucose homeostasis. Leptin, a multifunctional polypeptide primarily produced by adipocytes, favors energy expenditure increase and food intake reduction [26]. In recent years, increased evidence indicates that high levels of leptin may impair glucose-stimulated insulin secretion and induce apoptosis of β cells. Our study showed that *H. pylori* infection elevated the production of leptin among patients with T2DM, and thus might promote the development of diabetes.

DM is always a multifactorial metabolic disorder characterized by changes in the metabolism of carbohydrates, fats, and protein. A growing body of evidence has demonstrated a significant relationship between lipid profiles and thrombotic activation–related anti-thrombin (AT)-III and *H. pylori* infection [27]. In the present study, lipid profile was not significantly different between *H. pylori*–positive and *H. pylori*–negative groups, but LPa levels were found to be significantly increased in infected diabetic patients. LPa is a new novel marker of cardiac events, and serum elevated level of LPa is an independent risk factor for coronary heart disease (CHD) [28], so *H. pylori* infection has been hypothesized to predispose diabetics to cardiovascular and cerebral diseases.

In conclusion, our results demonstrated a significant association between *H. pylori* infection and impaired glycemic control in type 2 diabetic patients. Leptin appeared to mediate this effect. The relationship between *H. pylori* and LPa level remains speculative, but a greater understanding may give important insights into the cardiac events in diabetic patients. A few limitations warrant consideration: first, this was a single-center study, and the small sample size used in this study remains as a limitation. Further investigations are recommended, with a larger subject population, to validate the findings reported here. Second, we did not investigate the patients with *H. pylori* infection after treatment. More investigations will be required to evaluate the effects of *H. pylori* eradication on the metabolic status in diabetic patients.

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Compliance with ethical standards

Ethical approval Our research protocol was approved by ethical committee of Bengbu Medical College, and a written informed consent was signed by each participant according to national and institutional guidelines.

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

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ORIGINAL ARTICLE

Central obesity is associated with helicobacter pylori infection: a large-scale cross-sectional retrospective study in West China

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Abstract

Context The association between obesity and Helicobacter pylori (*H. pylori*) infection remains controversial.

Aims The objective is to investigate the relationship between obesity and *H. pylori* infection, as diagnosed on the basis of a 14C urea breath test (14C-UBT) and waist circumference (WC).

Settings and design A retrospective cross-sectional study was performed at Health management center of a Tertiary care teaching hospital in Southwest of China.

Materials and methods Clinical information of 76,915 individuals (46,003 men and 30,912 women) with 44.0 (35.0–51.0) years was extracted from medical record. *H. pylori* infection was diagnosed by a positive 14C-UBT, and obesity was defined as WC ≥ 90 cm in men and ≥ 80 cm in women.

Statistical analysis Descriptive statistics, Student's *t* test, Mann-Whitney *U* test, and chi-square test, followed by binary logistic regression were performed in SPSS.

Results The overall prevalence of *H. pylori* infection was 39.95%. The prevalence of *H. pylori* infection in subjects with central obesity was significantly higher than that in normal-WC subjects (42.20% vs. 39.10%, $p < 0.001$). WC was significantly higher in *H. pylori*-positive subjects compared with *H. pylori*-negative subjects ($p < 0.01$). There was a linear association between WC quintiles and *H. pylori* infection prevalence. After adjusting for confounders, central obesity was associated with *H. pylori* infection (OR = 1.052, 95% CI, 1.009–1.096, $p = 0.02$).

Conclusions Central obesity is associated with *H. pylori* infection after adjusting for multiple confounding factors such as age, gender, and lifestyle characteristics.

Keywords Helicobacter pylori · Central obesity · ^{14}C urea breath test · Waist circumference

Background

Helicobacter pylori (*H. pylori*) infection affects almost half of the world's population. *H. pylori* infection contributes to a variety of gastrointestinal diseases, such as chronic gastritis, peptic ulcer, gastric cancer, and gastric mucosa-associated lymphoma (MALT). Additionally, *H. pylori* infection also plays a role in extragastric diseases, such as cardiovascular and immunological systemic disorders. Recently, chronic spontaneous urticaria and insulin resistance [1] have also been found to be associated with *H. pylori* infection. On the other hand, obesity is also a growing global health problem. But the relationship between *H. pylori* infection and obesity remains controversial [2–7]. Some studies identified a positive correlation [3, 8–10], while others found a negative correlation [4, 7] or even no correlation [2, 11]. A meta-analysis of 18 observational studies involving over 10,000 subjects found a higher body mass index (BMI) among *H. pylori*-positive subjects compared with *H. pylori*-negative subjects [12]. It is known that BMI has significant limitations in the assessment of obesity [6, 13]. Obesity can be assessed by several methods such as BMI and waist circumference (WC). In the majority of prior studies that explored the association between *H. pylori* infection and obesity, obesity was defined by BMI. For the first time, we used WC to define obesity to evaluate such a relationship. Both BMI and WC are commonly used to estimate body fat. But BMI does not distinguish fat mass from lean mass, nor does it necessarily reflect body fat distribution, particularly in those with BMI < 30 kg/m^2 [14]. When used in

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older people or muscular individuals such as athletes, BMI may lead to an inappropriate diagnosis of obesity, because it generally overestimates adiposity on those with more lean body mass [14]. ^{14}C urea breath test (^{14}C-UBT) was a simple invasive methods with high sensitivity 0.96 (95% CI 0.95–0.97) and specificity 0.93 (95% CI 0.91–0.94) to detect *H. pylori* infection [15]. In order to explore the association between *H. pylori* infection diagnosed by ^{14}C-UBT and obesity diagnosed on the basis of WC, we conducted a large-scale cross-sectional retrospective study in West China.

Methods

Data collection

Subjects who underwent ^{14}C-UBT in the Health Management Center of West China Hospital were enrolled into the study from December 2013 to December 2014. The analyses were limited to the participants who had complete records of anthropometric, biochemical data and had results of *H. pylori* ^{14}C-UBT test. A total of 76,915 individuals (46,003 men and 30,912 women) with a medium (interquartile ranges) age of 44.0 (35.0–51.0) years were involved in the final analysis. All subjects had the data about their medical history (hypertension and diabetes mellitus), smoking (current smoking defined as ≥ 20 cigarettes per month for ≥ 6 months) and alcohol use (current alcohol use defined as at least once per week for ≥ 6 months).

The measurement of WC, body weight, blood pressure, height, and hip circumference was recorded and documented. The measurement was performed by trained nurses. The participants were in a standing position with light clothes. WC was measured at the level of the iliac crest. All participants were asked to stand upright with arms hanging freely in normal clothing without shoes. All measurements were measured using the non-elastic tape on bare skin. WC was measured horizontally at the midpoint between the last rib and the iliac crest to the nearest 0.1 cm [16]. The average of three measurements was taken. Hip circumference was measured at the broadest part of the buttock. Height was measured by an inelastic ruler to the nearest 0.5 cm. Weight was measured to the nearest 0.1 kg in light clothing and without shoes using Weight scale (Wujin, RGT-120, Wujin Medical Equipment Co. Ltd., Jiangsu, China). BMI was calculated as weight/height^2 (kg/m^2). Blood pressure was measured with the sitting position by a sphygmomanometer (Yuyue, GB 3053-93, Yuyue Medical Equipment Co., Ltd., Jiangsu, China) on the right arm after a 5-min rest. The mean of two measurements at least 1 min apart was recorded.

Diagnosis of *H. pylori* infection

H. pylori infection was diagnosed by a positive ^{14}C-UBT. To avoid false-negative results, subjects were asked not to take any antibiotics for at least 4 weeks or any proton pump inhibitors or H_2 antagonists for at least 1–2 weeks before the test. The subjects took a ^{14}C-labeled urea containing tablet (Shanghai Xinke Pharmaceutical Co., Ltd., Shanghai, China) with 120-mL water after an overnight fast. The dose of radiation was as small as 1 μCi. After 15 min, the subject breathed out into a dry breath card (Anhui Yanghe Pharmaceutical Co., Ltd., Anhui, China) until the color of the card indicator changed from blue to white, which took about 2–5 min. Thereafter, the card was inserted into the YH04D machine (Anhui Yanghe Pharmaceutical Co., Ltd., Anhui, China) to detect the presence of *H. pylori*. Results were labeled as *H. pylori* positive or negative.

Diagnosis of obesity

Obesity was assessed according to waist circumference. For men, a WC of < 90 cm was defined as normal WC, and a WC ≥ 90 cm as central obesity. For women, a WC < 80 cm was defined as normal WC, and a WC ≥ 80 cm as central obesity [17]. All the subjects were classified into quintiles according to their WC: WC ≤ 73 cm, 73–81 cm, 81–88 cm, and ≥ 88 cm for quintile 1, 2, 3, and 4, respectively.

Blood test

Blood samples were collected from the antecubital vein after an overnight fast and were analyzed without freezing. The samples were centrifuged at 3000 rpm for 10 min, and then analyzed. Fasting plasma glucose (FPG), total cholesterol (TC), high-density lipoproteins cholesterol (HDL-C), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GGT), serum uric acid (UA), serum creatinine (Scr), and blood urea nitrogen (BUN) were analyzed by a biochemical autoanalyzer (ROCHE cobas 8000, ROCHE Diagnostics, Basel, Switzerland) at the Department of Clinical Laboratory Diagnostics, West China Hospital Sichuan University. Plasma glucose concentrations were assayed by the hexokinase method. TGs in plasma were assayed by means of glycerol oxidation. TC was measured by enzymic method. ALT, AST, and γ-GGT were assayed by means of the enzyme rate method. UA, Scr, and BUN were assayed by means of enzyme coupling method. HDL-C was measured by homogeneous enzyme colorimetry. Low-density lipoproteins cholesterol (LDL-C) was calculated using the Friedewald formula [18], i.e., LDL-C = TC − (HDL-C) − (TG/5).

Statistical analysis

All tests were performed using the IBM SPSS Statistics 19.0 (IBM Corp, New York, NY, USA). Data normality was tested by Kolmogorov-Smirnov test. Continuous variables were

presented as mean ± standard deviation for normal distributions, and medians (25th to 75th percentiles) for skewed distributions. Categorical variables were presented as percentage (%). Statistical analysis of continuous variables was performed using the Student's *t* test and Mann-Whitney *U* test, while analysis of categorical variables were performed using the chi-squared test. Binary logistic regression models were used to explore the correlation between central obesity and *H. pylori* infection after adjustment for a variety of subject's characteristics. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. A two-tailed *p* value of < 0.05 was considered statistically significant.

Results

Clinical and demographic characteristics

A total of 76,915 subjects (46,003 men [mean age, 45.19 ± 12.52 years] and 30,912 women [mean age, 43.14 ± 12.39 years]) were enrolled into this cross-sectional retrospective study (Table 1) The overall prevalence of *H. pylori* infection was 39.95%, 39.10% in normal-WC subjects, and 42.20% in subjects with central obesity, respectively ($\chi^2 = 61.613$, OR = 1.138, 95% CI, 1.102–1.175).

The characteristics of subjects with normal WC and central obesity are summarized in Table 1. The prevalence of *H. pylori* infection in subjects with central obesity was significantly higher than that in normal-WC subjects (42.20% vs. 39.10%, $p < 0.001$). Other parameters including age, height, BMI, WC, hip circumference (HC), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure(PP), fasting blood glucose (FPG), LDL-C, triglycerides (TG), total cholesterol (TC), uric acid (UA), serum creatinine (Scr), blood urea nitrogen (BUN), aminotransferase (ALT), γ-glutamyltransferase (γ-GGT), aspartate aminotransferase(AST), and prevalence of smoking, alcohol use, hypertension, and diabetes mellitus were significantly higher, but the HDL-C level was significantly lower in subjects with central obesity compared with normal-WC subjects (all $p < 0.01$).

Table 1 Comparison of clinical and demographic characteristics between subjects with normal WC and central obesity

	Total *n* = 76,915		Male *n* = 46,003		Female *n* = 30,912	
	Normal WC *n* = 55,839	Central obesity *n* = 21,076	Normal WC *n* = 32,048	Central obesity *n* = 13,955	Normal WC *n* = 23,791	Central obesity *n* = 7121
Age (years)	42.92 ± 12.31	48.19 ± 12.21	44.49 ± 12.76	46.79 ± 11.8	40.8 ± 11.35	50.95 ± 12.52
Height (cm)	163.05 ± 7.56	164.78 ± 8.47	167.31 ± 5.95	169.03 ± 6.05	157.31 ± 5.4	156.43 ± 5.92
BMI (kg/m^2)	22.49 ± 2.6	26.93 ± 2.71	23.55 ± 2.42	27.69 ± 2.38	21.07 ± 2.13	25.45 ± 2.71
Waist circumference (cm)	76.63 ± 7.75	91.69 ± 6.58	81.27 ± 5.84	94.82 ± 4.66	70.38 ± 5.19	85.57 ± 5.38
Hip circumference (cm)	91.65 ± 4.9	99.17 ± 5.31	93.26 ± 4.57	100.7 ± 4.62	89.48 ± 4.48	96.18 ± 5.3
Systolic blood pressure (mmHg)	111.16 ± 14.71	120.38 ± 16.2	114.66 ± 14.44	121.62 ± 15.31	106.46 ± 13.72	117.95 ± 17.55
Diastolic blood pressure (mmHg)	72.81 ± 9.46	78.29 ± 10.46	75.21 ± 9.48	80.13 ± 10.33	69.57 ± 8.4	74.71 ± 9.75
Pulse pressure (mmHg)	38.36 ± 10.35	42.09 ± 11.94	39.44 ± 10.69	41.5 ± 11.34	36.89 ± 9.7	43.25 ± 12.97
Fasting blood glucose (mmol/L)	5.21 ± 1.01	5.65 ± 1.47	5.33 ± 1.2	5.74 ± 1.6	5.04 ± 0.65	5.48 ± 1.15
Triglycerides (mmol/L)	1.45 ± 1.17	2.12 ± 1.67	1.72 ± 1.34	2.38 ± 1.84	1.09 ± 0.74	1.62 ± 1.1
LDL-cholesterol (mmol/L)	2.78 ± 0.77	2.99 ± 0.79	2.9 ± 0.77	3 ± 0.78	2.61 ± 0.74	2.98 ± 0.79
HDL cholesterol (mmol/L)	1.55 ± 0.41	1.32 ± 0.34	1.4 ± 0.36	1.22 ± 0.28	1.74 ± 0.38	1.53 ± 0.36
Total cholesterol (mmol/L)	4.78 ± 0.89	5 ± 0.94	4.85 ± 0.9	5 ± 0.93	4.67 ± 0.87	5.01 ± 0.95
Serum uric acid (μmol/L)	336.51 ± 87.67	379.04 ± 93.36	384.93 ± 75.58	417.08 ± 81.74	271.28 ± 54.15	304.48 ± 65.66
Creatinine (μmol/L)	76.17 ± 16.2	77.99 ± 16.16	85.93 ± 12.65	85.31 ± 13.19	63.02 ± 9.95	63.64 ± 10.99
Blood urea nitrogen (mmol/L)	4.97 ± 1.29	5.18 ± 1.31	5.22 ± 1.29	5.28 ± 1.29	4.63 ± 1.2	5 ± 1.32
Alanine aminotransferase (IU/L)	25.01 ± 18.06	35.2 ± 24.66	30.38 ± 20.18	40.71 ± 26.06	17.77 ± 11.21	24.38 ± 17.09
Aspartate aminotransferase (IU/L)	145.58 ± 20.44	148.83 ± 20.78	155.08 ± 18.46	156.5 ± 18.92	132.78 ± 15.37	133.8 ± 15.35
γ-Glutamyltransferase (IU/L)	28.58 ± 41.07	45.84 ± 55.49	37.27 ± 49.78	55.81 ± 61.39	16.87 ± 19.53	26.31 ± 33.95
H. pylori infection (%)	39.10	42.20	39.60	42.20	38.50	42.30
Smoking (%)	30.00	37.50	51.20	55.90	1.40	1.60*
Alcohol use (%)	45.30	53.70	72.80	76.80	8.40	8.60*
Hypertension (%)	5.10	14.60	6.70	14.70	3.00	14.40
Diabetes mellitus (%)	2.20	4.80	3.10	5.30	0.80	3.90

*$p > 0.05$

As shown in Table 2, parameters including age, height, BMI, WC, HC, SBP, DBP, PP, FPG, LDL-C, TG, TC, UA, Scr, BUN, ALT, γ-GGT, AST, and prevalence of smoking, alcohol use, hypertension, and diabetes mellitus were significantly higher, but the HDL-C level was significantly lower in the *H. pylori*-positive subjects with central obesity compared with *H. pylori*-positive and normal-WC subjects in both total and male subjects (all $p < 0.01$), except alcohol use, smoking, and creatinine in female ($p > 0.05$). *H. pylori*-positive with central obesity participants were related to a more bad metabolic profile than *H. pylori*-positive and normal-WC subjects.

The characteristics of *H. pylori*-positive and *H. pylori*-negative subjects are shown in Table 3. Multiple parameters including age, WC, HC, SBP, DBP, FBG, TG, LDL-C, TC, UA, creatinine, and prevalence of central obesity, smoking, alcohol use, hypertension, and diabetes mellitus were significantly higher, but the HDL-C level was significantly lower in *H. pylori*-positive subjects compared with *H. pylori*-negative subjects (all $p < 0.01$). Height, PP, ALT, AST, γ-GGT, and BUN were comparable between *H. pylori*-positive and *H. pylori*-negative subjects. Parameters including age, height, WC, HC, SBP, DBP, FBG, LDL-C, TC, UA, Scr, AST, and prevalence of central obesity, smoking, hypertension, and diabetes mellitus were significantly higher, but the HDL-C level was significantly lower in *H. pylori*-positive subjects compared with *H. pylori*-negative subjects (all $p < 0.01$). Height, PP, ALT, γ-GGT, TG, alcohol use, and BUN were comparable between *H. pylori*-positive and *H. pylori*-negative subjects in male. Characteristics including age, height, WC, HC, FBG, LDL-C, TC, AST, ALT, TG and prevalence of central obesity, smoking, hypertension, and diabetes mellitus were significantly higher in *H. pylori*-positive subjects compared with *H. pylori*-negative subjects (all $p < 0.01$). SBP, DBP, PP, γ-GGT, HDL-C, UA, Scr, hypertension, and BUN were comparable between *H. pylori*-positive and *H. pylori*-negative subjects in female.

A linear association between WC quintiles and *H. pylori* infection prevalence was detected. The prevalence was 37.76% among the subjects with WC in the first quintile and increased to 39.48%, 40.77%, and

Table 2 The association between *H. pylori*-positive with normal WC and *H. pylori*-positive with central obesity

	Total *H. pylori*-positive $n = 30,726$		Male *H. pylori*-positive $n = 18,559$		Female *H. pylori*-positive $n = 12,167$	
	Normal WC $n = 21,831$	Central obesity $n = 8895$	Normal WC $n = 12,676$	Central obesity $n = 5883$	Normal WC $n = 9155$	Central obesity $n = 3012$
Age (years)	45.31 ± 12.59	47.23 ± 11.55	45.31 ± 12.59	47.23 ± 11.55	41.88 ± 11.16	50.95 ± 11.91
Height (cm)	167.16 ± 5.9	168.97 ± 5.97	167.16 ± 5.9	168.97 ± 5.97	157.17 ± 5.41	156.6 ± 5.82
BMI (kg/m^2)	23.58 ± 2.41	27.75 ± 2.37	23.58 ± 2.41	27.75 ± 2.37	21.12 ± 2.14	25.46 ± 2.66
Waist circumference (cm)	81.42 ± 5.76	94.93 ± 4.74	81.42 ± 5.76	94.93 ± 4.74	70.52 ± 5.18	85.52 ± 5.2
Hip circumference (cm)	93.22 ± 4.54	100.7 ± 4.64	93.22 ± 4.54	100.7 ± 4.64	89.5 ± 4.43	96.28 ± 5.32
Systolic blood pressure (mmHg)	115 ± 14.59	121.69 ± 15.22	115 ± 14.59	121.69 ± 15.22	106.44 ± 13.8	117.73 ± 17.39
Diastolic blood pressure (mmHg)	75.51 ± 9.58	80.16 ± 10.27	75.51 ± 9.58	80.16 ± 10.27	69.53 ± 8.46	74.78 ± 9.77
Pulse pressure (mmHg)	39.49 ± 10.69	41.53 ± 11.22	39.49 ± 10.69	41.53 ± 11.22	36.91 ± 9.79	42.95 ± 12.79
Fasting blood glucose (mmol/L)	5.35 ± 1.29	5.75 ± 1.6	5.35 ± 1.29	5.75 ± 1.6	5.05 ± 0.64	5.49 ± 1.18
Triglycerides (mmol/L)	1.72 ± 1.36	2.38 ± 1.82	1.72 ± 1.36	2.38 ± 1.82	1.09 ± 0.8	1.63 ± 1.14
LDL-cholesterol (mmol/L)	2.92 ± 0.78	3.01 ± 0.78	2.92 ± 0.78	3.01 ± 0.78	2.65 ± 0.74	2.98 ± 0.8
HDL cholesterol (mmol/L)	1.39 ± 0.36	1.2 ± 0.28	1.39 ± 0.36	1.2 ± 0.28	1.74 ± 0.39	1.52 ± 0.36
Total cholesterol (mmol/L)	4.87 ± 0.91	4.99 ± 0.92	4.87 ± 0.91	4.99 ± 0.92	4.72 ± 0.88	5.01 ± 0.93
Serum uric acid (μmol/L)	385.07 ± 76.69	416.59 ± 80.88	385.07 ± 76.69	416.59 ± 80.88	272.03 ± 53.88	303.07 ± 65.69
Creatinine (μmol/L)	86.19 ± 12.76	85.37 ± 13.55	86.19 ± 12.76	85.37 ± 13.55	63.17 ± 9.99	63.58 ± 10.74*
Blood urea nitrogen (mmol/L)	5.22 ± 1.3	5.27 ± 1.29	5.22 ± 1.3	5.27 ± 1.29	4.65 ± 1.2	4.98 ± 1.29
Alanine aminotransferase (IU/L)	30.06 ± 19.74	40.31 ± 25.48	30.06 ± 19.74	40.31 ± 25.48	18.11 ± 11.8	24.42 ± 16.52
Aspartate aminotransferase (IU/L)	154.91 ± 18.41	155.98 ± 19.67	154.91 ± 18.41	155.98 ± 19.67	132.3 ± 15.83	133.87 ± 15.34
γ-Glutamyltransferase (IU/L)	37.09 ± 51.54	55.73 ± 63.99	37.09 ± 51.54	55.73 ± 63.99	17.07 ± 19.47	25.98 ± 28.57
Smoking (%)	31.30	37.90	52.70	56.40	1.60	1.80*
Alcohol use (%)	45.90	53.90	72.80	76.80	8.70	9.50*
Hypertension (%)	5.40	14.90	6.90	15.60	3.20	13.60
Diabetes mellitus (%)	2.40	5.10	3.30	5.60	1.10	4.20

*$p > 0.05$

Table 3 Comparison of clinical and demographic characteristics between *H. pylori*-positive and *H. pylori*-negative subjects

	Total $n = 76,915$		Male $n = 46,003$		Female $n = 30,912$	
	H. pylori-negative $n = 46,189$	*H. pylori*-positive $n = 30,726$	*H. pylori*-negative $n = 27,444$	*H. pylori*-positive $n = 18,559$	*H. pylori*-negative $n = 18,745$	*H. pylori*-positive $n = 12,167$
Age (years)	43.8 ± 12.67	45.21 ± 12.22	44.69 ± 12.64	45.92 ± 12.3	42.49 ± 12.59	44.13 ± 12.01
Height (cm)	163.54 ± 7.88	163.5 ± 7.82*	167.9 ± 6.06	167.73 ± 5.98	157.16 ± 5.55	157.03 ± 5.52
BMI(kg/m^2)	23.63 ± 3.28	23.83 ± 3.31	24.74 ± 3.05	24.9 ± 3.08	22.01 ± 2.92	22.19 ± 2.95
WC (cm)	80.49 ± 10.02	81.16 ± 10.03	85.16 ± 8.3	85.7 ± 8.32	73.65 ± 8.24	74.23 ± 8.29
Hip circumference (cm)	93.62 ± 6.02	93.84 ± 6.05	95.47 ± 5.7	95.59 ± 5.75	90.92 ± 5.43	91.18 ± 5.51
SBP (mmHg)	113.48 ± 15.61	114 ± 15.77	116.53 ± 15.01	117.12 ± 15.12	109.03 ± 15.41	109.23 ± 15.55*
DBP (mmHg)	74.15 ± 9.98	74.55 ± 10.13	76.51 ± 9.98	76.98 ± 10.04	70.7 ± 8.93	70.83 ± 9.09*
Pulse pressure (mmHg)	39.33 ± 10.94	39.45 ± 10.95*	40.02 ± 10.95	40.14 ± 10.9*	38.32 ± 10.85	38.41 ± 10.93*
FBG (mmol/L)	5.31 ± 1.14	5.35 ± 1.22	5.44 ± 1.3	5.48 ± 1.41	5.14 ± 0.8	5.16 ± 0.83
TG (mmol/L)	1.62 ± 1.34	1.65 ± 1.38	1.91 ± 1.53	1.93 ± 1.55*	1.2 ± 0.83	1.22 ± 0.92
LDL-cholesterol (mmol/L)	2.82 ± 0.78	2.86 ± 0.78	2.92 ± 0.78	2.95 ± 0.78	2.67 ± 0.76	2.74 ± 0.77
HDL cholesterol (mmol/L)	1.49 ± 0.4	1.47 ± 0.41	1.35 ± 0.35	1.33 ± 0.35	1.7 ± 0.39	1.69 ± 0.39*
Total cholesterol (mmol/L)	4.82 ± 0.91	4.86 ± 0.91	4.89 ± 0.91	4.91 ± 0.92	4.72 ± 0.9	4.79 ± 0.9
Serum uric acid (μmol/L)	347.35 ± 91.18	349.39 ± 91.38	394.43 ± 78.55	395.06 ± 79.41	278.42 ± 58.77	279.72 ± 58.58*
Creatinine (μmol/L)	76.48 ± 16.13	76.96 ± 16.32	85.62 ± 12.68	85.93 ± 13.02	63.09 ± 10.21	63.27 ± 10.18*
Blood urea nitrogen (mmol/L)	5.02 ± 1.3	5.04 ± 1.3*	5.24 ± 1.29	5.24 ± 1.3*	4.71 ± 1.24	4.73 ± 1.23*
ALT (IU/L)	27.73 ± 20.75	27.91 ± 20.36*	33.65 ± 22.89	33.31 ± 22.24*	19.05 ± 12.91	19.67 ± 13.4
AST (IU/L)	146.57 ± 20.43	146.32 ± 20.83*	155.68 ± 18.47	155.25 ± 18.83	133.22 ± 15.14	132.69 ± 15.72
γ-GGT (IU/L)	33.11 ± 45.17	33.6 ± 47.53*	42.82 ± 52.69	43 ± 56.46*	18.9 ± 24.94	19.28 ± 22.41*
Central obesity (%)	26.40	28.90	59.70	40.30	21.90	24.80
Smoking (%)	31.30	33.20	51.80	53.90	1.30	1.70
Alcohol use (%)	4.20	48.20	73.90	74*	8.10	8.90
Hypertension (%)	7.50	8.10	8.80	9.70	5.50	5.80*
Diabetes mellitus (%)	2.70	3.20	3.60	4	1.35	1.80

*$p > 0.05$

42.05% in quintile 2, 3, and 4, respectively (p for trend < 0.001). These results in Table 4 showed that individuals with a higher WC had an increased *H. pylori* infection risk.

As shown in Table 5, after adjusting for age, gender, HC, SBP, DBP, FBG, TG, LDL-C, HDL-C, TC, UA, Scr, smoking, alcohol use, hypertension, and diabetes mellitus, central obesity was still found to be positively correlated with *H. pylori* infection (OR = 1.052, 95% CI, 1.009–1.096, $p = 0.02$).

As shown in Tables 6 and 7, after adjusting for age, gender, HC, SBP, DBP, FBG, TG, LDL-C, HDL-C, TC, UA, Scr, smoking, alcohol use, hypertension, and diabetes mellitus, age, central obesity, SBP, hypertension, diabetes mellitus, alcohol use were associated with *H. pylori* infection in female, while age, central obesity, SBP, DBP,TG, HDL cholesterol, and smoking were associated with *H. pylori* infection in male.

Central obesity was still found to be positively correlated with *H. pylori* infection in male (OR = 1.049, 95% CI, 1.004–

Table 4 Association of WC with prevalence rate of Helicobacter pylori infection

WC	Total	*H. pylori* infection	*H. pylori* prevalence rate (%)	*H. pylori* prevalence ratio	χ^2	p value
Quintile 1	20,044	7569	37.76	1.00	79.04	0.000
Quintile 2	19,710	7782	39.48	1.05		
Quintile 3	19,576	7981	40.77	1.08		
Quintile 4	17,585	7394	42.05	1.11		

Table 5 Analysis of the relationship between *H. pylori* infection and central obesity: univariate and multivariate analysis

	Unadjusted			Adjusted		
	p value	OR	95% CI	*p* value	OR	95% CI
Age	0.00	1.009	1.008–1.010	0.00	1.010	1.009–1.011
Gender	0.01	0.960	0.932–0.989	0.00	1.126	1.066–1.19
Central obesity	0.00	1.138	1.102–1.175	0.02	1.052	1.009–1.096
Systolic blood pressure	0.00	1.002	1.001–1.003	0.00	0.996	0.995–0.997
Diastolic blood pressure	0.00	1.004	1.002–1.005	0.00	1.005	1.003–1.007
Triglycerides	0.01	1.015	1.004–1.026	0.02	0.971	0.948–0.995
HDL cholesterol	0.00	0.888	0.857–0.921	0.00	0.844	0.784–0.907
Smoking	0.00	1.091	1.058–1.125	0.00	1.083	1.043–1.125
Alcohol use	0.01	1.041	1.012–1.072	0.02	1.048	1.008–1.09
Hypertension	0.00	1.096	1.039–1.156	0.07	0.946	0.892–1.004
Diabetes mellitus	0.00	1.069	1.026–1.113	0.02	1.050	1.007–1.095

OR odds ratio, *CI* confidence interval

1.096, $p = 0.032$) and female (OR = 1.075, 95% CI, 1.011–1.143, $p = 0.02$), respectively.

Discussion

Our study found that the prevalence of *H. pylori* infection was significantly higher in subjects with central obesity (42.20%) compared with that in normal-WC subjects (39.10%). Similarly, a small-sample study of 103 obese subjects by Arslan et al [19] reported that the prevalence of *H. pylori* infection was greater in obese subjects (57.2%) versus the control group (27%). An association between central obesity and *H. pylori* infection was observed in our study, which was also consistent with previous findings. A study involving 2913 Danish adults [20] found that the seroprevalence of *H. pylori* infection was increased in persons with high BMI. Another study from Iceland [21] also revealed that *H. pylori* infection was associated with overweight. Moreover, several studies [3, 8–10, 22] based on Chinese populations have also drawn a similar conclusion that obesity is linked to *H. pylori* infection. A study conducted in Central China indicated that the prevalence of *H. pylori* infection increased with BMI. Yang et al [10] showed that *H. pylori* infection confirmed by gastric biopsy pathology was associated with obesity assessed by BMI in an elderly Chinese population. Guo et al. [9] reported that waist circumference played an important role in *H. pylori* infection which was detected by serous *H. pylori* IgG antibodies; and the vacuolating cytotoxin gene A (VacA) was the most common strain in Northern China. However, some studies found that *H. pylori* infection had no association with BMI. A case-control study [23] in patients with morbid obesity from Taiwan revealed an opposite connection between morbid obesity and *H. pylori* seropositivity. A study [24] including 370 severely obese patients showed that the BMI of *H. pylori*-positive subjects did not differ from that of *H. pylori*-negative subjects. Ioannou et al. [25] noted that the status of *H. pylori*/CagA antibody was not related with obesity. A study from Greece [26] revealed that the risk of *H. pylori* infection was not increased among obese young individuals. Another study [27] consisting of 801 healthy university students showed that there was no significant difference in the BMI between subjects with and without *H. pylori*

Table 6 Multivariate analysis of the relationship between *H. pylori* infection and central obesity in female

	p	OR	95% CI
Age (years)	0.000	1.013	1.011–1.016
Central obesity	0.020	1.075	1.011–1.143
SBP (mmHg)	0.000	0.994	0.992–0.996
Hypertension	0.031	0.885	0.792–0.989
Diabetes mellitus	0.015	1.288	1.051–1.578
Alcohol use	0.024	1.102	1.013–1.199

Table 7 Multivariate analysis of the relationship between *H. pylori* infection and central obesity in male

	p	OR	95% CI
Age (years)	0.000	1.009	1.007–1.011
Central obesity	0.032	1.049	1.004–1.096
SBP (mmHg)	0.024	0.998	0.996–1.000
DBP (mmHg)	0.000	1.006	1.003–1.008
TG (mmol/L)	0.005	0.961	0.934–0.988
HDL cholesterol (mmol/L)	0.000	0.762	0.695–0.835
Smoking	0.000	1.082	1.041–1.124

infection based on the presence or absence of specific IgG *H. pylori* antibodies in urine. But our study has shown that the BMI and WC in *H. pylori*-positive subjects were higher than in *H. pylori*-negative subjects ($p < 0.05$) and central obesity is associated with *H. pylori* infection after adjusting for multiple confounding factors such as age, gender, and lifestyle characteristics. On the one hand, obesity is known to increase the risk of infections [28]. Immune cell dysfunction due to obesity may result in weakened host defense [29]. Many studies in animal models and human subjects have shown that obesity can change both innate and adaptive immunity. Excess adiposity is associated with impaired immune response. Central obesity may be a predisposing factor to *H. pylori* infection. On the other hand, as virus can lead to infectobesity, *H. pylori* may be a contributing factor to obesity. The virus induces obesity probably by means of interacting with the hypothalamic-pituitary-adrenal axis [30], while the possible mechanisms of *H. pylori*-induced obesity include interruption of leptin secretion, glucose uptake, and inflammation [31], *H. pylori* infection can induce chronic low-grade inflammation, and then prolonged, low-level immune stimulation induces hypertrophy of adipose tissue and increases shunting of energy to host defense mechanisms [32]. Our study found that the FPG, TG, LDL-C, and TC levels were higher in *H. pylori*-positive subjects than those in *H. pylori*-negative subjects. Thus, we speculate that *H. pylori* infection contributes to obesity by disrupting glycometabolism and lipid metabolism.

These conflicting findings may be explained by differences in the diagnostic methods of *H. pylori* infection, sample size, target populations, ethnic groups, geographic regions, and *H. pylori* strains. Most of these published studies diagnosed *H. pylori* infection by serology which has a low diagnostic sensitivity (85%) and specificity (79%), only useful to rule out *H. pylori* infection. The urine *H. pylori* antibody test [33] only detects exposure status rather than active infection, with a sensitivity of 86% and a specificity of 91%. In our study, *H. pylori* infection was defined by ^{14}C-UBT, the most reliable noninvasive method that detects active *H. pylori* infection with a sensitivity of 96% and a specificity of 93%.

In terms of sample size and target population, most prior studies only enrolled hundreds of or no more than ten thousand participants, but in our study, a large population of 76,915 individuals (46,003 men and 30,912 women) with a medium (interquartile ranges) age of 44.0 (35.0–51.0) years were recruited. We focused on the adult population in West China, while some other studies [34] targeted at children and adolescents. It has been shown that race, geographic region and *H. pylori* strain may be important factors affecting the association between obesity and *H. pylori* infection. A US study [25] found that the prevalence of *H. pylori* infection and CagA strains differed among non-Hispanic blacks, whites, and Mexican-Americans.

One of the strengths of our study is that a large population consisting of 76,915 subjects from West China was included in this cross-sectional retrospective survey. All of these participants underwent ^{14}C-UBT and we controlled for multiple covariates to explore the association between obesity and *H. pylori* infection. Nevertheless, there are also several limitations of our study. Firstly, as this is a cross-sectional retrospective study, the casual relationship between *H. pylori* infection and central obesity cannot be assessed. Secondly, due to the very small but calculable dose of radiation, ^{14}C-UBT is not appropriate for use in children [35]. Consequently, data on children is not available in the present study, and our findings could not be generalized to the entire population. Thirdly, the *H. pylori* strain was not determined in this study, and thus the association between different *H. pylori* strains and obesity cannot be explored.

Conclusion

Our study based on a large Chinese population in West China reveals that central obesity is associated with *H. pylori* infection after adjusting for multiple confounding factors such as age, gender, and lifestyle characteristics.

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Contributorship statement Qinqin Wu designed the study, carried out the study, analyzed the results, and contributed to the discussion of results and drafting of the manuscript. Ken Qin analyzed and interpreted the data in the revised version. Youjuan Wang contributed to designing the study and discussion of results, and the final manuscript. All authors have read and approved the final manuscript.

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

Compliance with ethical standards

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

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

The study was permitted by the Ethics Committee of West China, Sichuan University.

Consent for publication Not applicable.

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ORIGINAL ARTICLE

The prevalence of prediabetes and associated conditions in Ahmedabad population

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Abstract

Objectives Prediabetes is the borderline of diabetes, the most common disease worldwide, and the prevalence appears to be increasing with weight. Thus, the present study was planned to study the prevalence of prediabetes and its association with body mass index, blood pressure and lipid abnormalities in apparently healthy school, college-going students and adult population.

Methods It was a population-based cross-sectional study, conducted on 2412 subjects of Ahmedabad of age 12 to 55 years. Body mass index, blood pressure and prediabetes were estimated using standard protocol. Lipid profile, vitamin D, insulin and C-reactive protein were estimated by a trained lab technician. Subjects with fasting blood sugar level ≥ 100 mg/dL and ≤ 125 mg/dL were identified as prediabetics. The data was analysed using SPSS 20 by Pearson's chi-square test and one-way analysis of variance.

Results Prediabetes prevalence was 5.09% in 12–17 years age group followed by 28.81% and 33.19% in 18–35 and 36–55 years age group, respectively. Prediabetes was found higher amongst overweight, obese, pre-hypertensive and hypertensive participants than healthy participants. The prevalence of prediabetes was found higher amongst participants who do not indulge in exercise. Junk food and sweet eating frequencies, stress level and socioeconomic status, lipid abnormalities, vitamin D deficiency, hyperinsulinemia and elevated C-reactive protein were found significantly associated with prediabetes. Prediabetes is a lifestyle disorder and its prevalence has increased at an alarming rate with age.

Conclusion Prediabetes and its associated risk factors were found to be common in the city of Ahmedabad which suggests the need for greater public awareness programmes on these morbidities.

Keywords Prediabetes · Body mass index · Hypertension · Lipid abnormalities · Vitamin D deficiency · Hyperinsulinemia · Ahmedabad

Introduction

Globally, the number of people with diabetes is increasing from 451 million in 2017 to 693 million in 2045 [1]. According to International Diabetes Federation in Diabetes Atlas 2006, in India, currently, more than 50 million people had diabetes, and it is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken [2]. Studies showed an increasing prevalence of type 2 diabetes amongst adults in all the regions like in Kerala, Chennai, Bangalore, Hyderabad, Kolkata and Mumbai [3]. Because of the increasing burden of the disease and its complications, efforts are required to prevent its complications with earlier diagnosis and treatment. Prediabetes broadly refers to an intermediate stage between complete normal blood glucose level and the clinical stage of type 2 diabetes mellitus by considering two parameters, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). The American Diabetes Association (ADA) defined the same value for impaired glucose tolerance (140 to 200 mg/dL), whereas the WHO defined a lower value for impaired fasting glucose (100–125 mg/dL). ADA has one more additional criteria to define prediabetes is hemoglobin

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A1c (HbA1c) level of 5.7 to 6.4% [4]. The prevalence of prediabetes is expected to increase to 471 million globally by 2035 as reported by an International Diabetes Federation [5]. The conversion rate of diabetes from IFG alone in the Hoorn study, the Paris prospective study and an Italian study was respectively 33%, 2.7% and 9.1%, whereas that of diabetes from IFG and IGT was 64.5%, 14.9% and 44.4% [6]. Anjana et al. reported prediabetes to type 2 diabetes mellitus progression rate of 78.9 per 1000 person-years in a cohort of 299 prediabetes individuals followed for 9.1 years, from southern India [7]. Similarly, Dutta et al. found 71.52 per 1000 person-years in a cohort of 144 individuals with prediabetes, followed for 32 months from eastern India [8]. The conversion rate into diabetes was found highest (72.7%) in a Brazilian-Japanese population with IGT and IFG over 7 years [9]. The transition from prediabetes to diabetes is higher and by characterising established metabolic, and other markers accompanying transition to overt type 2 diabetes should yield a better understanding of the precipitating risk factors amongst those with prediabetes [10]. Thus, early screening of prediabetes is of great significance for early detection and reducing the incidence of diabetes. The data on the prevalence of prediabetes is scanty, and there are hardly any studies providing the prevalence of prediabetes and its association with risk factors in the Indian population. Therefore, the objective of our study was to determine the prevalence of prediabetic status and its associated risk factors in the Ahmedabad population.

Methodology

This cross-sectional, multi-centric, observational study was conducted amongst the 2412 subjects of both gender in the age group of ≥ 12 to 55 years, residing in the urban community in Ahmedabad, to find out prevalence of prediabetes and its association with body mass index, blood pressure, dietary type, participation in exercise, stress level, socioeconomic status, lipid abnormalities, vitamin D deficiency, hyperinsulinemia and elevated C-reactive protein from December 2013 to December 2016. The subjects were divided into three groups, i.e. school-going children (12 to 17 years), young aged (18 to 35 years) and adult aged (36 to 55 years). Subjects from different zones in the city were included to avoid bias and to get an equal distribution of subjects by socioeconomic state, ethnic variability and gender.

The sample size for this study was calculated from the ICMR-INDIAB study phase I conducted by Anjana et al. in 2011 on the rural and urban population of four states; Tamil Nadu, Maharashtra, Jharkhand and Chandigarh [11] using a single cross-sectional survey formula [12]. The current study has considered the prevalence of Tamil Nadu state (8.3%), and the sample size was calculated by a single cross-sectional survey formula. The estimated sample size was 2030.

The study excluded subjects with any previously diagnosed chronic ailments such as diabetes, hypertension, chronic obstructive pulmonary disease, asthma, cancer, hyperparathyroidism, hypocalcemia, sarcoidosis, chronic kidney disease, a significant chronic medical condition that would interfere with study participation, pregnant or lactating women or subjects who are under any drug therapy.

The participants were not forced for their participation in the study. Height and weight were measured with a stadiometer and a digital weighing scale, respectively; body mass index (BMI) was calculated. Blood pressure (BP) measurements were measured in a sitting position by an auscultatory method using a standard mercury sphygmomanometer. The proforma was prepared with close-ended questions. The questions were explained to all the participants by the investigator to gather information regarding contained details about the participation in exercise, indoor games, outdoor games, playing on laptop or mobile and watching television with either yes or no answer. Dietary type was classified as a vegetarian (vegetables only), non-vegetarian (vegetables, eggs and non-vegetables) and eggetarian (vegetables and eggs) diet. Frequencies of junk food and sweet eating habit were categorised as every day, once in a week, once in 15 days and once in a month. Stress level was categorised as no stress at all, less level of stress, medium level of stress and high level of stress. Socioeconomic class was defined using education, occupation and monthly family income score as described by Gururaj et al. [13]. Clinical examination of the subjects was carried out by taking their blood samples. We have referred the seventh report of the joint national committee on prevention, detection and treatment of high blood pressure (JNC 7) criteria for pre-hypertension and hypertension. A systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80–89 mmHg was/were determined as a pre-hypertension case whilst systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg was considered as hypertension [14]. For serum lipids, we referred to NCEP-ATP III (National Cholesterol Education Program) guidelines. According to these standard guidelines, hypercholesterolemia is defined as total cholesterol more than 200 mg/dL, hypertriglyceridemia as triglycerides more than 150 mg/dL and HDL-C less than 40 mg/dL in men and less than 50 mg/dL in women. Dyslipidemia is defined by the presence of hypercholesterolemia and low HDL-C levels [15]. Subjects with vitamin D level less than 20 ng/dL were identified as vitamin D deficiency in this study. High-sensitivity C-reactive protein (hs-CRP) concentration was assayed on the Roche Cocas c111 analyser (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Subjects with CRP more than 3 mg/L were considered as having elevated CRP. We referred "Williams Textbook of

Endocrinology, 12th edition by Melmed S et al published in 2011" for hyperinsulinemia. A fasting insulin level < 25 mIU/L (< 174 pmol/L) is considered normal [16].

Statistical analysis

The collected data were thoroughly cleaned and entered in MS Excel spreadsheets for analysis. The statistical analysis was done using SPSS software version 20. Comparison of categorical data between groups was done by Pearson's chi-square test, whilst the comparison of mean values between groups was tested by one-way analysis of variance (ANOVA). Significance for each analysis is set at a p value of 0.05.

Result

A total number of 2412 subjects with age group between 12 and 55 years from different zones of Ahmedabad were selected for the study. Out of 2412 subjects, 456 participants were school-going children and age of these school-going children was between 12 and 17 years; 1010 participants were of 18–35 years age group and 946 participants were of 36–55 years age group. These subjects underwent for laboratory investigations. Prevalence of prediabetes in school-going children (12–17 years) was 5.09% followed by 28.81% and 33.19% in 18–35-years and 36–55 years age group, respectively.

Association of prediabetes with blood pressure, body mass index, family history of diseases, participation in exercise, participation in indoor and outdoor games, playing on laptop/mobile, watching television, dietary type, stress level and socioeconomic class

In the present study, the higher prediabetes prevalence was found amongst pre-hypertensive and hypertensive participants compared with participants with normal blood pressure, and the association between prediabetes and higher blood pressure was found significant amongst all three age groups. The prevalence of prediabetes amongst overweight and obese participants was noted higher when compared to healthy weight participants amongst all three age groups. None of the underweight participants reported having prediabetes in this study. The mean BMI amongst prediabetes participants of 12–17 years, 18–35 years and 36–55 years age group was documented 25.6 ± 0.688 kg/m^2, 25.8 ± 0.147 kg/m^2 and 25.7 ± 0.220 kg/m^2, respectively. In 12–17 years age group, the prevalence of prediabetes was noted higher amongst participants with a positive family history of obesity, whilst the prevalence was higher amongst participants with a negative family history of diabetes and hypertension. In 18–35 years age groups, 48.44%, 43.75%, 34.75% and 46.46% subjects with prediabetes had a positive family history of diabetes, obesity, thyroid and hypertension, respectively. Similarly, in 36–55 years age group, the prevalence of a family history of diabetes was 37.10%, hypertension was 43.70%, obesity was 38.79% and thyroid was 10.78% amongst prediabetes participants. The positive significant association was noted between participation in exercise and prediabetes demonstrating the higher prevalence of prediabetes amongst participants who denied to indulge in exercise; 7.89%, 64.22% and 37.63% amongst 12–17, 18–35 and 36–55 years age groups, respectively. In 12–17 years age group, the present study identified a higher prevalence of prediabetes amongst subjects who participated in the indoor game whereas the indistinguishable prevalence of prediabetes was found amongst subjects who participated in outdoor games. Whereas, in the age group of 18–35 years and 36–55 years, the prevalence of prediabetes was higher amongst participants who did not participate in indoor (30.24% and 29.55%, respectively) and outdoor games (34.16% and 33.98%, respectively) compared to those who participate in indoor and outdoor games. All the study participants agreed to play on laptop/mobile and watch television for more than 1 h in a day. Amongst all the dietary types, the prevalence of prediabetes was noted higher amongst non-vegetarian participants of 12–17 years (24.00%), 18–35 years (56.16%) and 36–55 years (66.07%). However, the association between dietary type and prediabetes was not found significant in 12–17 years age group. We noted that frequency of junk food had no significant association with incident prediabetes in 12–17 years age group, whilst the greater percentage of prediabetes participants of 18–35 years and 36–55 years age groups reported to eat junk food once in a week. The prevalence of prediabetes was reported higher amongst participants who eat sweets every day in all three age groups; 7.76%, 35.84% and 42.79% amongst 12–17, 18–35 and 36–55-year age group, respectively. Furthermore, frequencies of eating sweets are found to have a significant association with risk of developing prediabetes amongst all three age groups in the current study. The level of stress is found to be significantly associated with the risk of prediabetes amongst all three age groups, showing a higher prevalence of prediabetes amongst participants with a medium level of stress than no stress at all. Socioeconomic class such as upper, upper middle, lower middle, upper lower and lower was found significantly associated with prediabetes amongst participants of 12–17 years, 18–35 years and 36–55 years of age groups demonstrating a higher prevalence of prediabetes amongst upper socioeconomic class (Table 1).

Table 1 the association of prediabetes with blood pressure, body mass index, participation in exercise, dietary type, stress level and socioeconomic class

Parameters		Age groups (in years)		
		12–17	18–35	36–55
Blood pressure (mmHg)	Normal	2 (0.53%)	92 (18.4%)	64 (17.43%)
	Pre-hypertension	13 (23.21%)	124 (44.44%)	123 (44.40%)
	Hypertension	8 (34.78%)	75 (32.46%)	127 (42.05%)
	Statistical analysis (*p* value)	0.018*	0.002*	< 0.001*
BMI	Underweight	0	0	0
	Healthy weight	11 (3.94%)	159 (22.64%)	146 (24.25%)
	Overweight	5 (6.75%)	103 (55.08%)	138 (55.64%)
	Obese	7 (28.00%)	29 (55.76%)	30 (58.82%)
	Statistical analysis (*p* value)	0.012*	< 0.001*	< 0.001*
Family history of diabetes	Present	6 (3.70%)	187 (48.44%)	259 (73.78%)
	Absent	17 (5.70%)	104 (16.66%)	55 (9.24%)
	Statistical analysis (*p* value)	0.022*	< 0.001*	< 0.001*
Family history of obesity	Present	14 (7.10%)	182 (43.75%)	229 (62.39%)
	Absent	9 (3.47%)	109 (18.35%)	85 (14.68%)
	Statistical analysis (*p* value)	0.297	< 0.001*	< 0.001*
Family history of thyroid	Present	2 (5.55%)	49 (34.75%)	41 (40.19%)
	Absent	21 (5.00%)	242 (27.84%)	273 (27.84%)
	Statistical analysis (*p* value)	< 0.0001*	< 0.001*	< 0.001*
Family history of hypertension	Present	5 (3.03%)	184 (46.46%)	283 (68.35%)
	Absent	18 (6.18%)	107 (17.42%)	31 (17.42%)
	Statistical analysis (*p* value)	0.0007*	< 0.001*	< 0.001*
Participation in exercise (1–3 h/week)	Yes	11 (3.61%)	47 (14.32%)	37 (17.61%)
	No	12 (7.89%)	244 (13.77%)	277 (37.63%)
	Statistical analysis (*p* value)	0.043*	< 0.001*	< 0.001*
Participation in indoor games (1–3 h/week)	Yes	9 (7.37%)	23 (18.54%)	11 (18.64%)
	No	14 (4.19%)	268 (30.24%)	303 (34.16%)
	Statistical analysis (*p* value)	0.297	< 0.001*	< 0.001*
Participation in outdoor games (1–3 h/week)	Yes	13 (5.05%)	43 (25.14%)	19 (24.35%)
	No	10 (5.02%)	248 (29.55%)	295 (33.98%)
	Statistical analysis (*p* value)	0.532	< 0.001*	< 0.001*
Playing on laptop/mobile (> 1 h/day)	Yes	23 (5.04%)	291 (28.81%)	311 (40.23%)
	No	0	0	3 (1.73%)
	Statistical analysis (*p* value)	< 0.001*	< 0.001*	< 0.001*
Watching television (> 1 h/day)	Yes	23 (5.04%)	291 (28.81%)	314 (33.19%)
	No	0	0	0
	Statistical analysis (*p* value)	< 0.001*	< 0.001*	< 0.001*
Dietary pattern	Vegetarian	12 (3.16%)	126 (21.53%)	198 (29.50%)
	Non-vegetarian	6 (24.0%)	41 (56.16%)	37 (66.07%)
	Eggetarian	5 (9.61%)	124 (35.27%)	79 (36.07%)
	Statistical analysis	0.153	< 0.001*	< 0.001*

Table 1 (continued)

Parameters		Age groups (in years)		
		12–17	18–35	36–55
	(*p* value)			
Junk food eating frequency	Everyday	1 (1.58%)	20 (14.38%)	47 (46.07%)
	Once in a week	10 (4.34%)	263 (41.87%)	232 (47.05%)
	Once in 15 days	7 (7.86%)	8 (4.30%)	26 (12.20%)
	Once in a month	5 (6.75%)	0	9 (6.52%)
	Statistical analysis (*p* value)	0.059	< 0.001*	< 0.001*
Sweet eating frequency	Everyday	8 (7.76%)	95 (35.84%)	98 (42.79%)
	Once in a week	11 (5.09%)	160 (29.79%)	187 (40.74%)
	Once in 15 days	3 (3.84%)	28 (20.14%)	22 (11.82%)
	Once in a month	1 (1.69%)	8 (11.59%)	7 (9.72%)
	Statistical analysis (*p* value)	0.012*	< 0.001*	< 0.001*
Stress level	No stress	16 (4.13%)	197 (26.30%)	156 (28.51%)
	Low level	5 (9.25%)	68 (35.05%)	144 (39.13%)
	Medium level	2 (13.33%)	26 (38.80%)	14 (45.16%)
	Statistical analysis (*p* value)	< 0.001*	< 0.001*	< 0.001*
Socioeconomic status	Upper	9 (10.97%)	117 (33.81%)	117 (38.48%)
	Upper middle	13 (4.54%)	145 (30.72%)	174 (33.65%)
	Lower middle	1 (4.34%)	14 (28.57%)	7 (25.0%)
	Upper lower	0	15 (13.15%)	14 (18.18%)
	Lower	0	0	2 (10.0%)
	Statistical analysis (*p* value)	< 0.001*	< 0.001*	< 0.001*

Analysed by one-way chi-square test for goodness of fit. * indicates statistical significance at *p* value less than 0.05

Association of prediabetes with various biochemical parameters

None of the subjects in the age group of 12–17 years found to have any of the lipid abnormalities. However, the mean level of cholesterol and triglycerides were higher whilst mean HDL-C was lower amongst prediabetes participants than participants with a normal blood sugar level. Similarly, mean cholesterol and triglycerides levels were found to be significantly higher whilst the low level of HDL-C amongst prediabetes participants than non-prediabetes participants in both 18–35 years and 36–55 years age group. Additionally, the prevalence of lipid abnormalities such as hypercholesterolemia, hypertriglyceridemia, low HDL-C level and dyslipidemia in prediabetes subjects was 34.02%, 29.89%, 44.32% and 27.83%, respectively, amongst the 18–35 years old participants. Whereas prediabetes participants of aged 36–55 years reported having 39.17% prevalence of hypercholesterolemia, 36.6% hypertriglyceridemia, 45.22% low HDL-C and 35.98% dyslipidemia. The prevalence of vitamin D deficiency amongst 12–17 years, 18–35 years and 36–55 years old participants was 86.95%, 88.65% and 84.39%, respectively. We further determined that the mean vitamin D level amongst all age groups was found significantly low amongst prediabetes participants compared with normal participants, demonstrating an association between prediabetes and vitamin D deficiency. None of the participants of 12–17 years age group found to have hyperinsulinemia, whilst the prevalence of hyperinsulinemia was 34.02% in 18–35 years, and 49.36% in 36–55 years age groups was noted in the current study. The mean insulin level was found significantly elevated amongst prediabetes participants than normal participants, and the association of prediabetes with hyperinsulinemia was found significant in 12–17, 18–35 and 36–55 years age groups. The prevalence of elevated C-reactive protein amongst 12–17 years, 18–35 years and 36–55 years was 8.69%, 28.86% and 41.71%, respectively. The mean value to C-reactive protein amongst prediabetes participants of age group 12–17, 18–35 and 36–55 years was significantly higher in comparison to prediabetes free participants (Table 2).

Table 2 The levels of vitamin D, insulin, CRP and lipid amongst subjects with prediabetes and normal (non-prediabetes) subjects

Parameters		Age groups (in years)		
		12–17 Mean ± SD	18–35	36–55
Vitamin D (ng/dL)	Prediabetes subjects	18.7 ± 0.563	16.9 ± 0.126	16.9 ± 0.186
	Normal subjects	21.8 ± 0.235	21.6 ± 0.183	20.8 ± 0.175
	Statistical analysis (p value)	0.002*	< 0.001*	< 0.001*
Insulin (mIU/L)	Prediabetes subjects	17.4 ± 0.605	23.2 ± 0.305	25.1 ± 0.316
	Normal subjects	14.2 ± 0.125	19.4 ± 0.160	20.2 ± 0.151
	Statistical analysis (p value)	< 0.001*	< 0.001*	< 0.001*
CRP (mg/L)	Prediabetes subjects	3.35 ± 0.041	2.12 ± 0.059	2.55 ± 0.056
	Normal subjects	0.84 ± 0.019	1.35 ± 0.022	1.7 ± 0.036
	Statistical analysis (p value)	< 0.001*	< 0.001*	< 0.001*
Cholesterol (mg/dL)	Prediabetes subjects	177.9 ± 3.503	191.1 ± 1.512	203.3 ± 1.479
	Normal subjects	146.9 ± 0.831	171.1 ± 0.742	179.6 ± 0.609
	Statistical analysis (p value)	< 0.001*	< 0.001*	< 0.001*
Triglycerides (mg/dL)	Prediabetes subjects	128.5 ± 2.523	147.1 ± 1.155	155.6 ± 1.665
	Normal subjects	119.1 ± 0.519	128.9 ± 0.552	133.2 ± 0.728
	Statistical analysis (p value)	< 0.001*	< 0.001*	< 0.001*
HDL-C (mg/dL)	Prediabetes subjects	47.7 ± 0.897	41.8 ± 0.592	37.9 ± 0.547
	Normal subjects	50.3 ± 0.250	44.8 ± 0.306	43.1 ± 0.342
	Statistical analysis (p value)	< 0.001*	< 0.001*	< 0.001*

Analysed by unpaired t test. * indicates statistical significance at p value less than 0.05

Discussion

Prediabetes is the term used to classify those at risk of developing type 2 diabetes, if not diagnosed early. Prediabetes is commonly associated with metabolic syndrome and increases the risk of cardiovascular events. The concept of prediabetes is new in India and very few studies demonstrating the prevalence of prediabetes and its association are available to date.

Pre-hypertension and prediabetes are the paramount risk factors of cardiovascular disease. Coexisting pre-hypertension and prediabetes might have more solemn consequences regarding cardiovascular diseases than expected with either pre-hypertension or prediabetes alone. The present study documented a higher prevalence of pre-hypertension and hypertension in all three age groups, pointing towards the close association between the two important cardiovascular risk factors. Supporting the findings of the current study, Muthunarayanan et al. (2015) reported a significantly higher prevalence of prediabetes and diabetes amongst 20-year-old participants of Tamil Nadu with systolic hypertension (> 140 mmHg) [17]. Additionally, Anjana et al. (ICMR-INDIAB study) and Balagopal et al. found a significant association between prediabetes, diabetes and hypertension [11, 18], which explains that hypertension is one of the paramount risk factors for the development of prediabetes [19].

The present study found that body weight higher than the normal is significantly associated with risk of developing prediabetes in all the age groups. The findings of the current study are in support with many studies within India [11, 20, 21] and outside India [22, 23], pointing the positive relationship between body mass index and prediabetes. However, contradictory findings stating no significant association between body mass index and prediabetes were reported by Lee et al. [24] and Gupta et al. [25].

It is ubiquitously accepted that physical activity in any form is considered beneficial to the prevention of many chronic diseases. The current study found physical exercise decreases the risk of prediabetes in all age groups, which is supported by Aune et al. (2015) [26]. Moreover, several studies have shown the benefits of physical activity as it improves insulin sensitivity and glucose tolerance and delays the onset of diabetes in prediabetes subjects [27, 28].

Rare studies reporting the association of prediabetes with dietary type have been published to date. The current study

found a significantly higher prevalence of prediabetes amongst participants with non-vegetarian dietary type in all three age groups. In contrast to the findings of the current study, Muthunarayanan et al. reported prediabetes participants to consume vegetable less than 3 days in a week [17] and Zhao et al. [29] in China found prediabetes participants consume a high-fat diet. However, in both studies, the association between dietary type and developing prediabetes was not significant.

It has long been noted that consumption of junk food has become a global phenomenon. Scanty data establishing the risk of prediabetes due to junk food and eating sweets are available. Astrup et al. reported that individuals who eat more than two fast-food meals per week were at risk of obesity and had a greater risk of insulin resistance [30]. The current study did not find an association between frequencies of junk food and incidence prediabetes in 12–17 years age group, whereas, in 18–35 years and 36–55 years age groups, once in a week frequency of eating junk food was significantly higher amongst prediabetes participants.

Research has indicated that stressful conditions have an adverse impact on diabetes [31]. No reports demonstrating an association between stress and prediabetes have been published to date. In the present study, it was apparent that the level of stress was significantly interlinked with the risk of prediabetes in all age groups.

The findings of the present study demonstrated that the prevalence of prediabetes was significantly higher amongst the upper, upper-middle and lower-middle socioeconomic class. The possible reasons for this could be high income, sedentary lifestyle and westernisation of developing country like India. To the best of our knowledge, none of the studies has been reported showing an association between prediabetes and socioeconomic class. However, many studies have reported a strong association between diabetes with socioeconomic class, whilst Mudhaliar et al. [32] in the rural setting of India and Kim et al. [33] in Korea have found an inverse association between socioeconomic status and prevalence of diabetes.

More cardiovascular disease occurs in patients with either type 1 or 2 diabetes. However, the link between diabetes and atherosclerosis is not completely understood [34]. Additionally, cardiovascular disease is the leading cause of death amongst adult diabetic patients [35]. In the present study, none of the participants of 12–17 years age group found to have any of the lipid abnormalities. However, in 18–35 years and 36–55 years age groups, the prevalence of hypercholesterolemia (34.02% and 39.17%, respectively), hypertriglyceridemia (29.89% and 36.6%), low HDL-C (44.32% and 45.2%, respectively) and dyslipidemia (27.83% and 25.98%, respectively) was noticeably higher amongst prediabetes participants than normal participants. With regard to the studies of Kansal [36] (Wardha) and Balgi (Mysore) [37], in the present study, the mean cholesterol and triglycerides level was higher amongst participants with prediabetes than the control group, and the mean level of HDL-C was lower amongst participants with prediabetes.

Vitamin D deficiency affects either insulin sensitivity and beta cell function or both which contributes as an important risk factor for the pathogenesis of type 2 diabetes mellitus [38]. The present study found a significant higher prevalence of vitamin D deficiency amongst prediabetes participants than normal participants. Comparing the findings of the current study, Srinath et al. (South India) [39] and Zhang et al. [40] (Kuwaiti adults) reported less prevalence of vitamin D deficiency amongst prediabetes participants (72.5% and 53.9%, respectively). Supporting the findings of the current study, Shankar et al. and Dutta et al. found a positive association between lower serum vitamin D level and prediabetes [41, 8].

To the best of our knowledge, none of the studies has been published to date showing an association between hyperinsulinemia and prediabetes. However, Salazar et al. and Haffner et al. demonstrated that individuals with prediabetes were more insulin resistant than those with normal fasting glucose [42, 43], suggesting a possible association between higher insulin level and prediabetes, which is in favour of the findings of the current study.

C-reactive protein, a major acute-phase protein, acts inevitably as a marker of inflammation. Studies have reported inflammation in glucose imbalance which was demonstrated by an elevated level of inflammatory biomarkers like C-reactive protein [44]. In the present study, the mean level of C-reactive protein amongst prediabetes participants of 12–17 years, 18–35 years and 36–55 years age group was 3.35 ± 0.041 mg/L, 2.12 ± 0.059 mg/L and 2.55 ± 0.056 mg/L, respectively. The mean level of C-reactive protein amongst prediabetes participants in the current study was similar to the findings of Lin et al. [45], whilst Sabanayagam et al. reported a moderately higher level of C-reactive protein than the current study [46]. Moreover, the current study provides evidence for a positive association of elevated C-reactive protein level with prediabetes which was supported by Jaiswal et al. [47].

Strength of the study

The cross-sectional study includes both men and women, which provides the opportunity to display separate estimates based on gender. A large sample increases the statistical power of the study to detect the decrease in risk of prediabetes. The prevalence of prediabetes in school children, young aged and adult aged groups was alarming.

Limitation of the study

In the present study, waist to hip ratio and abdominal obesity were not analysed, which are associated with prediabetes and lipid abnormalities.

Conclusion

In conclusion, the prevalence of prediabetes has increased at an alarming rate. In an urban area, major risk factors considered for prediabetes and associated conditions are current lifestyle methods such as physical inactivity, unhealthy eating habits, stress and BMI. Early diagnosis of prediabetes and associated conditions as well as lifestyle modifications may help from diabetes and cardiovascular risk.

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Compliance with ethical standards

Ethical approval The study was approved by the Institutional Ethics Committee of Nirma University, Ahmedabad, with protocol number IEC/NU/V/IP/01. All the participants were informed about the purpose of the study and were ensured strict confidentiality, and then, written informed consent was taken from each of them before the total procedure. For school-going children, written consent of their parents or legally acceptable representative was taken.

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

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ORIGINAL ARTICLE

A cross-sectional study to evaluate diabetes management, control and complications in 1631 patients with type 2 diabetes mellitus in Vietnam (DiabCare Asia)

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Abstract

Aims To assess diabetes care delivery and prevention of short- and long-term diabetes-related complications in patients with type 2 diabetes mellitus (T2DM) in Vietnam.

Methods DiabCare Asia is an observational, non-interventional, cross-sectional study of hospital-based outpatient care for patients diagnosed with T2DM.

Results A total of 1631 patients (mean age 62.7 years; 58.9% female) participated in the study. The percentage of patients with HbA1c < 7.0% (< 53 mmol/mol) was 36.1% and mean (standard deviation) HbA1c was 7.9 ± 1.8% (63 ± 19 mmol/mol). The proportion of patients using insulin was 40%, at a mean total daily dose of 35.4 U. Apart from dyslipidemia (81.2%) and hypertension (78.4%), the most common diabetes-related complications were peripheral neuropathy (37.9%) and eye complications (39.5%). Current insulin therapy was associated with peripheral vascular disease (odds ratio [OR] = 2.28 [95% confidence interval (CI) 1.68; 3.09]) and eye complications (OR = 1.70 [95% CI 1.37; 2.11]).

Conclusion In this sample of patients with T2DM in Vietnam, the majority had poor glycemic and metabolic control. Concerted efforts are needed to optimize control and prevent complications in these patients. Trial registration: NCT02066766

Keywords Diabetes mellitus · Hospital care · Prevention · Diabetes complications · Treatment adherence · Hypoglycemia

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Introduction

In 2017, there were 425 million people diagnosed with diabetes worldwide. The Southeast Asia region is home to approximately 82 million people living with diabetes, and there are approximately 159 million people living with diabetes in the Western Pacific region. The prevalence of diabetes in both regions already exceeds that of Europe, South and Central America, and Africa, and, by 2045, the prevalence in the Western Pacific region is expected to reach 183 million [1]. Furthermore, undiagnosed diabetes represents a significant health problem [2], leading to an increase in the burden of disease, which includes diabetes-related complications. Therefore, diabetes presents significant challenges to developing countries in the above regions [2–5]. Between 1990 and 2010, the total disability-adjusted life years (DALYs), a measure of overall disease burden, attributable to diabetes increased by nearly 70%, while DALYs attributed to cardiovascular disease (CVD) and cancer each increased by approximately 25% in the Asia-Pacific region [5].

In Vietnam, the prevalence of diabetes has almost doubled within the past 10 years and has consequently been recognized as a major public health burden. In 2012, the prevalence of diabetes was 5.4%, with an additional 13.7% of individuals exhibiting impaired glucose tolerance. Recent studies have reported a diabetes prevalence of 7.2% in central Vietnam [6–8]. Factors contributing to this increase in prevalence of diabetes in Vietnam include a change in the economic landscape toward a more industrial-based economy, urbanization, a change in dietary habits (i.e., an increase in meat and fat intake), aging of the population, increased tobacco smoking, and an increased prevalence of hypertension [6, 9–11].

Severe micro- and macrovascular complications are common in patients with diabetes, particularly in those with poor glycemic control [12, 13]. There were 480,000 diabetes-related deaths associated with the above complications in people < 60 years of age in the Western Pacific region in 2017 [1]. Treatment costs for diabetes-related complications can be significant, with a recent Vietnamese study reporting that type 2 diabetes mellitus (T2DM) therapy costs 246.10 USD annually per patient [14]—an appreciable cost within the context of the 150 USD average Vietnamese monthly salary.

The series of cross-sectional, observational DiabCare studies aimed to assess diabetes management, control, and diabetes-related complications in patients with T2DM [15–23]. The studies also evaluated both primary and secondary preventative efforts, and patients' treatment adherence to inform healthcare policy and modify diabetes management programs [15–23]. In 1998, DiabCare Asia conducted the first regional survey that included Vietnam [15, 16]. Since this survey, only local and smaller studies have been conducted. Thus, there is a need for national data to inform the treatment and prevention of T2DM and to help raise awareness of diabetes among Vietnamese healthcare professionals (HCPs), as well as to plan educational programs and the provision of medical insurance. The current DiabCare Asia study aims to report on diabetes management, glycemic and metabolic control, and prevalence of diabetes-related complications in patients with T2DM in Vietnam.

Methods

Study design

DiabCare Asia was an observational, non-interventional, cross-sectional study conducted in Bangladesh, Indonesia, Malaysia, Philippines, Sri Lanka, and Vietnam (Clinicaltrials.gov registration number: NCT02066766). The current manuscript reports data from the Vietnam cohort only, involving diabetes clinics/units of 43 districts, provincial, and central hospitals in Vietnam between April 2015 and August 2015. Due to the observational nature of this study, there were no study-specific visits or investigational products and patients were treated according to routine clinical practice at the discretion of their physician.

Study participants

Patients routinely visiting the centers during the study period were screened for eligibility. Adults > 18 years with T2DM receiving non-pharmacological or pharmacological treatment at a particular center for ≥ 1 year and who had visited the center within the last 3–6 months were included. Patients who had suspected or confirmed pregnancy, or who were unable to comply with protocol requirements (any procedure related to recording of data, including patient interview and completion of questionnaires), were excluded from the study. Patients could withdraw from the study at any time. All patients meeting the inclusion criteria during the recruitment period were enrolled in the study.

Study endpoints

The primary endpoint of the study was the proportion of patients with glycosylated hemoglobin (HbA1c) < 7.0% (< 53 mmol/mol). Secondary endpoints included duration of diabetes, duration and type of antidiabetic treatment, measures of glycemic control and lipid control, and presence of known risk factors or diabetes-related complications. Potential risk factors were analyzed for their relationship with diabetes-related complications. Full details of secondary endpoints and potential risk factors are detailed in Supplementary Methods.

Assessments

Relevant data were collected by patient interview and from patients' medical records and recorded in study-specific case report forms. Data collected from the medical records included demography, medical history, diabetes-related complications, eye and foot examinations, diabetes management, and most recent laboratory investigations performed in the previous year. Blood samples obtained from all patients at study entry were assessed centrally for HbA1c. Patients completed a treatment adherence questionnaire, a hypoglycemia questionnaire, and the EQ-5D health-related quality of life questionnaire [24], as detailed in Supplementary Methods.

Patient data were kept confidential and stored according to local data protection regulations.

Sample size

The prevalence estimates and sample size were estimated based on published data [25]; local external experts advised on the list of clinics visited by most patients for diabetes care, to ensure a representative national sample to fulfill the objectives of the study.

Prevalence of CVD was used as a basis for the sample size target as available literature suggests that this is the least prevalent of all diabetes-related complications and its use confers the maximum possible representativeness to the sample size estimate. Assuming a CVD prevalence of 1%, a sample of 2000 patients from Vietnam was required to attain a 5% level of significance and a 30% margin of error.

Statistical analysis

The full analysis set included all patients enrolled in the study with at least one data point, and was used for all analyses. Missing data were not replaced. Continuous variables were summarized using descriptive statistics (*n*, mean, SD). Categorical variables were presented as number and percentages of patients (%). The number of missing observations is displayed and percentages are calculated based on the total number of patients in each category. For diabetes-related complications, patients with missing values were assumed not to have the complication in question.

The association between potential risk factors and diabetes-related complications was analyzed as detailed in Supplementary Methods.

Results

Patient characteristics

In total, 1631 patients participated in the study. Mean patient age was 62.7 years and the majority of patients (58.9%) were female. Mean duration of diabetes was 9.5 years. A large proportion of patients (39.4%) had a family history of diabetes, 49.2% of patients led a sedentary lifestyle, and 14.1% were current smokers (Table 1).

Diabetes management

The majority of patients (86.3%) were receiving oral or non-insulin injectable therapies, and 40.0% were on insulin treatment at a mean total daily dose of 35.4 U (Table 2).

Table 1 Patient characteristics

Variable	
Age (years)	
N	1631
Mean (SD)	62.7 (10.4)
Gender, *N* (%)	
Male	671 (41.1)
Female	960 (58.9)
Race, *N* (%)	
Vietnamese	1626 (99.7)
Danish	2 (0.1)
German	1 (0.1)
Missing	2 (0.1)
Body weight (kg)	
N	1620
Mean (SD)	59.7 (10.4)
BMI (kg/m^2)	
N	1609
Mean (SD)	23.9 (3.5)
Duration of diabetes (years)	
N	1630
Mean (SD)	9.5 (6.5)
Duration of treatment (years)	
N	1630
Mean (SD)	9.3 (6.4)
Duration of OAD treatment (years)	
N	1589
Mean (SD)	8.6 (6.0)
Duration of insulin treatment (years)	
N	674
Mean (SD)	3.8 (3.9)

Demographic parameters and clinical and treatment history data were collected from medical records

BMI, body mass index; *OAD*, oral antidiabetic drug; *SD*, standard deviation

Table 2 Antidiabetic therapies

Variable	
Receiving current oral or non-insulin injectable therapy	
N (%)	1407 (86.3)
Missing	0 (0.0)
Antidiabetic therapy, *N* (%)	
Metformin	1213 (86.2)
Sulfonylurea	980 (69.7)
Glucosidase inhibitor	208 (14.8)
Thiazolidinedione	8 (0.6)
Herbal	17 (1.2)
Glinide	7 (0.5)
DPP-4 inhibitor	198 (14.1)
GLP-1RA	2 (0.1)
Missing	1 (0.1)
Receiving current insulin therapy	
N (%)	653 (40.0)
Missing	1 (0.1)
Insulin injection type, *N* (%)	
Pen	420 (64.3)
Vial/syringe	231 (35.4)
Missing	2 (0.3)
Insulin regimen, *N* (%)	
Basal + OAD	97 (14.9)
Basal–bolus	38 (5.8)
Premix BID	416 (63.7)
Premix OD	17 (2.6)
Premix TID	42 (6.4)
Other	41 (6.3)
Missing	2 (0.3)
Number of daily injections	
N	651
Missing	2
Mean (SD)	2.1 (0.7)
Total daily insulin dose (U)	
N	638
Missing	15
Mean (SD)	35.4 (18.1)

Treatment history data were collected from medical records

BID, twice daily; *DPP-4*, dipeptidyl peptidase-4; *GLP-1RA*, glucagon-like peptide-1 receptor agonist; *OAD*, oral antidiabetic drug; *OD*, once daily; *TID*, three times daily

Table 3 Glycemic and metabolic parameters

Variable	
HbA1c	
N	1622
Mean (SD), %	7.9 (1.8)
Mean (SD), mmol/mol	63 (19)
HbA1c categories, *N* (%)	
< 7.0% (< 53 mmol/mol)	589 (36.1)
7.0–< 8.0% (53–< 64 mmol/mol)	397 (24.3)
8.0–< 9.0% (64–< 75 mmol/mol)	282 (17.3)
9.0–< 10.0% (75–86 mmol/mol)	158 (9.7)
≥10.0% (≥ 86 mmol/mol)	196 (12.0)
Missing	9 (0.6)
HbA1c tested in last year	
N (%)	1499 (91.9)
Missing	7 (0.4)
Number of HbA1c tests in previous year	
N	1437
Mean (SD)	2.8 (1.2)
SMBG tested in last year	
N (%)	773 (47.4)
Missing	2 (0.1)
Number of SMBG tests in previous year	
N	764
Mean (SD)	6.2 (8.6)
FPG (mmol/l)	
N	1592
Mean (SD)	8.0 (2.7)
PPG (mmol/l)	
N	492
Mean (SD)	11.6 (4.0)
Systolic blood pressure (mmHg)	
N	1618
Mean (SD)	129.1 (15.9)
Diastolic blood pressure (mmHg)	
N	1618
Mean (SD)	76.6 (8.6)
Total cholesterol (mmol/l)	
N	1436
Mean (SD)	7.6 (27.4)
HDL cholesterol (mmol/l)	
N	1394
Mean (SD)	1.9 (6.8)
LDL cholesterol (mmol/l)	
N	1377
Mean (SD)	5.0 (20.6)
Triglycerides (mmol/l)	
N	1443
Mean (SD)	5.4 (12.7)

Blood samples were obtained from all patients at study entry for HbA1c assessment by a central laboratory. Clinical history and data from most recent laboratory investigations within the past year were collected from medical records

FPG, fasting plasma glucose; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *PPG*, postprandial plasma glucose; *SD*, standard deviation; *SMBG*, self-measured blood glucose

The most commonly used oral antidiabetic drug (OAD) was metformin (86.2%) and the most commonly prescribed insulin regimen was premix twice-daily (BID) (63.7%). Most patients had been evaluated for HbA1c (91.9%) in the previous year (Table 3), with a mean of 2.8 tests per year.

Treatment adherence

A large proportion of patients only partially adhered to clinical recommendations regarding diet (47.8%) and exercise (37.8%; Supplementary Table 1). Many patients never self-tested (40.6%), 9.0% of patients did not fully adhere to their prescribed medications, and 10.7% did not completely adhere to scheduled appointments with HCPs.

Glycemic and metabolic endpoints

Mean HbA1c was 7.9% ± 1.8% (63 ± 19 mmol/mol; Table 3). HbA1c target of < 7.0% (< 53 mmol/mol), as recommended by the American Diabetes Association (ADA) [25], was met by 36.1% of patients, with 51.3% of patients having HbA1c of 7.0–< 10.0% (53–< 86 mmol/mol) and 12.0% of patients having HbA1c ≥ 10.0% (≥ 86 mmol/mol).

A high proportion of patients (78.4%) had hypertension (defined as [i] currently taking medication for hypertension, or [ii] systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg), and 74.9% were receiving anti-hypertensive medication. There was also a high proportion of patients (81.2%) with dyslipidemia (defined as [i] low-density lipoprotein [LDL] cholesterol > 2.6 mmol/l, or [ii] high-density lipoprotein [HDL] cholesterol < 1.0 mmol/l in males and < 1.3 mmol/l in females, or [iii] triglycerides > 1.7 mmol/l or currently taking medication for dyslipidemia), and 69.8% were on dyslipidemia medication.

Diabetes-related complications and management

Aside from hypertension and dyslipidemia, the most prevalent complications were peripheral neuropathy (37.9%) and eye complications (39.5%; Table 4). One-third of patients (33.4%) had cardiovascular complications, of which angina was the most frequently reported (19.9%), and almost a quarter of patients had renal complications (24.1%).

Anti-hypertensive treatment (angiotensin-converting-enzyme inhibitors and/or angiotensin-receptor blockers) was the most common intervention for primary and secondary prevention of complications (47.8% and 46.8%, respectively), followed by lipid-lowering treatment (statins; 47.1% and 40.3%, respectively), and anti-platelet treatment (aspirin; 22.8% and 22.7%, respectively). A small proportion of patients were on primary and secondary foot ulcer prevention programs (9.3% and 5.7%, respectively), or foot ulcer special care treatment (8.9%). Over the previous 2 years, 62.0% and 46.4% of patients had been screened for eye complications and peripheral neuropathy, respectively.

In the multivariate analysis assessing potential risk factors for diabetes complications, dyslipidemia was independently associated with age (adjusted OR, 1.01), male gender (OR, 1.33), and fasting plasma glucose (FPG) (OR, 0.95; Table 5). Hypertension was independently associated with use of multiple OADs (OR, 0.71), insulin therapy (OR, 0.37), body mass index (BMI) (OR, 0.85), and age (OR, 0.92).

Diabetes duration was associated with peripheral vascular disease (OR, 1.03), diabetic nephropathy (OR, 1.05) and eye complications (OR, 1.06). Current treatment with insulin, and hypertension were both independently associated with higher odds of cardiovascular complications (OR, 1.46 and 2.88, respectively), peripheral vascular disease (OR, 2.28 and

Table 4 Proportion of patients with diabetes-related complications

Complication, *N* (%)	All subjects (*N* = 1631)
Dyslipidemia[a]	1324 (81.2)
Missing	1 (0.1)
Hypertension[b]	1278 (78.4)
Missing	0 (0.0)
Any recorded eye complications	644 (39.5)
Missing	10 (0.6)
Cataract	467 (28.6)
Missing	11 (0.7)
Non-proliferative diabetic retinopathy	213 (13.1)
Missing	16 (1.0)
Diabetic retinopathy	189 (11.6)
Missing	15 (0.9)
Proliferative diabetic retinopathy	73 (4.5)
Missing	16 (0.1)
Severe vision loss	52 (3.2)
Missing	10 (0.6)
Macular edema	42 (2.6)
Missing	16 (0.1)
Peripheral neuropathy	618 (37.9)
Missing	4 (0.2)
Erectile dysfunction[c]	223 (33.2)
Missing	2 (0.3)
Any recorded cardiovascular complications	544 (33.4)
Missing	1 (0.1)
Angina	324 (19.9)
Missing	2 (0.1)
Peripheral vascular disease	188 (11.5)
Missing	1 (0.1)
Left ventricular hypertrophy	158 (9.7)
Missing	1 (0.1)
Stroke	71 (4.4)
Missing	1 (0.1)
Myocardial infarction	45 (2.8)
Missing	1 (0.1)
Congestive heart failure	15 (0.9)
Missing	2 (0.1)
Atrial fibrillation	10 (0.6)
Missing	1 (0.1)
Any recorded renal complication	393 (24.1)
Missing	3 (0.2)
Microalbuminuria	343 (21.0)
Missing	67 (4.1)
Gross proteinuria	183 (11.2)
Missing	55 (3.4)
End-stage renal disease	26 (1.6)
Missing	3 (0.2)
Dialysis	5 (0.3)
Missing	3 (0.2)
Any recorded foot complications	103 (6.3)
Missing	1 (0.1)
Healed ulcer	82 (5.0)
Missing	2 (0.1)
Ulcer infection	51 (3.1)
Missing	1 (0.1)

Table 4 (continued)

Complication, *N* (%)	All subjects (*N* = 1631)
Active ulcer	22 (1.3)
Missing	1 (0.1)
History of amputation	14 (0.9)
Missing	1 (0.1)

[a] Dyslipidemia: (i) LDL cholesterol > 2.6 mmol/l or (ii) HDL cholesterol < 1.0 mmol/l in males and < 1.3 mmol/l in females, or (iii) TG > 1.7 mmol/l or currently taking medication for dyslipidemia

[b] Hypertension: (i) currently taking medication for hypertension, or (ii) systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg

[c] Based on the total number of male patients (*n* = 671). Diabetes-related complication data were collected from medical records. Patients with missing observations were assumed not to have the complication in question

HDL, high-density lipoprotein; *LDL*, low-density lipoprotein; *TG*, triglycerides

1.93, respectively), diabetic nephropathy (OR, 1.94 and 1.67, respectively), and eye complications (OR, 1.70 and 1.27, respectively). HbA1c was associated with cardiovascular complications (OR, 1.07), peripheral vascular disease (OR, 1.09), and eye complications (OR, 1.06).

Hypoglycemic episodes

Symptoms of mild, moderate, severe, and nocturnal hypoglycemia within the previous 3 months were reported by 39.7%, 2.8%, 1.3%, and 8.2% of patients, respectively (Supplementary Table 2). Among patients who had experienced hypoglycemia, most (88.6%) did not check their blood glucose or only checked it occasionally, and only 10.6% of patients always measured their blood glucose when a hypoglycemic event occurred. Most patients (97.1%) who had experienced hypoglycemia did not visit or only occasionally visited a hospital during an event. Following a hypoglycemic episode, 88.8% snacked between meals and 8.2% of patients skipped or reduced their diabetes medications. Most patients (59.8%) indicated that they were not worried about hypoglycemia.

EQ-5D VAS and health status questionnaire

Patients reported a healthy state of overall well-being on the EQ-5D visual analogue scale (VAS), with a mean (± SD) score of 71.3 ± 16.9 (on a scale of 0–100). However, in the EQ-5D health status questionnaire, many patients reported moderate pain or discomfort (32.6%), and moderate anxiety or depression (26.8%; Supplementary Table 3).

Discussion

This study provides an overview of the status of diabetes care in patients with T2DM treated in various hospital care settings in Vietnam in 2015. With regard to the primary endpoint, 36.1% of patients had HbA1c < 7.0% (< 53 mmol/mol) and the mean HbA1c was 7.9% (63 mmol/mol). This study was similar in design to the DiabCare Asia 1998 study, which also recruited all eligible patients with T2DM treated at hospital or referral clinics in Vietnam. The DiabCare Asia 1998 study group reported that 18% of patients in Vietnam had HbA1c < 7.0% (< 53 mmol/mol) and a mean HbA1c of 8.9% (74 mmol/mol) [15]. Thus, the current data indicate that glycemic control has improved from 1998 to the present day and suggest an improvement in the standard of care for patients with T2DM in Vietnam.

While these data indicate that the proportion of patients with T2DM in Vietnam achieving recommended glycemic targets has increased over time, the majority of the cohort (i.e., around 63%) had HbA1c > 7.0% (> 53 mmol/mol), with a notable minority (12%) of patients presenting with HbA1c ≥ 10.0% (≥ 86 mmol/mol). Several possible reasons may underlie this suboptimal glycemic control. Firstly, the frequency of testing was lower than that recommended by the ADA [26], with many patients not monitoring their blood glucose levels at all in the past year. This lack of testing may be attributable to public health factors such as no local provision for testing and a lack of health insurance cover; health insurance was only introduced in Vietnam in the last two decades and is still not universal [27]. Secondly, a high proportion of patients did not adhere to treatment-related advice and a substantial proportion did not adhere to their prescribed treatment regimens. Thirdly, about half of the patient cohort led a sedentary lifestyle with no exercise. Finally, the prevalence of diabetes in Vietnam is increasing due to an aging population, and changes in lifestyle and dietary patterns, resulting in a significant public health burden [6].

The Vietnamese government has unveiled a national strategy for non-communicable diseases for 2015–2025 that explicitly includes the control and prevention of diabetes. In order for this goal to be achieved, the current data indicate that more work is required within diabetes treatment services in Vietnam. In particular, these findings emphasize the need to promote frequent HbA1c testing and improve patient adherence to lifestyle modification and medication. To improve the quality of care, the ADA advocates that diabetes services should follow the chronic care model [27]. Moreover, recruitment of specialist staff such as nutritionists and diabetes specialist nurses may be needed in Vietnam. Educational programs delivered by diabetes educators would also likely improve outcomes in Vietnamese patients diagnosed with T2DM.

Patients with T2DM are predisposed to developing hypertension and dyslipidemia, which are known to increase the risk of late complications such as end-stage renal failure and cardiovascular events [28–31]. In this study, the high proportion of patients

Table 5 Risk factors for diabetes complications using univariate and multivariate logistic regression analysis

Variables	Age	Gender	Duration of diabetes	BMI	Receiving insulin therapy	Use of multiple OADs	HbA1c	SMBG testing	Hypertension	FPG
Univariate analysis										
Cardiovascular complications	1.03 (1.02;1.04) ***	0.92 (0.74;1.13)	1.04 (1.02;1.06)***	1.01 (0.98;1.04)	2.01 (1.63;2.48) ***	0.77 (0.60;0.98)*	1.09 (1.03;1.16)**	0.99 (0.80;1.22)	3.63 (2.65; 4.97)***	1.03 (0.99;1.07)
Peripheral vascular disease	1.03 (1.01;1.05)***	1.27 (0.94;1.72)	1.05 (1.03;1.08)***	0.95 (0.91;1.00)*	3.26 (2.37;4.49)***	0.64 (0.44;0.92)*	1.13 (1.05;1.22)**	1.05 (0.77;1.42)	2.38 (1.49;3.81)***	1.01 (0.96;1.07)
Diabetic nephropathy	1.04 (1.03;1.05)***	0.98 (0.77;1.23)	1.10 (1.08;1.12)***	1.00 (0.97;1.03)	2.99 (2.37;3.78)***	0.87 (0.66;1.14)	1.11 (1.04;1.18)***	1.34 (1.07;1.69)*	2.50 (1.79;3.48)***	1.08 (1.04;1.13)***
Eye complications	1.07 (1.06;1.08)***	0.89 (0.73;1.09)	1.10 (1.08;1.12)***	0.96 (0.93;0.99)**	2.40 (1.96;2.95)***	0.80 (0.63;1.02)	1.11 (1.05;1.17)***	1.54 (1.26;1.88)***	2.34 (1.79;3.05)***	1.03 (0.99;1.07)
Dyslipidemia	0.99 (0.98;1.00)	0.74 (0.57;0.95)*	0.99 (0.97;1.01)	1.03 (0.99;1.07)	0.74 (0.58;0.95)*	0.72 (0.52;0.99)*	1.00 (0.94;1.08)	1.04 (0.81;1.33)	0.90 (0.66;1.22)	1.02 (0.98;1.08)
Hypertension	1.08 (1.06;1.09)***	0.71 (0.56;0.91)**	1.08 (1.05;1.10)***	1.15 (1.10;1.19)***	2.07 (1.60;2.68)***	0.99 (0.75;1.31)	1.04 (0.97;1.12)	1.27 (1.00;1.61)*	–	1.01 (0.97;1.06)
Multivariate analysis										
Cardiovascular complications	1.01 (1.00;1.02)**	1.15 (0.96;1.38)	1.00 (0.99;1.02)	–	1.46 (1.19;1.81)***	–	1.07 (1.02;1.14)*	0.80 (0.67;0.95)*	2.88 (2.22;3.74)***	–
Peripheral vascular disease	1.01 (0.99;1.02)	1.57 (1.19;2.06)**	1.03 (1.01;1.05)*	0.92 (0.89;0.97)***	2.28 (1.68;3.09)***	–	1.09 (1.01;1.18)*	–	1.93 (1.28;2.92)**	–
Diabetic nephropathy	1.04 (1.02;1.05)***	1.17 (0.95;1.43)	1.05 (1.03;1.07)***	–	1.94 (1.56;2.41)***	–	–	–	1.67 (1.25;2.23)***	1.08 (1.05;1.13)***
Eye complications	1.06 (1.05;1.07)***	1.01 (0.84;1.22)	1.06 (1.04;1.08)***	0.96 (0.94;0.99)**	1.70 (1.37;2.11)***	–	1.06 (1.00;1.12)*	1.24 (1.04;1.48)*	1.27 (1.00;1.62)*	–
Dyslipidemia	1.01 (1.00;1.02)*	1.33 (1.08;1.65)**	1.01 (1.00;1.03)	–	–	–	–	–	–	0.95 (0.91;0.99)*
Hypertension	0.92(0.91;0.93)***	0.99 (0.81;1.22)	0.99 (0.97;1.01)	0.85 (0.83;0.88)***	0.37 (0.28;0.50)***	0.71 (0.53;0.95)*	–	–	–	–

Hypertension: (i) currently taking medication for hypertension, or (ii) systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg. Dyslipidemia: (i) LDL cholesterol > 2.6 mmol/l or (ii) HDL cholesterol < 1.0 mmol/l in males and < 1.3 mmol/l in females, or (iii) TG > 1.7 mmol/l or currently taking medication for dyslipidemia

BMI, body mass index; *CI*, confidence interval; *FPG*, fasting plasma glucose; *OAD*, oral antidiabetic drug; *SMBG*, self-measured blood glucose; *TG*, triglyceride

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Data are presented as odds ratios (95% CI)

with dyslipidemia (81.2%) and hypertension (78.4%) irrespective of treatment for these conditions is a cause for concern. Indeed, the prevalence of hypertension was higher in the current study (78.4%) than reported in DiabCare Asia 1998 (61.0%) [15]. Interestingly, it was found that treatment with multiple OADs, current use of insulin therapy, BMI, and age were associated with hypertension in the patient cohort. This corroborates previous research indicating that older age and OAD use is associated with poor blood pressure control in patients with diabetes [32]. The relationship between insulin and hypertension may require further analysis, as insulin use can indicate late-phase diabetes in which hypertension may be more common compared with earlier stages in the disease pathway [33]. Age, male gender, and FPG were significantly associated with dyslipidemia. The lack of a significant association between HbA1c and dyslipidemia was perhaps surprising based on previous research [34–36].

Diabetes-related complications were prevalent, with the most common (excluding hypertension and dyslipidemia) being eye complications and peripheral neuropathy—both of which, encouragingly, were screened for in a relatively high proportion of patients (e.g., 62.0% of patients had been screened for eye complications). The previous DiabCare Asia 1998 study reported the most common complications in patients with T2DM were cataract, neuropathy, and retinopathy [15]. Neuropathy was only slightly more common in the current study compared with DiabCare Asia 1998, occurring in 37.9% and 35.0% of patients, respectively [15]. Eye complications were significantly associated with the most common risk factors observed in the current study (age, diabetes duration, BMI, current insulin therapy, HbA1c, and hypertension). Microalbuminuria occurred in 21% of patients in the current study compared with 45% of patients in DiabCare Asia 1998. This finding could be explained by the increased use of medications that reduce albuminuria, such as inhibitors of the renin-angiotensin-aldosterone system. These findings emphasize the importance of early identification of patients at risk of developing diabetes-related complications and implementing more effective preventative efforts—primarily targeted at improving overall glycemic and metabolic control—in Vietnam [12, 13, 37].

It is now well recognized that hypoglycemia can have deleterious physical and psychological consequences that can significantly impact the functioning of patients with diabetes [38, 39]. In the current study, patient responses to the hypoglycemia questionnaire showed that the majority did not check or only occasionally checked their blood glucose during a hypoglycemic event. Furthermore, around one-third of patients skipped or reduced their diabetes medications following an episode. These findings call for strategies to increase patients' awareness of the benefits of diabetes treatment, and for the development of measures to avoid and/or mitigate the impact of hypoglycemia in patients with T2DM in Vietnam.

There are several limitations to the current study. Due to the cross-sectional, observational design of the study, it was not possible to draw conclusions on the cause-and-effect relationship between risk factors and dyslipidemia, hypertension, and various diabetes-related complications. As all centers offered specialized diabetes care services, patients attending these centers who were eligible for study enrollment may not be representative of the Vietnamese T2DM patient population. The relatively high prevalence of CVD may reflect that patients attending these centers had more advanced disease than the general Vietnamese T2DM population. Treatment adherence, hypoglycemia, and EQ-5D were self-reported and, consequently, the estimates may have been subject to recall and/or desirability bias. Also, due to the retrospective collection of laboratory findings (aside from HbA1c), it was not possible to fully assess the glycemic control and lipid control status in the entire study cohort. Some important aspects of diabetes management, including patient literacy, were also not assessed, and it would have been interesting to ascertain the number of HCPs adhering to national guidelines on diabetes care. Lastly, the patient sample size was smaller than planned due to administration delays and financial constraints; nevertheless, the relatively large number of patients allow for valid observations to be drawn.

Conclusion

While glycemic control in patients with T2DM has improved in Vietnam from 1998 to the present day, most patients still have unsatisfactory glycemic and metabolic control, with a high prevalence of diabetes-related complications and suboptimal treatment adherence. Improvements in diabetes services are likely needed to better these outcomes. Finally, future studies are needed to continue to monitor diabetes care in Vietnam and to direct and improve diabetes management.

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Data accessibility The subject level analysis data sets for the research presented in the publication are available from the corresponding author on reasonable request.

Author contributions KTN revised the protocol before disseminating it to the sites and followed up all the data collecting process of the study in all sites before the analysis. All authors contributed to subject recruitment, data collection, review and discussion of the trial report, and review and discussion of the manuscript.

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Compliance with ethical standards

Ethical approval The study was conducted in accordance with the Declaration of Helsinki [40] and the Guidelines for Good Pharmacoepidemiology Practice (GPP) [41].

Informed consent Before any study-related activity, eligible patients were provided with oral and written study information, and their informed consent was obtained.

Conflict of interest Dr. KTN has received honoraria for lectures and meeting chairs from Abbott, Astra Zeneca, Aventis, Boehringer Ingelheim, MSD, Novo Nordisk, Sanofi, and Servier. Dr. BTTD, Dr. VKDN, Dr. HVL, Dr. KQT and Dr. NQT have nothing to disclose.

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ORIGINAL ARTICLE

Factors associated with silent myocardial ischemia, autonomic or peripheral neuropathies, and survival in diabetes mellitus type 2 patients without cardiovascular symptoms

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Abstract

Introduction Complications from diabetes mellitus (DM) include cardiovascular system, peripheral neuropathy (PN), and autonomic dysfunction (AD). Goal: Assess the association of silent myocardial ischemia, AD, and PN in cardiovascular asymptomatic type 2 diabetics.

Methods As part of a multicenter project, 40 patients with type 2 DM were studied, with > 5 years of known disease and a baseline electrocardiogram non suggestive of coronary artery disease. Myocardial SPECT was performed with exercise stress test measuring corrected QT interval (QTc) and heart rate recovery (HRR) post-exercise (abnormal QTc ≥ 450 ms at rest and HRR < 14 beats at the first minute in maximum exercise). After 3 years, it was possible to re-study 32 cases. PN was evaluated with Michigan Neuropathy Screening Instrument (MNSI). Logistic regression analysis was performed to determine associated factors for AD, PN, SI, and survival analysis.

Results Thirty-four percent of the group had ischemia in SPECT; QTc was prolonged in 23.3%; 31% fulfilled criteria of PN; and 25% of AD due to HRR alteration. With bivariate and multivariate analyses, associations were observed between lipid, glycemic parameters, ischemia, PN, and AD. The follow-up (mean 119 months) consigned 4 cardiac-related deaths; ischemia, glycemic control parameters, and microalbuminuria had significant value in bivariate analysis.

Conclusion In our small sample of asymptomatic cardiovascular type 2 DM patients, myocardial ischemia, glycemic control, and microalbuminuria have influence on survival, requiring a more intensive global therapeutic approach.

Keywords Diabetes mellitus · Autonomic dysfunction · Peripheral neuropathy · Silent myocardial ischemia · SPECT

Introduction

Diabetes mellitus (DM) is a recognized cardiovascular risk factor (CVRF) leading to heart failure and accelerated atherosclerosis, associated with endothelial dysfunction and insulin resistance, as well as with nonspecific inflammatory markers [1].

In type 2 DM, coronary artery disease (CAD) and silent ischemia are common and related with the presence of CVRF [2, 3]. However, the current recommendations are against routine cardiac screening; patients with higher risk must be under intensive medical therapy [4]. Myocardial perfusion single photon tomography (SPECT) is a non-invasive method for cardiovascular risk stratification; the extent of ischemia relates to survival [5]. The Detection of Ischemia in Asymptomatic Diabetics (DIAD) study demonstrated SI in 22% of type 2 DM patients [6, 7], although at almost 5-year follow-up there was a low rate of accumulated cardiac events [8]. Another

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study observed silent ischemia evaluated with exercise stress test in 26% vs. 14% in non-diabetic controls ($p < 0.001$) [9]. On the other hand, in that important experience, there was no association between traditional CVRF and silent ischemia, being cardiac autonomic dysfunction a strong predictor of ischemia.

Autonomic dysfunction (AD) is one of several chronic DM complications, related to poor metabolic control and longer period since initial diagnosis. It may involve the cardiovascular, gastrointestinal, or urogenital systems. Cardiovascular involvement varies between 2.5 and 50%, determined mainly by the diagnostic criteria used [10, 11], presenting with electrocardiographic abnormalities such as heart rate variability at rest and also prolongation and dispersion of the QT interval and post-exercise delay in the heart rate recovery (HRR). A prolonged corrected QT interval (QTc) has been associated with phenomena of ischemic origin as independent factor for mortality in DM [1, 12]. Abnormal response to exercise is also associated with lower survival; HRR reduction should be considered to stratify cardiovascular risk in DM [13]. Decrease of pain threshold determined by AD may explain myocardial silent ischemia. More than three decades ago, no differences were found in abnormal exercise tests in asymptomatic coronary DM patients, with and without peripheral neuropathy, but those with a positive stress test developed clinical CAD in the 4-year follow-up [14]. Typical diabetic neuropathy (PN) is a chronic, symmetric, sensory-motor polyneuropathy, associated with chronic hyperglycemia, oxidative stress, dyslipidemia, and CVRF. Microangiopathy, similar to that observed in retinopathy and nephropathy, may be associated with PN.

The aim of our work was to assess the association of myocardial silent ischemia, AD, and PN in patients with asymptomatic cardiovascular type 2 DM and to evaluate their survival in the medium term.

Methods

Our patients belonged to a multicenter prospective study of the International Atomic Energy Agency [9] from which we published our local experience [15]. The study was approved by the Scientific Ethics Committee of the Clinical Hospital of the University of Chile. Informed consent was obtained from all individual participants included in the study. We initially evaluated 40 type 2 DM patients, over 45 years old and diagnosed over 5 years. They were asymptomatic for CAD, with other CVRFs and normal electrocardiogram or only nonspecific abnormalities. Exclusion criteria were the known CAD, cerebrovascular stroke, inability to perform exercise stress test, and abnormal electrocardiograph at rest (arrhythmias, complete branch block left, Q wave, or other CAD suspect). Beta-blockade was suspended by protocol. Patients with known renal failure were not included. A total of 32 patients had a 3-year follow-up, presented in this work.

Acquisition, processing, and interpretation of SPECT images with exercise stress test were according to previously described protocol.

At 3-year follow-up, a lipid profile, creatininemia, fasting glycaemia, glycosylated hemoglobin (HbA1c), microalbuminuria, and ultrasensitive C-reactive protein (CRP) were obtained, as well as a myocardial perfusion SPECT. Survival was verified through data from the National Civil Registry and clinical files available up to 11 years after inclusion. There was no directed pharmacological intervention, and patients and/or physicians in-charge received the results of the exams.

Electrocardiogram analysis. Baseline and 3-year QTc interval were measured at rest, corrected by heart rate according to Bazett's formula [16], considering abnormal ≥ 450 ms [17, 18]. The maximum post-exercise HRR was calculated, considering abnormal less than 14 beats in the first minute [13].

Michigan Neuropathy Screening Instrument (MNSI). Consists of self-administered questionnaire and objective medical measurements, including inspection, reflexes, and perception of 128-Hz vibrations in both extremities. The score was abnormal at 4 out of 10 points in the questionnaire and equal or greater than 2 out of 10 points in the medical evaluation.

Statistical Analysis

Student's *t* and Wilcoxon's tests were used to compare averages, chi-square according to data distribution, and Cohen's kappa. To estimate factors associated with silent ischemia, PN, and AD, a bivariate and multivariate logistic regression model was generated. The adjustment of the model was checked through the Hosmer-Lemeshow test. Aikike Information Criteria was compared by means of the likelihood ratio (LR) test and areas under the Receiver Operating Characteristics (ROC) curve were obtained. To estimate factors associated with mortality, bivariate and multivariate Cox regression analyses were applied. The condition of proportional hazards was checked by the Schoenfeld method. Multivariate model for logistic and Cox regression analyses was performed using the steps method incorporating variables with p values < 0.05 and eliminating those < 0.2. Stata v12.1 statistical program was used for all the analysis.

Results

General

Upon admission to protocol, the mean number of CVRF was 1.7 ± 1.3, excluding DM; 72% were hypertensive, 56% smoked, 75% had known dyslipidemia, and 66% had some abnormality in their lipid profile. Table 1 shows their clinical, laboratory, functional parameters, and main medical therapy.

Table 1 Clinical and laboratory characteristics in 32 type 2 diabetic patients

	Baseline	Control 3rd year	*p*
BMI (kg/m^2)	28.4 ± 4.0	27.5 ± 4.5	ns
Total cholesterol (mg/dL)	187 ± 36	181 ± 46	ns
LDL (mg/dL)	107 ± 30	106 ± 36	ns
HDL (mg/dL)	45 ± 12	46 ± 11	ns
Triglycerides (mg/dL)	172 ± 85	167 ± 106	ns
HbA1c (%)	8.2 ± 2.3	8.7 ± 2.6	ns
HbA1c > 7.5%	48%	43.7%	ns
Fasting glycemia (mg/dL)	161 ± 64	175 ± 67	ns
Creatininemia (mg/dL)	0.83 ± 0.94	0.90 ± 0.5	ns
Resting heart rate (lat/min)	84.9 ± 13	88.2 ± 14	ns
Metabolic equivalents (METs)	8.7 ± 2.4	8.0 ± 2.1	0.0203
Maximal heart rate in stress test (lat/min)	157.8 ± 9.7	151 ± 10.7	< 0.0001
Theoretical maximal heart rate (%)	98.8 ± 5.6	94.6 ± 8.9	0.0009
Stress test duration (min)	6.9 ± 2.4	6.4 ± 2.5	ns
Medications			
Statins	35.5%	40.0%	ns
Diuretics	12.5%	28.1%	ns
Beta-blockers	15.6%	25.0%	ns
Angiotensin inhibitors	50.0%	53.1%	ns
Acetyl salicylic acid	28.1	37.5%	ns
ARA II	9.4%	6.2%	ns
Insulin	21%	21%	ns
Metformin	84%	72%	ns
Sulphonylureas	47%	43%	ns
SPECT ischemia [SSS > 3]	34%	19%	ns
QTc interval Bazett	425.1 ± 37.4	429.6 ± 33.9	ns
QTc ≥ 450 ms	23.3%	23.3%	ns
HRR 1st min (beats)	18.9 ± 5.8	17.4 ± 7.2	ns
abnormal HRR (< 14 beats)	9%	25%	ns
MNSI patient's questionnaire (≥ 4 points)	–	38%	–
MNSI medical score (≥ 2 points)	–	54.8%	–
CRP (mg/dL)	–	7.3 ± 7.1	–
Microalbuminuria (mg/L)	–	78 ± 196	–
(> 30 mg/L)		28%	

HRR heart rate recovery, *MNSI* Michigan Neuropathy Screening Instrument, *CRP* C-reactive protein, *HbA1c* glycosylated hemoglobin, *NS* not significant

Our type 2 DM patients were recruited between years 2006 and 2008; regarding their main oral medications were metformin in most and sulphonylureas, a few of them associated to insulin.

Baseline electrocardiographic stress test was negative in all patients, achieving 85% or more of their theoretical maximum heart rate; at 3-year follow-up, the test was positive in 2/32. The initial myocardial SPECT showed 34% of silent ischemia and one case with necrosis, left ventricular dilation, and decreased systolic function, without significant changes at 3 years (Table 1). After 3 years of follow-up, the heart rate at rest had a trend to increase in the subgroup with abnormal HRR ($p = 0.061$), and the maximum obtained at stress decreased significantly ($p = 0.0141$); in the group with adequate HRR, the baseline heart rate did not change ($p = 0.76$) and the maximum decreased significantly ($p = 0.0017$). The concordance in the 2 components of MNSI (questionnaire and score) was 77.4% with a Cohen's kappa of 0.558.

Bivariate analysis Table 2 shows the most important associations between the various parameters analyzed including biochemical, CVRF, therapeutic, electrocardiographic (QTc at rest and HRR at the first-minute post maximal stress as an AD variable), myocardial silent ischemia, and PN.

Table 2 Bivariate analysis of dichotomic parameters to estimate factors associated with silent ischemia, peripheral neuropathy, and autonomic dysfunction

Dependent variables	Parameter	OR (95% CI)	*p*
CVRF (no DM)	MNSI questionnaire	0.45 (0.21;0.97)	0.043*
Baseline serum creatinine	MNSI questionnaire	0.003 (0.00001;0.72)	0.038*
Baseline HbA1c	MNSI questionnaire	1.49 (1.02;2.18)	0.040*
Control HbA1c	MNSI questionnaire	1.66 (1.08;2.55)	0.020*
Control HDL	MNSI questionnaire	0.91 (0.83;0.99)	0.028*
	MNSI score	0.91 (0.84;0.98)	0.019*
BMI	Baseline HRR	1.35 (1.00;1.82)	0.047*
Baseline HDL	Control HRR	0.88 (0.78;0.99)	0.033*
Baseline triglycerides	Baseline HRR	1.02 (1.00;1.03)	0.016*
Control triglycerides	Baseline HRR	1.02 (1.00;1.05)	0.041*
Control HbA1c	Baseline HRR	1.54 (0.94;2.54)	0.083
	Control HRR	1.46 (1.01;2.12)	0.044*
Control insulin use	Control HRR	8.4 (1.19;59.49)	0.033*
Microalbuminuria	Baseline HRR	1.01 (0.99;1.02)	0.074
Control glycemia	Baseline myocardial SPECT	1.02 (1.00;1.04)	0.013*
Control HbA1c	Baseline myocardial SPECT	1.41 (0.99;2.01)	0.055
Baseline myocardial SPECT	Control QTc	7.08 (1.07;46.67)	0.042*
	Baseline TG	4.33(0.88;21.30)	0.071
Control myocardial SPECT	Control HRR	8.4 (1.19;59.49)	0.033*
Control HRR	Baseline HDL	8.00 (1.32;48.64)	0.024*
	Control HDL	4.5(0.87;23.34)	0.042*
MNSI score	RFC 1 control	1.44 (0.98;2.11)	0.064
	HDL basal	3.93(0.88;17.56)	0.073
MNSI questionnaire	Microalbuminuria	5.33 (1.0;28.43)	0.050
	Control HRR	5.33 (1.00;28.43)	0.050
	Baseline HDL	4.33(0.93;20.24)	0.062
	Control HDL	3.92(0.84;18.21)	0.081
	Microalbuminuria	5.33(1.0;28.43)	0.050
Control QTc	Control QTc	1.05 (0.99;1.09)	0.050
	Control myocardial SPECT	1.04 (0.99;1.08)	0.078
	Cholesterol control	4.8(0.79;28.89)	0.087

CVRF cardiovascular risk factors, *OR* odds ratio, *CI* confidence interval, *HRR* heart rate recovery, *MNSI* Michigan Neuropathy Screening Instrument, *HbA1c* glycosylated hemoglobin

*$p < 0.05$

Multivariate analysis The parameters used in the models to predict silent ischemia, PN, altered QTc, or AD with their odds ratio (OR), confidence intervals (CI), and areas under the ROC curve (AUC) are shown in Table 3.

Follow-up

The average follow-up corresponded to 119.3 months. There were 4 cardiac-related deaths (12.5%) in male patients according to death certificate. There were no deaths from other causes. The bivariate survival analysis showed a significant association between silent ischemia parameters, metabolic control, and microalbuminuria at 3-year follow-up. The multivariate analysis showed a trend to associate with microalbuminuria and QTc (Table 4).

Discussion

We found an association between lipid parameters, glycemic metabolism, myocardial silent ischemia, PN, and AD in type 2 DM patients. We also observed that silent ischemia, glycemic control parameters, and microalbuminuria have significant value in survival of asymptomatic coronary type 2 DM

Table 3 Multivariate analysis for all parameters to predict silent ischemia, peripheral neuropathy, altered QTc, or autonomic dysfunction

Factor	Parameter	OR (95% CI)	*p*	Area under ROC curve (AUC)
Baseline myocardial SPECT	Control serum glucose	1.03 (1.00;1.05)	0.027*	86.36%
	QTc2	11.04 (0.87;140.65)	0.064	
Control myocardial SPECT	Control HRR	14.00 (1.74;112.55)	0.013*	77.08%
Baseline HRR	BMI	1.54 (1.01;2.35)	0.045*	96.43%
	Microalbuminuria	1.01 (0.99;1.02)	0.152	
Control HRR	Control myocardial SPECT	141.18 (1.49;13,413.39)	0.033*	92.86%
	Baseline HDL	0.74 (0.58;0.96)	0.20	
MNSI score	Control HDL	0.91 (0.84;0.98)	0.019*	76.08%
MNSI questionnaire	Microalbuminuria	4.54 (0.79;25.85)	0.088	73.68%
	Control HDL	3.28 (0.64;16.67)	0.152	
QTc control	Baseline myocardial SPECT	7.48 (0.99;56.44)	0.051	79.5%
	Control Cholesterol	5.14 (0.69;38.32)	0.110	

OR odds ratio, *CI* confidence interval, *MNSI* Michigan Neuropathy Screening Instrument, *HRR* heart rate recovery

*$p < 0.05$

patients. It is interesting that these findings were present despite the small sample available. The QTc interval has been related to CAD and to increased risk of sudden cardiac death in DM. QTc reached statistical significance associated with the perfusion alterations and confirms published data considering it an ischemic variable more than a DA one [1]. The cut-off values for abnormality are controversial (longer in women); in general normal < 440 ms; over 500 ms with a high risk of arrhythmias and sudden death, with hyperglycemia and severe CAD being the predictors of this greater interval [19].

DA parameters have independent predictive value of adverse cardiovascular events in type 2 DM, independent of impaired myocardial perfusion [20, 21]. Abnormal HRR would represent vagal alteration and parasympathetic attenuation; 21 beats are considered adequate in the first minute after graduated effort; abnormal values have lower limit, which is why we prefer < 14 in our work [22–24]. The CARDIA study suggested that DA in combination with poor physical condition could be a mechanism associated with early glucose alteration and DM development; fasting hyperglycemia would cause peripheral nerve damage [24, 25]. The ACCORD study showed that DA was associated with higher mortality in subjects with type 2 DM at high cardiovascular risk; in the presence of AD at the start of follow-up, mortality was similar in those with intense or standard glycemic metabolic therapy [26].

Another method to quantify DA using cardiac adrenergic function imaging is by means of metaiodo-benzylguanidine (MIBG) labeled with Iodine-123; observed discrepancy between HR variability and MIBG uptake [27] and also that QTc is not a sensitive parameter for predicting AD in DM [28].

The standard for diagnosis of distal symmetric polyneuropathy is the neurological examination and electrophysiology, although other techniques of lower performance and cost are used for annual screening. The MNSI is simple, non-invasive, and presents

Table 4 Bivariate and multivariate survival analyses

Analysis	Bivariate		Multivariate	
Factor	HR (95%CI)	*p*	HR (95%CI)	*p*
Baseline triglycerides	1.01 (0.99;1.02)	0.086		
Baseline myocardial SPECT	7.27 (0.75;70.76)	0.088		
Control myocardial SPECT	17.79 (1.83;172.93)	0.013*		
Control HbA1c	2.28 (1.10;4.75)	0.027*		
Control HRR	0.86 (0.74;1.00)	0.051		
Control serum creatinine	2.66 (1.00;7.06)	0.050		
Control serum glucose	1.02 (1.00;1.03)	0.011*		
Microalbuminuria	1.00 (1.001;1.005)	0.010*	1.01 (0.99;1.02)	0.075
Control QTc	1.02 (0.99;1.04)	0.096	37.58 (0.63;2227.83)	0.082

HR hazard ratio, *CI* confidence interval

*$p < 0.05$

relative good agreement with other methods if clinical parameters such as vibration sensitivity are used together [29–31].

A recent report showed that silent ischemia was strongly associated with PN, using the Neuropathy Disability Score, even in type 2 DM without cardiovascular history or AD, which could help to investigate cardiovascular risk [32]. The origin of the alterations in long-standing DM is multifactorial and complex; association with endothelial dysfunction has been demonstrated, measured with flow-mediated dilation in the brachial artery and circulating inflammation biomarkers including cell adhesion cytokines, with FRCV and NP [33]. On the other hand, PN is different between type 1 and 2 diabetic patients, measured with magnetic resonance neurography, due to alterations in lipid metabolism [34] with proximal damage that seems to get worse with therapies that produce rapid low blood sugar levels [35].

There are different approaches to diagnose diabetic cardiomyopathy in addition to perfusion SPECT: positron tomography (PET) with absolute flow measurement to investigate microvascular disease and magnetic resonance imaging. Lately, echocardiography with speckle tracking technique has been used [36]. In a multivariate analysis, the complications of DM, hypertriglyceridemia, and overweight or obesity were closely associated with the initial stage of left ventricular longitudinal systolic dysfunction in asymptomatic DM with preserved LVEF, which would be an early marker of cardiomyopathy of this origin [37].

The strength of this work is its prospective nature and represents our current status in asymptomatic DM management. The main weaknesses are the small sample obtained and the lack of other tests to diagnose the presence of AD.

We conclude that in this sample of cardiovascular asymptomatic type 2 DM patients, myocardial silent ischemia is more frequent than expected, similar to published international experience. In our cases, that class of ischemia was associated with PN and AD, enhancing the importance of their clinical evaluation, especially in patients with longer duration of the metabolic disorder. Serum glucose control and microalbuminuria were related to survival, which requires an early global confrontation and intensified therapy.

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Compliance with ethical standards

The study was approved by the Scientific Ethics Committee of the Clinical Hospital of the University of Chile. Informed consent was obtained from all individual participants included in the study

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

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ORIGINAL ARTICLE

Frequency and risk factors of diabetic retinopathy in patients with type 2 diabetes presenting at a tertiary care hospital

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Abstract

Objective To evaluate frequency and risk factors of diabetic retinopathy in patients with type 2 diabetes.

Methodology This prospective observational study was conducted from January 2017 to June 2017 at the outpatient department of Baqai Institute of Diabetology and Endocrinology (BIDE) and Baqai Medical University a tertiary care diabetes hospital of Karachi, Pakistan. Convenience sampling was done. Demographic, anthropometric, clinical, and biochemical data were collected, and ophthalmic screening was performed by funduscopy at a slit lamp biomicroscopy with the help 90 D fundus lens. Grading of diabetic retinopathy was done according to the modified Early Treatment Diabetic Retinopathy Disease Severity Scale (ETDRS) criteria.

Result Retinopathy was present in 17.5% of patients. Non-sight-threatening retinopathy was found in 15.2% and sight-threatening retinopathy was found in 17.6%. In table 4, logistic regression analysis determined the relationship between retinopathy and its possible risk factors. In univariate logistic regression model, Age, poor glycemic control and longer duration of diabetes were found to be significant risk factors for developing retinopathy. In multivariate logistic regression model, duration of diabetes remained significantly associated with the development of retinopathy. Additionally, gender was not significantly associated in univariate analysis but it became significant after adjustment in multivariate model.

Conclusion An early screening program reduces the risk of incidence of diabetic retinopathy. Hence, screening of retinopathy should be done once in a year.

Keywords Diabetes · Funduscopy · Retinopathy · Risk indicators

Introduction

Diabetes mellitus is becoming the major cause of chronic diseases virtually all around the world [1]. More than 425 million people are affected globally; it may rise to 693 million in 2045 [1]. The National Diabetes Survey of Pakistan (NDSP) 2016–2017 reported that 26.3% of people suffered from diabetes [2]. The epidemic of diabetes pays significant attention to public health and philanthropists through diabetic complications; it may affect the eyes leading to diabetic retinopathy (DR) [3], and immense attention is needed on its effects [4]. For every three diabetic people, one was affected with diabetic retinopathy classified into proliferative and non-proliferative retinopathy and one out of ten developed the sight-threatening type of the disease [5]. In 2015, the prevalence of DR was estimated at 145 million; around 45 million people suffered from vision-threatening DR; 35% were persons with any type of retinopathy, whereas 7% were affected with proliferative retinopathy [3]. In patients with type 2 diabetes (T2DM), diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) are the primary causes in the defect of vision [6]. It gives rise to blindness in adult age making a bad mode of living. Cardiovascular risk factors and the prevalence of cardiovascular disease are associated with severity of DR [7]. Previously, many published studies were conducted to demonstrate the threat from diabetes and its

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complication from countries with a large population including China and India so that they implemented national plans to reduce the problems of diabetes [8, 9]. In Pakistan, more than 200 million are expected to have a large number of DR, with no strategy to reduce the consequences [5]. Present data is scarce for highlighting the crisis of DR to policy makers demonstrating diabetes-related blindness and evaluating strategies and management for primary prevention of diabetes. There is deficient precise data highlighting the crisis of diabetes and DR to evaluate national programs for policy makers demonstrating diabetes-related blindness [4]. Dyslipidemia of the metabolic syndrome is commonly associated with DR and known to be a risk factor for DR and DME [10]. On the other hand, in some studies, the three major risk factors of DR were observed: duration of diabetes, blood pressure control, and A1C levels [11].

The overall decline in the incidence and prevalence of DR depends on the management of T2DM. The aim of this study is to determine the frequency of DR in patients with type 2 diabetes mellitus at a tertiary care diabetes hospital.

Methodology

This prospective observational study was conducted from January 2017 to June 2017 at Baqai Institute of Diabetology and Endocrinology (BIDE) and Baqai Medical University a tertiary care diabetes hospital of Karachi, Pakistan. This study was ethically approved by the Institutional Review Board of BIDE. All patients attending the diabetes outpatient department were screened by an ophthalmologist. After giving informed consent, demographic and clinical parameters were collected from patients (age, gender, BMI) and baseline biochemical parameters (HbA1c, cholesterol, HDL, LDL, and triglyceride) were extracted from the health management system (HMS) of BIDE.

Previous history of an eye surgery or laser treatment was also taken from each patient record. Every individual having type 2 DM was included in this study. History of glaucoma; opaque cornea; other preexisting retinal diseases like age-related macular degeneration/retinopathy, central retinal artery occlusion (CRAO), branch retinal artery occlusion (BRAO), central retinal vein occlusion (CRVO), anterior ischemic optic neuropathy(AION), etc.; and patients on lipid-lowering medications were excluded from the study. Screening was performed by funduscopy at a slit lamp biomicroscopy with the help 90 D fundus lens. The presence of a sign of retinopathy was taken as sufficient evidence to classify the patient in the retinopathy group. A patient with normal fundus was referred for follow-up and also excluded from the study.

According to the modified Airlie House Classification, grading of diabetic retinopathy was done with the modified Early Treatment Diabetic Retinopathy Disease Severity Scale (ETDRS). Mild and moderate non-proliferative diabetic retinopathy (NPDR) without clinically significant macular edema (CSME) was categorized into the non-sight-threatening diabetic retinopathy (NSTDR) group. ACSME alone or in combination with NPDR or PDR and advanced diabetic eye disease (ADED) were categorized into sight-threatening DR (STDR) as well as proliferative diabetic retinopathy (PDR). Patients with advanced diabetic eye disease (ADED) were referred to a tertiary care hospital for pars-plana vitrectomy. Patients with severe non-proliferative diabetic retinopathy without CSME were considered clinically on an individual basis and decided either for follow-up.

t test and chi-square test were also applied to determine the significance. A logistic regression analysis was conducted to determine the risk factor of diabetic retinopathy. Analysis was done using SPSS version 20.0. A p value < 0.05 was considered statistically significant. Frequency and percentage were calculated for quantitative variable.

Results

A total number of 709 patients with diabetes were screened, out of which 394 were males and 315 females. Overall mean age was 49.93 ± 12.51 (years) and duration of diabetes was 9.01 ± 7.71 (years). Mean body mass index was 29.12 ± 5.88 (kg/m^2) along with high-density lipoprotein, low-density lipoprotein, and cholesterol 32.11 ± 9.76, 108.75 ± 40.35, 175.93 ± 51.78 (mg/dl), respectively (Table 1).

Diabetic retinopathy was found through funduscopy in 124 (17.5%) subjects. Eighty-eight (81.4%) were mild to moderate mon-proliferative diabetic retinopathy (NPDR) and 20 (18.5%) were moderate to severe while 21 (3%) were proliferative diabetic retinopathy (PDR), as shown in Table 2. Non-sight-threatening retinopathy (NSTDR) was found in 15.2% and sight-threatening retinopathy (STDR) was found in 17.6% (Fig. 1).

The frequency of NPDR (along with CSME and without CSME) in male participants increases as compared to that in female; meanwhile, PDR increases in female (without CSME) and increases in male (with CSME) as well but the difference is not statistically significant (Table 3).

Risk factors age, HbA1c, and duration of diabetes were analyzed by univariate analysis and revealed that age was 1.03 times (95% CI 1.01–1.04) at risk to have diabetic retinopathy; diabetic patients' HbA1c 1.11 times (95% CI 1.01–1.23) more likely to have retinopathy; and patients with duration of diabetes more than 10 years 5.18 times (95% CI 1.01–1.23) to develop retinopathy (Table 4).

Multivariate logistic regression analysis revealed that male gender possesses 2.28 times (95% CI 1.14–4.59) more likely to develop retinopathy than female. A patient with duration of

Table 1 Baseline characteristics of studied population

Parameters		Male	Female	*p* value	Overall
n		394	315	–	709
Age (years)		50.96 ± 12.26	48.64 ± 12.73	0.014	49.93 ± 12.51
Duration of diabetes		8.98 ± 7.84	9.03 ± 7.56	< 0.0001	9.01 ± 7.71
Marital status	Single	56 (14.2%)	60 (19%)	0.084	116 (16.4%)
	Married	338 (85.8%)	255 (81%)		593 (83.6%)
Smoking habit	No	290 (73.6%)	312 (99%)	N/A	602 (84.9%)
	Yes	60 (15.2%)	3 (1%)		63 (8.9%)
	Ex-smoker	44 (11.2%)	0 (0%)		44 (6.2%)
Drinking habit	No	387 (98.2%)	315 (100%)	N/A	702 (99%)
	Yes	7 (1.8%)	0 (0%)		7 (1%)
Systolic blood pressure (mmHg)		127.47 ± 17.09	128.16 ± 18.86	0.614	127.78 ± 17.89
Diastolic blood pressure (mmHg)		80.71 ± 8.71	79.63 ± 9.27	0.115	80.23 ± 8.97
Family history of DM (diabetes mellitus)	No	72 (28.1%)	43 (21.1%)	0.083	115(25%)
	Yes	184 (71.9%)	161 (78.9%)		345(75%)
BMI (body mass index) (kg/m^2)		28.25 ± 5.48	30.2 ± 6.2	< 0.0001	29.12 ± 5.88
Hba1c (%)		9.41 ± 2.21	9.64 ± 2.29	0.333	9.5 ± 2.24
Cholesterol (mg/dl)		166.99 ± 47.82	188.7 ± 54.67	< 0.0001	175.93 ± 51.78
HDL (high-density lipoprotein)(mg/dl)		29.97 ± 8.44	35.11 ± 10.69	< 0.0001	32.11 ± 9.76
LDL (low-density lipoprotein) (mg/dl)		103.63 ± 38.74	115.82 ± 41.59	0.005	108.75 ± 40.35
Triglyceride (mg/dl)		218.17 ± 128.57	225.96 ± 118.8	0.589	221.41 ± 124.46
Serum creatinine (mg/dl)		1.23 ± 0.59	1.12 ± 0.75	0.118	1.18 ± 0.66

Data presented as mean ± SD or *n* (%)

$p < 0.05$ considered to be statistically significant

DM ≥ 10 years shows significant value 1.12 times (95% CI 1.07–1.18) than patient with ≤ 10 years of diabetes, whereas age (95% CI 0.97–1.04) and poor glycemic control (HbA1c ≥ 7%) (95% CI 0.99–1.34) were not significantly associated with the development of diabetic retinopathy (Table 4).

Discussion

Diabetic retinopathy is rapidly spread around the world particularly in low- and middle-income countries [10]. The present study found that the frequency of DR was 17.5% in

Table 2 Stages of diabetic retinopathy

Parameters	Male	Female	*p* value	Overall
n	394	315	–	709
Diabetic retinopathy (DR)				
No	317 (80.5%)	268 (85.1%)	0.107	585 (82.5%)
Yes	77 (19.5%)	47 (14.9%)		124 (17.5%)
Non-proliferative diabetic retinopathy (NPDR)				
Mild to moderate NPDR	55 (78.5%)	33 (86.8%)	0.291	88 (81.4%)
Moderate to severe NPDR	15 (21.5%)	5 (13.2%)		20 (18.5%)
Clinically significant macular edema (CSME)				
No	365 (92.6%)	299 (94.9%)	0.216	664 (93.7%)
Yes	29 (7.4%)	16 (5.1%)		45 (6.3%)
Proliferative diabetic retinopathy (PDR)				
No	382 (97%)	306 (97.1%)	0.883	688 (97%)
Yes	12 (3%)	9 (2.9%)		21 (3%)

Data presented as *n* (%)

$p < 0.05$ considered to be statistically significant

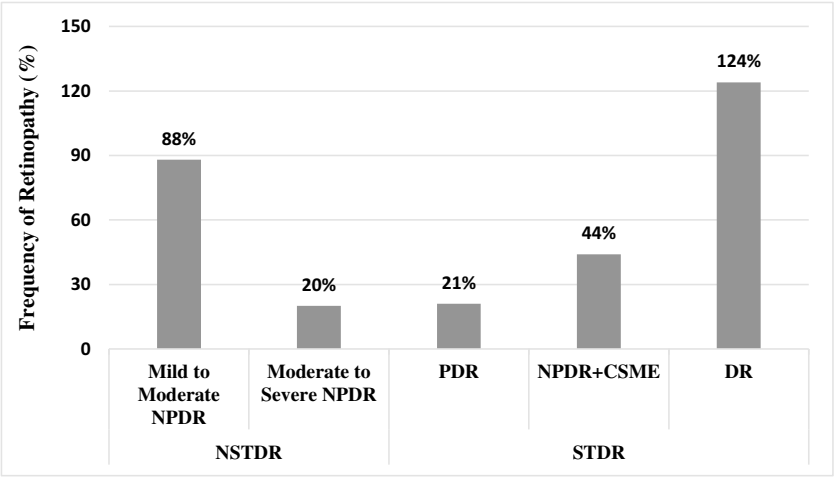

Fig. 1 Frequency of various stages of retinopathy among type 2 diabetic retinopathy patients according to the modified Airlie House Classification grading of diabetic retinopathy, modified by the early treatment research group (ETRG)

patients with type 2 diabetes. The prevalence of DR was reported to be 34.08% in China [12], in Scotland 19.3%, and in Wales 30.2% respectively [13, 14]. The variations reported in the frequency may be due to the inclusion of both types of diabetes and usage of different screening methods and grading systems.

Therefore, the high frequency of retinopathy has suggested for ophthalmic examination and screening as per international guidelines of patient with type 2 diabetes [15].

In this study, duration of diabetes more than 10 years and male gender were more likely to have diabetic retinopathy, so duration of diabetes and male gender were shown as important predictors for the severity of retinopathy, as endorsed by previous studies [16]. People who suffered from diabetes at an earlier age get more chance to develop retinopathy. Male gender has greater risk due to the presence of factors like obesity and hypertension; however, the reason is not clear and further investigation is required. In this study, HbA1c was shown as a risk indicator of DR [17]. Other studies showed the value of HbA1c at an increasing trend as severity of diabetic retinopathy increases. The poor glycemic control was significantly associated with severity of retinopathy [18]. In previous studies, logistic regression analysis showed that males are 3.5 times more prone to develop DR due to some confounding factors such as obesity or hypertension [19] Duration of diabetes is also a risk indicator for developing retinopathy [17].

Regarding the grades of retinopathy in this study, the proportion of mild to moderate type of retinopathy is 88 (81.4%), which is elevated than that reported from Saudi Arabia and Oman [20]. Moderate to severe NPDR was reported in 18.5% of patients which is also higher by 4% than previous study results in Oman [21], while proliferative diabetic retinopathy (PDR) was 3%. The globally documented prevalence of PDR at 6.9% was lower by 50.5% than that documented in West India [22, 23]. Non-sight-threatening retinopathy (NSTDR) and sight-threatening retinopathy (STDR) were found to be 15.2% and 17.6%, respectively. As compared to previous screening in Liverpool and Wales, STDR was reported at

Table 3 Association of NPDR, PDR with CSME/ without CSME according to Gender distribution

	NPDR	Male	Female	*p* value	Overall
n		394	315	–	709
Without CSME	No	323 (88.5%)	277 (92.6%)	0.071	600 (90.4%)
	Yes	42 (11.5%)	22 (7.4%)		64 (9.6%)
With CSME	No	1 (3.4%)	0 (0%)	N/A	1 (2.2%)
	Yes	28 (96.6%)	16 (100%)		44 (97.8%)
	PDR				
Without CSME	No	354 (98.6%)	289 (98%)	0.526	643 (98.3%)
	Yes	5 (1.4%)	6 (2%)		11 (1.7%)
With CSME	No	28 (80%)	16 (80%)	0.99	44 (80%)
	Yes	7 (20%)	4 (20%)		11 (20%)

Data presented as *n* (%)

$p < 0.05$ considered to be statistically significant

Table 4 Risk indicators of diabetic retinopathy explored by logistic regression analysis

Parameters	Crude OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Age (years)	1.03 (1.01–1.04)	< 0.0001	1.00 (0.97–1.04)	0.652
Married	1.65 (0.91–3.01)	0.096	2.38 (0.67–8.43)	0.178
Positive family history of DM	1.12 (0.64–1.98)	0.673	1.09 (0.50–2.37)	0.827
HbA1c ≥ 7%	1.11 (1.01–1.23)	0.030	1.15 (0.99–1.34)	0.066
Obesity	0.71 (0.45–1.10)	0.129	1.80 (0.79–4.11)	0.161
Male	1.38 (0.93–2.06)	0.108	2.28 (1.14–4.59)	0.020
Duration of DM ≥ 10 years	5.18 (3.04–8.82)	< 0.0001	1.12 (1.07–1.18)	< 0.0001
Hypertension	1.39 (0.92–2.09)	0.112	1.07 (0.53–2.13)	0.841

Obesity: BMI ≥ 25 kg/m^2 ; adjusted for the variables present in the table OR odds ratio; CI confidence interval; $p < 0.05$ considered to be statistically significant

6.0% and 2.9% respectively among patients with type 2 diabetes [14, 24]. Both results are lower than the findings of this study; lifestyle and environmental differences may contribute to the discrepancy.

On the other hand, studies on population with DR had CSME 36% which is higher than others (19.2%, 6.27%, 15.1%, and 5.78%) previously reported by Shrestha, et al., Raman et al., Benarous et al., and Thapa et al., respectively [25–28]. Similarly, the sight-threatening CSME with severe NPDR was 47.8% [29]. CSME with moderate and severe NPDR and PDR reported a significant correlation [19]. According to gender, NPDR (with CSME and without CSME) was associated with male, whereas PDR was associated with female (without CSME), and CSME in male was more prevalent. This showed a close association of CSME with severity of DR in male gender, whereas previous studies have shown that a significant association of CSME in patient with diabetic retinopathy was found in females (58%) as compared to males (42%) [30].

Consequently, the burden of diabetes emphasizes the population communities as well as health resources to enhance lifestyle, resist the trend of increasing prevalence, and reduce the possibility of complications.

Limitations

This study design was prospective observational; it has a limitation of having a small sample size and no control group. More population-based studies and surveillance should be carried out to address the actual magnitude of the problem.

Authors' contributions SS: Concept and design, researched data, wrote and reviewed the manuscript
AF: Concept and design, edited and reviewed the manuscript
ARK: Researched data, edited and reviewed the manuscript
AB: Wrote and reviewed the manuscript
NAS: Edited and reviewed the manuscript
AB: Concept and design, edited and reviewed the manuscript

Compliance with ethical standards

Ethical approval for the study was obtained from the institutional review board (IRB) of BIDE.

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

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ORIGINAL ARTICLE

The tear VEGF and IGFBP3 in healthy and diabetic retinopathy

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Abstract

Background/ purpose Diabetic retinopathy is an important problem worldwide. The purposes of this study were to measure and analyze VEGF and IGFBP3 level changes in tears of type 2 diabetics with and without retinopathy.

Methods In a case–control study, tear samples of 30 diabetes patients without diabetic retinopathy (DNR) were collected, as well as tears of 30 patients with diabetes retinopathy (DR), and 30 healthy subjects without diabetes as the control group by Schirmer's standard strip, who were matched in terms of age and sex with the other groups. VEGF and IGFBP3 concentration was measured by ELISA method. HbA1c, VEGF, and IGFBP3 were measured in all 3 groups and statistically analyzed.

Results The percentage of HbA1C in the DNR group was clearly higher than the control group. Also, there was a significant difference in the percentage of HbA1C between DR and DNR groups. The concentration of VEGF in tears statistically increased in comparison with control ($P < 0.05$) but the IGFBP3 level did not change.

Discussion The VEGF level in tears is clearly linked with the process of DR; however, there wasn't any significant relationship between the level of IGFBP3 in tears and diabetic retinopathy. It is concluded that VEGF can be used as a noninvasive method for the diagnosis of diabetic retinopathy.

Keywords Diabetes · Diabetic retinopathy · VEGF · IGFBP3 · Schirmer's strip

Introduction

Diabetes is an important pathological condition involving small blood vessels and large vessels. The involvement of small vessels includes retinopathy, nephropathy, and neuropathy, while the large vessel diseases involve cardiovascular disease, strokes, and decreased blood flow in the legs. The reason for all these complications is the increased levels of blood sugar. In fact, chronic hyperglycemia plays the leading role in the onset of vascular complications of diabetes through creating structural and metabolic disorders. These structural and metabolic disorders, including abnormal activation of inflammatory signaling cascades, increased production of oxygen free radicals, and abnormal stimulation and regulation of the hemodynamic system [1, 2].

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Diabetic retinopathy (DR) is the most prominent cause of blindness and visual impairment. DR is the long-term complication of diabetes and poor control which is the most important cause of blindness in the working population in developed countries [3].

One-hundred fifty million people worldwide suffer from this complication and WHO predicts that this figure will be doubled within 10–15 years [4]. Based on the presence or absence of new abnormal vessels, diabetic retinopathy is divided into two types: proliferative (PDR) and non-proliferative (NPDR) [5]. Several studies report the prevalence of any kind of retinopathy in various studies over 30% [6–8]. Chronic hyperglycemia leads to metabolic and hemodynamic disorders such as vascular disruption, thickening of the capillary basement membrane, formation of microaneurysms, microvascular cell death, and ultimately reduced perfusion and extensive internal retinal ischemia [9], which ultimately all these factors lead to disrupted response in a collection of cells, including neurons, glial cells, and capillaries cells [10]. While the exact pathogenesis of DR is not clear; currently, inflammation and other related processes are considered causes of lesions in small vessels, neurons, and glial cells [10–12]. However, the role of inflammation as the cause of DR has not been clearly explained yet [13, 14].

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein with 45-KDa molecular weight. It is a powerful endothelial mitogen for endothelial cell growth, angiogenesis, and increased vascular permeability [15, 16]. In recent years, the basic documentation about the role of cytokines, inflammatory cells, growth factors, and angiogenic factors was proven in the pathogenesis of DR [17–20]. In some studies, increased VEGF levels in the serum and tear of diabetic patients with retinopathy have been reported compared with the controls [21–23]. In addition, anti-VEGF monoclonal antibody is one of the best drugs for prevention of angiogenesis and metastasis.

Insulin-like growth factor-binding protein 3 (IGFBP3) is a secretory N-linked glycosylated phosphorylated protein with known pro-apoptotic function and inhibition of cell proliferation [24]. In a study on laboratory mouse samples, IGFBP3 suppressed the destruction of vessels by oxygen and re-growth of blood vessels leading to DR [25]. A reduced level of IGFBP3 in the serum of insulin-dependent diabetes with DR has also been reported [26].

However, simultaneous changes in the levels of VEGF and IGFBP3 in tears of patients with DR had not been studied in human subjects up to now. In the current study, changes in the levels of VEGF and IGFBP3 in the tears of patients with type 2 diabetes with and without retinopathy were measured and analyzed.

Materials and methods

Study subjects

In a case–control study, 60 diabetic patients and 30 healthy controls were enrolled after giving a full description with details on the study methods and objectives. The diagnosis of type 2 diabetes was in accordance with the America Diabetes Association (ADA) Guidelines [27]. In this research, the aim and methods of study were explained for patients and they were satisfied for blood sampling. No therapeutic intervention was performed in patients. Written informed consent was obtained from all subjects. This study was conducted under ethical approvals from the local ethical committee and according to Helsinki declaration.

Sixty diabetic patients included 30 patients with DR and 30 without DR. DR identification was carried out on the basis of ETDRS criteria [28]. This study was conducted in the center of eye diseases in Bu-Ali-Sina Hospital, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Mazandaran, Iran. The control group consisted of volunteers who referred to the ophthalmology clinic for routine eye exams or prescription of glasses. The exclusion criteria from the study included history of intraocular surgery, intravitreal therapy, photocoagulation, trauma, vitreous hemorrhage, isolated retinal, and ocular inflammation such as uveitis during the past 6 months and other systemic disorders. Before entering the study, all participants in the study were visited and ophthalmology tests including best corrected visual acuity (BCVA) measured by means of standard ETDRS, measuring intraocular pressure with tonometry, and fundus eye examination by a qualified ophthalmologist using slit lamp bio microscopy using 75D lens and indirect ophthalmoscopy with a 20D lens were done. Peripheral retinal examination was done to rule out other pathologies. HbA1c levels were measured (Nyco Card HbA1c test, Italy) in all subjects participating in the study.

Tear sampling

Samples of unstimulated tear from inferior meniscus between 6 o'clock status and lateral canthus were collected by Schirmer's standard strip. Immediately after sampling, the strip was placed into a sterile labeled tube and stored at − 40 °C until analysis of samples. The wet part of the Schirmer strip was divided into smaller parts and every 1 mm of the strip was soaked in 10 ml of phosphate buffered saline for 3 h in order to free its proteins. The test was done at the same time of the day for all patients.

Cytokine assay

VEGF and IGFBP3 were measured using a 96-well plate coated with antibody specific for human VEGF (Ebioscience, Vienna, Austria) and IGFBP3 (Human IGFBP-3 ELISA Kit Biovendor, Mediagnost, Aspenhausttr.25, D-71770 Reutlingen, Germany) according to manufacturer's instructions.

Statistical analysis

Data were expressed as mean standard error (SE) and standard deviation (SD) and analyzed using the statistical package for the social sciences (SPSS version18) for Windows. Groups' homogeneity in terms of age and gender was assessed with ANOVA and chi square tests. The parameters that were statistically significant different between groups were analyzed using Mann–Whitney *U* test or Student *t* test. All tests were performed at an error level of 5%.

Results

Age, gender, HBA1C, diabetes duration, VEGF, and IGFBP3 levels in tear are shown in Table 1. With regard to age and sex distribution, no significant difference was observed between the study groups.

Table 1 Demographic and laboratory data of participants in the study

	Controls	DNR	DR	p value
No. of subjects	30	30	30	N.S[a]
				N.S[b]
				N.S[c]
Age (mean ± SD) (years)	56.9 ± 8	58.8 ± 7.8	62.2 ± 7	N.S[a]
				N.S[b]
				N.S[c]
Gender (M/F)	(15/15)	(16/14)	(14/16)	N.S[a]
				N.S[b]
				N.S[c]
HbA1c (mean ± SD) (%)	5.4 ± 0.2	8 ± 1	9 ± 1.4	< 0.001[a]
				< 0.05[b]
				< 0.001[c]
DM duration (mean ± SD) (years)	Not applicable	10 ± 3.7	15.2 ± 3.9	< 0.001[a]
				< 0.001[b]
				< 0.001[c]
VEGF(mean ± SEM) (pg/ml)	58.7 ± 5.13	75.1 ± 18.77	235.42 ± 47.82	N.S[d]
				< 0.001[e]
				< 0.001[f]
IGFBP3(mean ± SEM) (ng/ml)	9.67 ± 1.56	12.5 ± 1.55	11.8 ± 1.37	N.S[d]
				N.S[e]
				N.S[f]

[a] *t* student test: DNR versus controls; [b] *t* student test: DNR versus DR; [c] *t* student test: DR versus controls; [d] Mann–Whitney 필 test: DNR versus controls; [e] Mann–Whitney 필 test: DNR versus DR; [f] Mann–Whitney 필 test: DNR versus controls

DM, diabetes mellitus; *SD*, standard deviation; *SEM*, standard error of the mean; N.S, not significant.

The HBA1C percentage was significantly higher in the group of DNR than in controls ($p < 0.001$). There was also a significant difference in the percentage of HbA1C between diabetic patients with and without retinopathy ($p < 0.05$). Duration of diabetes was significantly higher in patients with retinopathy ($p < 0.001$). The difference in tears' VEGF levels was not significant between controls and those in the DNR group. The tears' VEGF levels were significantly higher in the group of DR than those in the DNR group ($p = 0.001$). This proves that the level of VEGF in tears increases along DR (Fig. 1).

The difference in tears' IGFBP3 levels was not significant between controls and diabetic patients. Tears' IGFBP3 levels did not change significantly along diabetic retinopathy as shown in Fig. 2.

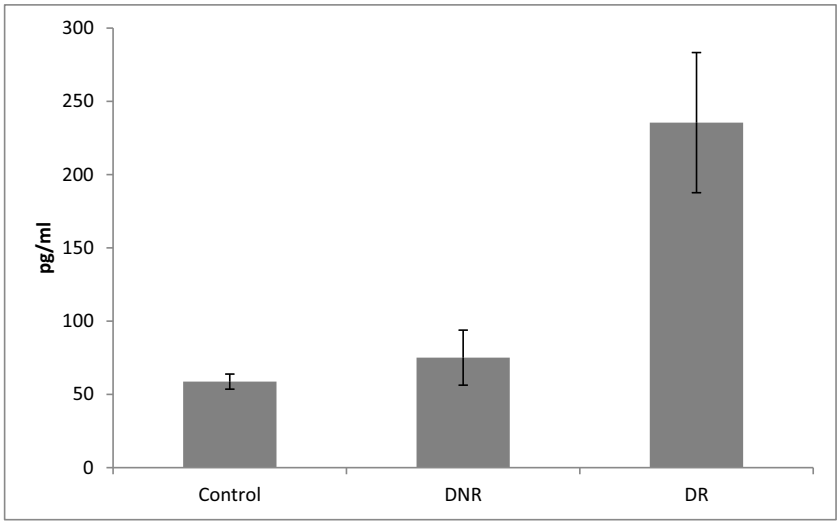

Fig. 1 VEGF levels in tears of the three different study groups. The data are given as mean and standard error of the mean

Discussion

VEGF levels in tears are strongly linked with the process of vascular injury in the retina but there was no significant correlation between the levels of IGFBP3 with DR. Our findings, along with other published research results, show that the proangiogenic factors emerge more in the tears of patients with DR compared with antiangiogenic factors.

Liu et al. demonstrated that levels of VEGF and other inflammatory cytokines increase in the tears of patients with DR which is indicative of the role of VEGF in the progression of DR. In this study, tear samples were taken using a capillary tube [22]. Also, other researchers realized the relationship between the level of VEGF in tears and diabetic retinopathy and nephropathy [21]. Other studies noted the suppressing role of signaling IGFBP3 on DR in animal models, but no study on human subjects has been done [25]. Janssen et al. demonstrated that fasting levels of free IGF-1, total IGF-1, and IGFBP3 in serum of insulin-dependent diabetes obviously decreased compared with the control group [26]. Also, Meyer-Schwickerath et al. demonstrated that levels of IGF1, IGF2, IGFBP2, and IGFBP3 increase in the vitreous of the eye neovascular diseases such as DR [29, 30]. Our findings endorse the important role of VEGF in the development and progression of DR, but there wasn't clear relationship between the level of IGFBP3 in tears with DR. A strong correlation between inflammatory biomarkers, such as VEGF with DR disease, has been proven by past studies.

In addition, another important finding is the significant correlation between HbA1C levels and duration of diabetes with VEGF levels in tear in the present study which confirms the role of VEGF in tears as a predictor of diabetic microvascular complications. All these findings confirm the role of VEGF in the development and progression of DR and thereby enabling the diagnosis and treatment of this factor. Early diagnosis and treatment of diabetes can reduce the risk of its microvascular complications. Several clinical trials proposed the benefits of early intervention in reducing microvascular complications of diabetes [31]. Screening for undiagnosed diabetes, especially in unsigned high-risk populations by sampling the serum of individuals, is the best practice for early detection of type 2 diabetes [32]. ADA has recommended that all nondiabetic people over 45 years of age to have diabetes screening every 3 years. Despite repeated screening and targeting high-risk populations and appropriate follow-up, abnormal results have been non-frequent and screening efficiency is still low [33]. DR early diagnosis is required, because early detection and treatment can prevent blindness. It has been proven that keeping blood glucose levels close to normal leads to a reduction of long-term complications of diabetes, such as DR [34]. In this way, we can reduce the number of diabetic patients with visual impairments [35, 36].

Diabetes causes a variety of ocular disorders due to metabolic disorders and peripheral neuropathy such as a dry eye, meibomian gland dysfunction, decreased corneal sensation, and other harmful side effects [37–39]. Tear IGFBP3 has been studied in other ocular problems like ROP and it was found to be low [40].

All these effects can change cytokine levels in tears. Further studies with more patients are needed to determine the impact of these eye diseases on changes in the level of cytokines. After rejecting these conditions, then VEGF can be used as a diagnostic biomarker for DR.

Although, in this study, the severity of DR was not implied but the criterion was that of suffering from the disease, this could be a possible cause for the lack of obvious changes in IGFBP3 levels between the groups under study. In addition, the other methods for tear collection are glass capillary and polyester rods. In this study, we have used a different method than that of other studies, that is, sampling was done using Schirmer strips. The Schirmer strip is a noninvasive method.

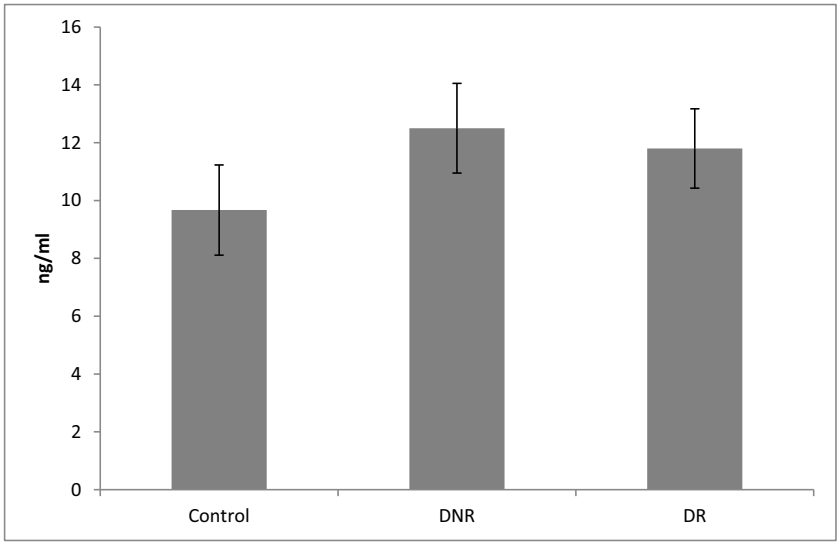

Fig. 2 IGFBP3 levels in tears of the three different study groups. The data are given as mean standard error of the mean

All these factors can affect the levels of cytokines in tears and confirm our results. As a result, data from this study showed that the level of VEGF in tear is related to DR disease. But there is no clear association between the levels of IGFBP3 in tears with DR. Since we did not have data regarding the subclassification of DR, we could not correlate levels of VEGF/IGFBP3 in non-proliferative and proliferative DR. Finally, it is concluded that VEGF and IGFBP3 levels can be inflammatory diagnostic markers for retinopathy in diabetes patients.

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Compliance with ethical standards

This study was approved by the local ethics committee at Mazandaran University of Medical Sciences and all participants provided the written informed consents. This research conforms to the latest revision of the Declaration of Helsinki.

Conflict of interest The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Association of the rs3758391 polymorphism in the SIRT1 gene with diabetic nephropathy and decreased estimated glomerular filtration rate (GFR) in a population from southwest Iran

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Abstract

Introduction Type 2 diabetes mellitus (T2DM) is a polygenic metabolic disorder. SIRT1 has an essential role in the insulin-signaling pathway and energy homeostasis. SIRT1 exerts protective effects in the kidney cells.

Objectives We aimed to investigate whether the rs3758391 variant was associated with diabetic nephropathy, measures of kidney function, and BMI in a population with and without diabetes in southwest Iran.

Methods The study comprised 132 patients with type 2 diabetes mellitus (T2DM) (with and without nephropathy). They were compared with 66 normal subjects. The subjects were genotyped for the rs3758391 polymorphism by the PCR–RFLP method. Fasting blood glucose, HbA1c, urea, creatinine, and urinary albumin were measured using a biochemistry analyzer. Serum cystatin C levels were measured by ELISA.

Results The genotype distribution and allele frequencies were significantly different between the entirely diabetic group and the healthy subjects (p value < 0.05). For T2DM, the odds ratios (ORs) for the TT genotype and the T allele carrier were 5.7 (95% confidence interval (CI) 2.2–14.9, $p < 0.001$) and 4.01 (95% CI 2.1–7.5, $p < 0.001$), respectively. For diabetic nephropathy, the ORs for the TT genotype and the T allele carrier were 3.96 (95% CI 1.5–10.0, $p = 0.003$) and 3.0 (95% CI 1.4–6.4, $p = 0.003$), respectively. For decreased eGFR below 60 mL/min/1.73m^2, the OR for TT was 2.9 (95% CI 1.1–7.5, $p = 0.02$).

Conclusion Our results confirm that the risk allele of the rs3758391 SNP in the SIRT1 gene is strongly associated with T2DM and diabetic nephropathy. The TT genotype is also associated with decreased eGFR.

Keywords Type 2 diabetes · Diabetic nephropathy · SIRT1 · rs3758391 polymorphism · eGFR

Introduction

Type 2 diabetes mellitus (T2DM) is a complex, multi-factorial, and polygenic metabolic disorder. Its pathogenesis is influenced by diverse environmental and genetic risk factors [1]. T2DM is characterized by hyperglycemia, with variable degrees of insulin resistance, impaired insulin secretion, and increased hepatic glucose production [1, 2]. Micro-vascular and macro-vascular problems are the main complications of diabetes. An unfavorable micro-vascular complication of diabetes is diabetic nephropathy.

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It is characterized by proteinuria, hypertension, and loss of renal function [3–5]. Diabetes is the main cause of chronic renal failure and end-stage renal failure [3]. In 2013, approximately 25% of chronic kidney disease (CKD) cases and 35% of end-stage renal disease (ESRD) cases were related to diabetes [4]. Over time, poor glycemic control contributes to the increased risk of renal complications in diabetic patients; however, many individuals with poorly controlled diabetes never develop CKD, while others with optimal glycemic control develop renal complications. Considering these findings, it is important to find out the genetic susceptibility to the development of diabetic nephropathy [6–9]. Both candidate gene approaches and genome-wide association studies (GWAS) have considered several candidate genes with potential effects on the renal complications of diabetes. SIRT1 belongs to a group of highly conserved NAD^+-dependent protein deacetylases (SIRT1–7). It can be found in the nucleus, cytoplasm, and mitochondria [10]. SIRT1 has an essential role in the insulin-signaling pathway and energy homeostasis [11]. Several studies have demonstrated that calorie restriction (CR) leads to a high expression of SIRT1 in various tissues such as the brain, liver, muscle, and kidney [11–13]. This upregulation may lead to CR-mediated protection against hypoxia in aged kidneys [14]. SIRT1 exerts protective anti-apoptotic, anti-oxidative, and anti-inflammatory effects via its regulation of mitochondrial biogenesis and autophagy in the kidney cells [15]. It seems that the polymorphism of the SIRT1 gene can influence BMI and obesity by suppressing the PPARG (peroxisome proliferator-activated receptor gamma) in adipose tissues [16]. The SIRT1 gene is located in chromosome 10 (10q21.3), and polymorphic variants of this gene have been studied in the susceptibility to several disorders like nephropathy [13]. The variety of SIRT1 expressions, which may be caused by promoter region variants, can lead to individuals being susceptible to certain diseases such as diabetes and CKD. One of the promoter region variants is the rs3758391 polymorphism of the SIRT1 gene that may lead to changed expression of SIRT1. Previous studies have reported the association of the rs3758391T allele with T2DM [17]. However, no study has yet evaluated the impact of the polymorphism on the risk of diabetic nephropathy. Considering the important role of SIRT1 in physiological processes creating protection against nephropathy, the aim of this study was to analyze the SIRT1 promoter region polymorphism rs3758391C/T in relation to the genetic susceptibility to diabetic nephropathy.

Materials and methods

Patients and sampling

The present case-control study was conducted on 66 diabetic patients without nephropathy, 66 diabetic patients with nephropathy, and 66 healthy control subjects. The patients were recruited from the Golestan Hospital in Ahvaz. Diabetes diagnoses were performed by an expert endocrinologist based on the American Diabetes Association (ADA) criteria. A fasting blood sugar (FBS) > 126 mg/dL or current use of hypoglycemic agents was used to diagnose the diabetic patients, while measurements of blood urea nitrogen (BUN), serum creatinine (Cr), and albuminuria determined the nephropathy. The study protocol was approved by the local ethics committee at the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1395.76). After obtaining informed consent from all participants, the peripheral blood samples were collected and divided into two tubes. One tube was used for serum separation and biochemical measurements. The other tube was used for DNA extraction and genotyping analysis. All samples were stored at − 80 °C until the final analysis. DNA extractions were performed simultaneously for all samples. We also collected 24-h urine samples from all subjects included in the study in order to investigate albuminuria.

Laboratory assays

The BMI values were calculated after anthropometric measurements. Fasting blood glucose, HbA1c, BUN, creatinine, and urinary albumin were measured using a biochemistry analyzer (BT3000, Italy) and Pars Azmun assay kits (Tehran, Iran).

Measurement of cystatin C

It has been suggested that serum cystatin C is an accurate endogenous marker of GFR in research and clinical practice [18]. Serum cystatin C levels were measured using an available ELISA kit (BioVendor Human Cystatin C ELISA, NC, USA) based on a sandwich enzyme immunoassay, using a specific capture antibody and an HRP-labeled antibody according to the manufacturer's protocol. All tests on samples were performed with the help of ELISA kits by technicians who were unaware of whether the sample belonged to cases or to controls.

Calculation of eGFR

GFR is the best overall index of kidney function. Based on the recommendation of the National Kidney Foundation, we used the CKD-EPI creatinine–cystatin C equation (2012) to estimate the GFR [19].

$$\mathrm{eGFR} = 135 \times \min\left(\frac{S_{\mathrm{Cr}}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{S_{\mathrm{Cr}}}{\kappa}, 1\right)^{-601} \times \min\left(\frac{S_{\mathrm{cys}}}{0.8}, 1\right)^{-0.375} \times \max\left(\frac{S_{\mathrm{cys}}}{0.8}, 1\right)^{-0.711} \times 0.995^{\mathrm{Age}} \times 0.969\ [\text{if female}] \times 1.08\ [\text{if black}]$$

Abbreviations/units: eGFR (estimated glomerular filtration rate) = mL/min/1.73m^2, S_{Cr} (serum creatinine) = mg/dL, S_{cys} (standardized serum cystatin C) = mg/L, $\kappa = 0.7$ (females) or 0.9 (males), $\alpha = -0.248$ (females) or -0.207 (males), min(S_{Cr}/κ or 1) indicates the minimum of S_{Cr}/κ or 1, max(S_{Cr}/κ or 1) indicates the maximum of S_{Cr}/κ or 1, min(S_{cys}/0.8, 1) indicates the minimum of S_{cys}/0.8, 1, max(S_{cys}/0.8, 1) indicates the maximum of S_{cys}/0.8, 1, age = years.

DNA preparation and genotyping

Genomic DNA was isolated from peripheral blood cells using the Yekta Tajhiz Azma (YTA) Genomic DNA Extraction Kit for Whole Blood (Cat No. YT 9040. Tehran, Iran). The SIRT1 rs3758391C/T polymorphism was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) methods. The following primers were used: forward, 5′GTCACGCAGGTAATTGATGCAG3′; reverse, 5′ GGCTTAGTGGAAAGCCCTTC3′ [20]. PCR was performed by premix 2x master mix red (Amplicon, Denmark) containing 1.5 mm $MgCl_2$, according to the recommended protocol. Next, 100 ng of template DNA, 0.25 μL of each primer (100 pmol/μL), 8 μL master mix, and DNase/RNase-free water were added to a final volume of 25 μL. The amplification reactions included 5 min at 94 °C followed by 30 cycles of 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 45 s, followed by a final extension for 5 min at 72 °C. PCR products were visualized using standard electrophoresis on a 2% agarose gel containing safe stain, and images were prepared using the gel documentation system (Labtech International Ltd., England). The amplicon size for the rs3758391C/T was 241 bp. PCR products were incubated overnight with restriction enzyme Hin1II (New England Biolabs, USA). Following digestion, an amplicon with the T allele of the rs3758391C/T polymorphism gave rise to two fragments of 146 and 95 bp, whereas an amplicon with the C allele at this position remained undigested as a 241-bp fragment (Fig. 1).

Statistical analyses

Statistical analyses were performed using the SPSS software for Windows version 18.0 (SPSS, Chicago, IL, USA). The data are presented as mean ± standard deviation (SD) for continuous variables in each group. The continuous variables of the case and control groups were compared using the Student's t test and the Mann–Whitney U test. Genotypes and allele frequencies in the groups were analyzed using the χ^2 test. Where appropriate, the ORs with corresponding 95% confidence intervals (CIs) were calculated for association between genotypes, and T2D and diabetic nephropathy. Logistic regression was performed for the analysis of independent risk factors of the TT genotype and the T allele for T2DM and diabetic nephropathy. A two-sided p value less than 0.05 was considered statistically significant.

Results

As shown in Table 1, there was a significant difference between diabetic patients (both with and without nephropathy) and the control group in terms of parameters such as BMI, FBS, HbA1c, eGFR, BUN, Cr, cystatin C, and albuminuria. Significantly higher values of these parameters were found in diabetic patients as compared with healthy subjects, except eGFR, which decreased in diabetic patients ($p < 0.001$).

We could not find any significant association between BMI values and rs3758391 genotypes in any of the groups. The OR for TT versus CC in an obese sub-group (BMI ≥ 30 kg/m^2) as compared with a non-obese sub-group was 1.054 (95% CI 0.5–2.3, $p = 0.89$).

Association of the rs3758391C/T polymorphism with T2DM

Genotype distribution and allele frequencies were significantly different between the entire diabetic group and the healthy subjects, as analyzed by the chi-square test (p value < 0.05: Table 2). The frequency of the minor allele T in the diabetic group was significantly higher than the control group (45.8% versus 20.4%, respectively). The ORs for TT homozygous genotypes in the diabetic group versus the healthy control group were 5.7 (95% CI 2.2–14.9, $p < 0.001$) and 6.4 (95% CI 2.4–17.3, $p < 0.001$), respectively (before and after adjusting for age, sex, and BMI). The OR was also significantly high for the TT/CT genotype versus the CC homozygous genotype after adjustment (OR 4.2, 95% CI 2.2–8.2, $p < 0.001$).

Association of the rs3758391C/T polymorphism with diabetic nephropathy

In order to analyze the association of the rs3758391C/T polymorphism with diabetic nephropathy, genotype distribution and allele frequency were evaluated in the sub-groups of diabetic patients with and without nephropathy. As shown in Table 3, the frequencies of the TT genotype and the T risk allele were higher in patients with nephropathy ($p < 0.05$). The ORs for the TT homozygous genotype in diabetic patients with nephropathy versus diabetic patients without nephropathy (before and after adjusting for age, sex, BMI, and HbA1c) were 3.96 (95% CI 1.5–10.0, $p = 0.003$) and 5.99 (95% CI 1.9–18.3, $p = 0.002$), respectively. The OR for the TT/CT genotype versus the CC homozygous genotype was also significant for diabetic nephropathy (OR 3.0, 95% CI 1.4–6.4, $p = 0.003$).

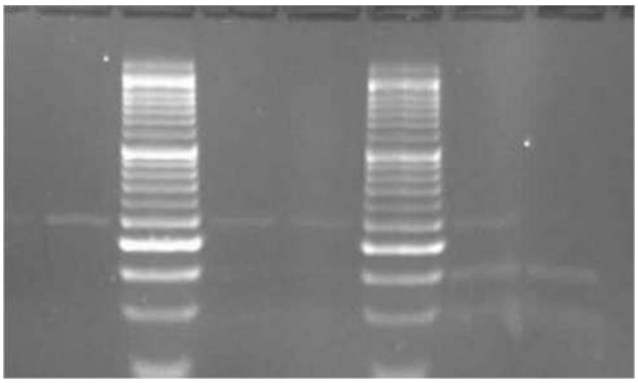

Fig. 1 PCR product electrophoresis photograph related to the SIRT1 rs3758391C/T polymorphism after PCR–RFLP. Sample 1: TT; samples 2 and 3: CT; samples 4 and 5: CC. The ladder was 50 bp

Association of the rs3758391C/T polymorphism with kidney function

We also evaluated the glycemic and kidney function parameters in subjects with TT and CC genotypes of the polymorphism in all groups. Different values of the parameters were found between these two genotypes. Higher values for FBS and HbA1c were observed in the TT variant as compared with the CC sub-groups. The differences were significant for FBS and HbA1c in the control and diabetic groups, respectively. Notably, the parameters of kidney function and nephropathy including Cr, cystatin C, and albuminuria showed significantly higher levels in diabetic nephropathy patients with the TT genotype as compared with those with the homozygous CC variant. Our results showed that eGFR significantly decreased in diabetic nephropathy patients with the TT genotype (Table 4). Additionally, we assessed the association of this polymorphism with decreased eGFR. We measured the OR for the TT homozygous in diabetic patients to get an eGFR below 60 mL/min/1.73m^2 (Table 5). The OR for TT versus CC was 2.9 (95% CI 1.1–7.5, $p = 0.02$).

Discussion

In this study, we attempted to investigate the relationship between the rs3758391 polymorphism of the SIRT1 gene, and the risk of T2DM and BMI changes. We found that the rs3758391 polymorphism is a notable susceptibility variant for T2DM in southwest Iran, where the majority of the population comprises people of Arab ethnicity. A significant association of the rs3758391 polymorphism with T2DM was observed in our study population. The effect of rs3758391 SNP of the SIRT1 gene as a susceptibility element for T2DM has been shown in other populations with different minor allele frequencies. The frequency of the risk T allele in T2DM patients in the present study was 49.6%, whereas it was 64.3% in Mexico City [17]. In our study, the frequency of the T allele in non-diabetic subjects was 20.4%, while it was 58.3% in Mexico City [17]. Our results showed that the frequency of the T allele in both control and diabetic subjects was lower in our subjects as compared with other populations [17]. Despite the lower frequency, the obtained OR for T allele in our study was higher than in previous

Table 1 Demographic and clinical profile of studied subjects

Variable	Control	T2DM	*p* value
Number	66	132	–
Male/female	38/28	53/79	–
Age	52 ± 8	55 ± 8	0.014
Diabetes duration	–	12 ± 4	–
Diabetic nephropathy, *n* (%)	–	66 (50)	–
BMI (kg/m^2)	26.9 ± 4.6	28.7 ± 4.9	0.015
FBS (mg/dL)	86.8 ± 12.3	227.8 ± 69.3	< 0.001
HbA1c % (mmol/mol)	4.8 ± 0.5 (29 ± 5.5)	7.9 ± 1.2 (63 ± 13.1)	< 0.001
eGFR (mL/min/1.73m^2)	79.5 ± 16.8	55.4 ± 31.9	< 0.001
BUN (mg/dL)	13.2 ± 2.3	27 ± 17.5	< 0.001
Cr (mg/dL)	0.83 ± 0.15	1.79 ± 1.38	< 0.001
Cystatin C (mg/mL)	1162 ± 350	1956 ± 1485	0.019
Albuminuria (mg/24 h)	6 ± 2	225 ± 244	< 0.001

Data are mean ± SD. *BMI* body mass index, *FBS* fasting blood sugar, *HbA1C* hemoglobin A1c, *eGFR* estimated glomerular filtration rate, *BUN* blood urea nitrogen, *Cr* creatinine

Table 2 Genotype and allele frequencies of SIRT1 rs3758391C/T variations between T2DM patients and controls

SIRT1 rs3758391C/T polymorphism	Controls	T2DM	OR (95% CI)	*p* value	Adjusted OR[a] (95% CI)	*p* value[a]
TT	6 (9.1%)	35 (26.5%)	5.7 (2.2–14.9)	< 0.001	6.4 (2.4–17.3)	< 0.001
CT	15 (22.7%)	51 (38.6%)	3.3 (1.6–6.7)	0.001	3.3 (1.5–6.9)	0.002
CC	45 (68.2%)	46 (34.8%)	1.0-ref		1.0-ref	
Dominant						
TT	6 (9.1%)	35 (26.5%)	3.6 (1.4–9.1)	0.004	4.5 (1.7–11.5)	0.002
CT+CC	60 (90.9%)	97 (73.5%)	1.0-ref		1.0-ref	
Recessive						
TT+CT	21 (31.8%)	86 (65.2%)	4.01 (2.1–7.5)	< 0.001	4.2 (2.2–8.2)	< 0.001
CC	45 (68.2%)	46 (34.8%)	1.0-ref		1.0-ref	
Alleles						
T	27 (20.45%)	121 (45.8%)				
C	105 (79.55%)	143 (54.2%)				

Data in *n* (%). [a] Adjusted for age, sex, BMI

reports. Sirtuins (silent information regulators of transcription) like the SIRT1 are a family of deacetylase enzymes involved in several signaling pathways [21]. Changes in SIRT1 expression have been reported in different physiological and pathological conditions such as diabetes, cardiovascular diseases, and CKDs. SIRT1 as a regulatory molecule in insulin signaling, inflammation, mitochondrial function, and circadian rhythms may provide anti-diabetic effects through insulin secretion and improvement of insulin resistance [22]. Our results did not show a significant influence of the SIRT1 variants on BMI. It has been shown that, under excessive energy intake, decreased SIRT1 activity may contribute to the development of obesity-related problems, including insulin resistance and T2DM [22]. In a Chinese cohort, Zheng et al. reported that rs10509291AA and rs10823116GG genotypes were associated with increased BMI but the rs7894483TT genotype showed a negative association with lower BMI values [23]. Based on a cross-sectional study on 4023 subjects, it was revealed that self-reported calorie restriction and diet modifications abrogated the genetic cooperation of rs7895833A and rs1467568G alleles of the SIRT1 gene for changes in BMI and long-term weight [11]. This might be true for our study as well.

In this study, we also aimed to investigate an association of the rs3758391 polymorphism of the SIRT1 gene with the risk of diabetic nephropathy. We found a significant association of

Table 3 Genotype and allele frequencies of SIRT1 rs3758391C/T variations between T2DM patients with and without nephropathy

SIRT1 rs3758391C/T polymorphism	T2DM without nephropathy	T2DM with nephropathy	OR (95% CI)	*p* value	Adjusted OR[a] (95% CI)	*p* value[a]
TT	12 (18.2%)	23 (34.8%)	3.96 (1.6–10)	0.003	5.99 (1.9–18.3)	0.002
CT	23 (34.8%)	28 (42.4%)	2.5 (1.1–5.7)	0.027	2.6 (1–6.3)	0.04
CC	31 (47%)	15 (22.7%)	1.0 (ref)		1.0 (ref)	
Dominant						
TT	12 (18.2%)	23 (34.8%)	2.41 (1.1–5.4)	0.03	3.2 (1.2–8.3)	0.02
CT+CC	54 (81.8%)	43 (65.2%)	1.0 (ref)		1.0 (ref)	
Recessive						
TT+CT	35 (53%)	51 (77.3%)	3.01 (1.4–6.4)	0.003	3.3 (1.5–7.5)	0.004
CC	31 (47%)	15 (22.7%)	1.0 (ref)		1.0 (ref)	
Alleles						
T	47 (35.6%)	74 (56%)				
C	85 (64.4%)	58 (44%)				

Data in *n* (%). [a] Adjusted for age, sex, BMI, HbA1c

Table 4 BMI and biochemical parameters measured in all subjects participating in the study

	Control subject			Diabetic patients					
				Without nephropathy			With nephropathy		
	CC	TT	*p* value	CC	TT	*p* value	CC	TT	*p* value
BMI (kg/m^2)	27 ± 4.5	27 ± 4.4	0.838	29.7 ± 5.5	28.7 ± 5.4	0.559	27.7 ± 3	26.5 ± 4	0.394
FBS (mg/dL)	86 ± 14	94 ± 6	0.024	207 ± 56	255 ± 75	0.04	205 ± 64	256 ± 71	0.021
HBA1c % (mmol/mol)	4.8 ± 0.5 (29 ± 5.5)	5.1 ± 0.2 (32 ± 2.2)	0.202	7.6 ± 1 (60 ± 10.9)	9.8 ± 1.5 (84 ± 16.4)	0.001	7.7 ± 0.9 (61 ± 9.8)	8.1 ± 1.04 (65 ± 11.4)	0.167
eGFR (mL/min/1.73m^2)	80 ± 18	77 ± 9	0.713	77 ± 16	85 ± 22	0.318	41 ± 24	23 ± 20	0.005
BUN (mg/dL)	13.3 ± 2.2	13.3 ± 2.8	0.929	13.2 ± 2.7	12.4 ± 2.4	0.476	34 ± 9	45 ± 17	0.124
Cr (mg/dL)	0.85 ± 0.13	0.83 ± 0.2	0.964	0.87 ± 0.17	0.82 ± 0.13	0.437	2.1 ± 1	3.4 ± 1.6	0.039
Cystatin C (mg/L)	1.1 ± 0.3	1.2 ± 0.15	0.275	1.1 ± 0.4	1.1 ± 0.4	0.774	2.1 ± 1.4	3.6 ± 1.8	0.011
Albuminuria (mg/24 h)	6.2 ± 1.9	6.7 ± 2.7	0.515	9.5 ± 3.2	9.4 ± 4.5	0.764	346 ± 105	509 ± 175	0.002

Data are mean ± SD

this SNP with diabetic nephropathy and decreased eGFR. Elevated BUN, Cr, cystatin C levels, and albuminuria, and decreased eGFR (as markers of kidney damage) were observed in people with the TT genotype as compared with other variants. This might show the higher risk of micro-vascular complications of T2DM for homozygous risk allele carriers. Changes in SIRT1 expression and activity might account for pathological conditions like diabetes and CKD. Decreased expression and activity of SIRT1 has been reported in patients with renal disease [21]. SIRT1 exerts protective anti-apoptotic, anti-oxidative, and anti-inflammatory effects via its regulation of mitochondrial biogenesis and autophagy [15]. SIRT1 enhances nitric oxide (NO) production in endothelial cells by deacetylating and activating NO synthase (eNOS). NO is a protective factor in vascular tissues including the kidneys. Impaired NO production, due to endothelial cell dysfunction, plays an important role in the pathophysiology of renal injuries and diabetic nephropathy [24]. In diabetic nephropathy, hyperglycemia and decreased SIRT1 expression lead to the apoptosis of podocytes in the kidneys [25]. Different polymorphisms in the SIRT1 gene could increase the risk of diabetic nephropathy. In a study by Maeda et al. on Japanese subjects with type 2 diabetes, it was shown that, among 11 SNPs related to the SIRT1, four SNPs including rs2236319, rs10823108, rs3818292, and rs4746720 had significant correlations with diabetes nephropathy [13]. In addition to an increased risk of T2DM by 5.7 and an augmented risk of diabetic nephropathy by 3.96 in the TT homozygous genotype of the rs3758391 polymorphism, we found a strong association of the TT homozygous genotype with decreased eGFR (eGFR < 60 mL/min/1.73m^2).

Based on our results and the mentioned mechanism for SIRT1, it is suggested that changes in SIRT1 expression on kidney cells (in relation to the TT genotype of the rs3758391 polymorphism) may account for the increased risk of kidney damage in these patients.

In conclusion, our results confirm that the T risk allele of the rs3758391 polymorphism of SIRT1 gene is significantly associated with T2DM and the development of diabetic nephropathy among a population of southwest Iran. This is even after making adjustments for risk factors.

Table 5 Association of TT genotype with eGFR < 60 mL/min/1.73m^2

SIRT1 rs3758391C/T polymorphism	eGFR < 60 mL/min/1.73m^2 (61)	eGFR > 60 mL/min/1.73m^2 (59)	OR (95% CI)	*p* value
TT	20 (32.8)	12 (20.3)	2.9 (1.1–7.5)	0.024
CT	25 (41)	19 (32.2)	2.3 (0.98–5.4)	0.054
CC	16 (26.2)	28 (47.5)	1.0 (ref)	
Alleles				
T	65 (53.28)	43 (36.44)		
C	57 (46.72)	75 (63.56)		

Data in n (%)

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Compliance with ethical standards

Human and animal rights The study has been approved by the appropriate local ethics committee at the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1395.76) and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ahvaz Jundishapur University of Medical Sciences research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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

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ORIGINAL ARTICLE

Arterial stiffness and matrix metalloproteinases: A correlation study in hypertensive type 2 diabetic subjects

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Abstract

Introduction Matrix metalloproteinases (MMP's) and tribbles 3 human homolog (Trb3) are implicated in atherosclerosis. Changes in the concentration of these biomolecules signal the risk of atherosclerosis in type 2 subjects (T2DM), with or without hypertension (HT), at an early stage.

Aim Our aim was to assess the relation between noninvasive arterial stiffness indices and circulating levels of MMP2, MMP9 and Trb3.

Methodology The study included 144 participants divided into 4 groups: T2DM > 5 years + HT, DM + HT ($n = 55$), T2DM < 2 years, DM ($n = 28$), HT ($n = 31$), and healthy controls (HC) ($n = 30$). Anthropometric measurements and blood biochemistry profiles were established using standard protocols. MMP2, MMP9 and Trb3 were estimated using ELISA. Pulse wave velocities (PWV) and arterial stiffness indices (ASI) were measured using PeriScope^{TM}. Results were analysed using SPSS 21.

Results MMP2, average Brachial Stiffness Index (ba ASI) (Pearson's $r = 0.235$, $p = 0.005$) and Ankle-Brachial Index (ABI) (Pearson's $r = 0.225$, $p = 0.007$) were positively correlated. Average Ankle ASI (Ank ASI) was positively correlated to Trb3 (Pearson's $r = 0.184$, $p = 0.028$), but negatively to MMP9 (Pearson's $r = -0.184$, $p = 0.027$). In multiple linear regression, MMP2 influenced ba ASI [adjusted $R^2 = 0.038$; $F(3) = 2.862$, $p = 0.039$] and ABI [adjusted $R^2 = 0.033$; $F(3) = 2.642$, $p = 0.052$]. MMP9 influenced Ank ASI [adjusted $R^2 = 0.058$; $F(3) = 3.912$, $p = 0.01$].

Conclusion Arterial stiffness indices and matrix metalloproteinases conform early risk of atherosclerosis in diabetic subjects.

Keywords Arterial stiffness · Matrix-metalloproteinases · Tribbles 3 · Atherosclerosis · T2DM

Introduction

Arteries carry blood to all parts of the body through a distribution network. Arteries are flexible and compliant to the systole and diastole of the heart. This natural elasticity of the arteries results in the maintenance of narrow pulse pressure. Age and some disease conditions reduce this elasticity of the walls of the arteries. The arteries become harder and less compliant to the systole and diastole. Thus, there is a reduction in the cardiac stroke volume, which results in increased diastolic pressure and increased heart rate. Arterial stiffness has been associated with atherosclerosis and cardiovascular complications [1]. The ease of using oscillometry-based method in clinics has enhanced the prognosis of various complications involved with the arteries [2]. A large number of diseases, particularly diabetes (DM) and hypertension (HT), have been reported to be associated with increasing arterial stiffness [3, 4]. The onset and a steady increase of arterial stiffness and macro vascular complications in these chronic diseases

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eventually results in cardiovascular complications. Arterial stiffness is hence an important factor which increases the risk of cardiovascular complications and associated mortality in T2DM and HT subjects [5].

Pulse wave velocity (PWV), Arterial Stiffness Index (ASI) and Ankle Brachial Index (ABI) are well established as independent markers for the assessment of the risk of atherosclerosis [6, 7]. The use of PeriScope™ is internationally validated, non-invasive diagnostic method used to assess arterial stiffness indices mentioned above [2]. Arterial stiffness indices can be monitored to evaluate the status of diseases and the success rate of therapies given to diabetic and hypertensive patients [8, 9].

Atherosclerotic plaque formation is a process associated with changes in the structure of the intimal layer of the arteries. These changes can be attributed to a large number of factors such as vascular smooth muscle cell migrations, altered metabolism of macrophages and deposition of cholesterol [10, 11]. Along with these factors, a large number of biomolecules circulating in the blood have been reported to play an important role in plaque formation. Plaques formed in the arteries occlude the luminal volume of the arteries. This results in altered flow dynamics of the blood. It also results in alteration of blood pressure. Atherosclerosis has been considered as an important prelude to the progression of cardiovascular complications.

Matrix metalloproteinases (MMP's) are proteins that cause alteration of the extra cellular matrix and also affect the intimal layer of arteries [12, 13]. Concentration of these molecules has been reported to be used as biomarkers in the serum of patients with cardiovascular complications [14]. Patients with HT and T2DM have increased concentrations of MMP2 and MMP9 in their serum [15, 16]. Matrix metalloproteinases have also been associated with arterial stiffness indices [17, 18], although there are conflicting reports about such changes in the levels of MMP2 and MMP9 and their association with arterial stiffness [19, 20].

Tribbles 3 human homolog (Trb3) is a pseudo-kinase associated with insulin-dependent glucose metabolism. It has been reported to inhibit the intracellular signal transducing AKT/PI3 pathway, which is activated by insulin [21]. Inhibition of this pathway induces insulin resistance in diabetic subjects [22]. It has also been correlated with increase in the oxidative stress found in metabolic conditions, like T2DM [23, 24]. Although there is an increasing evidence to support the role of Trb3 in manifestation of diabetic complications, there are not many reports which associate Trb3 with arterial stiffness indices.

Hence, the aim of our study was to identify association of markers of arterial stiffness and the biomolecules MMP2, MMP9 and Trb3 in diabetic subjects.

Materials and methods

Study design

Selection and description of participants One hundred forty-four subjects, age > 40 years, were selected for our study. The volunteers were divided into four groups: DM + HT = diabetic > 5 years with hypertension ($N = 55$); DM = diabetic < 2 years, without hypertension ($N = 28$); HT = hypertensive ($N = 31$); and HC = healthy controls ($N = 30$). The norms for designation of diabetes were followed according to American Diabetes Association (ADA) guidelines (FPG 100–125 mg/dL and HbA1c = 5.7–6.4%) [25]. Joint National Committee (JNC) 7 guidelines were followed for hypertension (systolic pressure of 120–139 mmHg are pre-hypertensive and systolic pressure > 140 mmHg are hypertensive) [26]. Healthy controls were recruited as individuals, male or female, with values of FPG, HbA1c and blood pressure within the normal ranges mentioned above.

Exclusion criteria

Subjects below 40 years, and with major systemic illness, chronic inflammatory diseases and autoimmune diseases, were excluded from the study. Subjects with diabetes history between 2 and 5 years were excluded from the study to understand the differences between early and late stages of the disease.

Methodology

Procurement of serum samples Venous blood was obtained using standard venepuncture procedures, and serum thus obtained was divided into set of five aliquots and stored at − 80 °C until further assay.

Physical parameters like body mass index (BMI), waist/hip ratio (WHR) and blood pressures (systolic and diastolic) were measured using standard protocols. Biochemical parameters of the volunteers were estimated using standard ready-to-use kit protocols (blood sugar by glucose oxidase method; Spin React, Spain; Triglycerides by GPO-POD method, Innoline- Merck India; Total cholesterol by CHOD-POD method, HDL cholesterol by enzyme selective protection method, Agappe Diagnostics, India, and Glycated Hemoglobin (HbA1c) by cation exchange resin method, Erba Diagnostics-Germany) and measured in Konelab 20 i, auto analyzer (Thermo Electron Corporation, Waltham US).

ELISA studies Ready-to-use ELISA kits were purchased and used for the estimation of MMP2, MMP9 (R&D systems Minneapolis, USA) and Trb3 (EiaAB, Wuhan, China). All

reagents and serum samples were brought to room temperature (freeze thaw cycles were avoided) and diluted as per kit instructions. Standards were prepared and ELISA assays were performed according to the instructions provided by the manufacturers. Serum samples were diluted (if required), to obtain absorbance values in agreement with the standard graph. Intra- and inter-assay controls were maintained in all ELISA experiments. Intra-assay CV (%) for MMP2, MMP9 and Trb3 were 2.3, 2.6 and 2.4, respectively, and inter-assay CV (%) for MMP2, MMP9 and Trb3 were 7.2, 7.5 and 6.8 respectively.

PeriScope™ data (Genesis Medical Systems Pvt. Ltd., India) was used to measure pulse wave velocities (PWV), Carotid-Femoral PWV (C-F PWV), Arterial Stiffness Index (ASI) and Ankle-Brachial Index (ABI). The participants were made comfortable in the supine position. A four limb BP cuffs and a four lead ECG cable were attached to the PeriScope™ hardware. After entering personal data of the subjects, the fully automated run for PeriScope™ was initiated. All the parameters like PWV, ASI and ABI were calculated automatically using the built-in DSP algorithm. The representative output of the data obtained by the PeriScope™ is shown in Fig. 1 [27].

Statistics Standard graphs for MMP2, MMP9 and Trb3 were plotted using Curve Expert 1.4 software, and concentrations of the unknown serum were determined using fourth degree polynomial regression and taking the dilutions used into account. The results were analysed using IBM SPSS software version 21. Mean ± SD or median and inter quartile range were reported for all parameters in the study groups after confirming normality of the data using Kolmogorov-Smirnov test. Distribution of values across the groups was confirmed using ANOVA (using post hoc Bonferroni test) or Kruskal-Wallis test. Inter-group comparisons were performed using the independent t test or Mann-Whitney U test 95% confidence interval limits. Correlations between the parameters in question and the groups in study were established using the Pearson's correlation coefficient or the Kendall rank correlation coefficient. Multiple linear regression was used to identify the parameters which possess greater contributing value to the dependent variables. All significant differences were considered for $p < 0.05$ at 5% level of significance.

Results

All differences mentioned were considered for $p < 0.05$ level of significance

As reported in Table 1, we found no differences in the BMI, WHR and HDL cholesterol levels across the different groups in our study. Increased systolic blood pressure was seen in the DM + HT, DM and HT groups when compared to healthy controls. There was a marked decrease in the diastolic blood pressure in the DM group when compared to the DM + HT and HT groups.

Fasting, post-prandial blood sugar and HbA1c levels were elevated in the DM + HT and DM groups.

Triglycerides, total cholesterol and HDL cholesterol levels were comparable in all groups, without significant deviations, but DM group had elevated levels of LDL cholesterol when compared to the HT group.

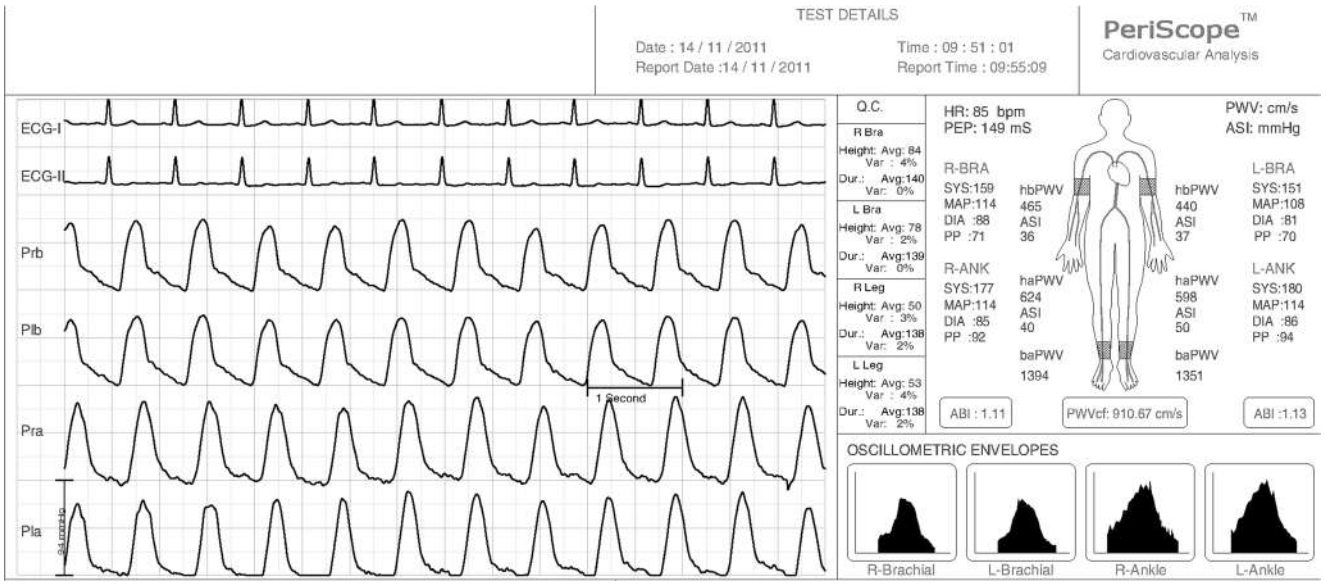

Fig. 1 Representation of various parameters using Periscope™. The above figure depicts various indices used to measure arterial stiffness. The data so obtained can be used directly for analysis

Table 1 Results of anthropometry and serum biochemistry of subjects in the study

Parameters	Group A1 DM + HT $N = 55$	Group A2 DM $N = 28$	Group B1 HT $N = 31$	Group B2 HC $N = 30$	p value 5% significance
Age (years)	59.3 ± 9.6	52.12 ± 10.49	55.16 ± 10.74	51.47 ± 9.33	0.000[a, d]
Male/female	30:25	13:15	16:15	16:14	NS
BMI (kg/m^2)	26.74 ± 4.3	24.99 ± 4.15	26.5 ± 4.83	26.05 ± 6.02	NS
Waist/hip	0.90 ± 0.07	0.90 ± 0.06	0.87 ± 0.06	0.87 ± 0.05	NS
Systolic pressure (mmHg)	145.56 ± 18.58	134.93 ± 10.35	138.77 ± 18.1	129.0 ± 13.3	0.000[a] 0.047[b] 0.031[c] 0.009 [d]
Diastolic pressure (mmHg)	83.45 ± 7.62	78.57 ± 5.2	82.71 ± 8.02	80.0 ± 6.6	0.004[d] 0.047[f]
Fasting blood sugar (FBS) (mg/dL)	149.4 ± 50.55	142.21 ± 56.69	102.90 ± 29.3	99.7 ± 20.5	0.000[a, b,e,f]
Post-prandial blood sugar (PPB) (mg/dL)	217.57 ± 74.37	178.89 ± 79.24	120.58 ± 44.78	107.27 ± 24.5	0.000[a,b,e] 0.019[d] 0.002[f]
$HbA1_c$ (mmol/L)	7.94 ± 1.78	6.21 ± 1.43	5.06 ± 0.72	5.05 ± 0.55	0.000[a,b,d,e,f]
Triglycerides (mg/dL)	143.09 ± 142.32	136.32 ± 66.26	107.42 ± 41.75	106.4 ± 47.4	NS
Total cholesterol (mg/dL)	171.2 ± 59.11	195.89 ± 53.86	160.16 ± 45.29	180.7 ± 43.5	NS
HDL cholesterol (mg/dL)	47.87 ± 13.36	52.48 ± 17.75	50.48 ± 23.08	48.5 ± 12.61	NS
LDL cholesterol (mg/dL)	95.38 ± 49.54	116.19 ± 42.28	87.78 ± 38.14	116.8 ± 25.6	0.044[c]

Values above are expressed as mean ± SD. Significance at 0.05 level between groups given as superscripts: a = A1 and B2, b = A2 and B2, c = B1 and B2, d = A1 and A2, e = A1 and B1, f = A2 and B1

NS not significant

Serum concentrations of MMP2, MMP9, Trb3 and arterial stiffness parameters are reported in Table 2.

MMP2 levels are elevated in DM + HT when compared to the DM and HT groups ($p = 0.005$ and 0.012, respectively). The MMP2 levels in the DM + HT group were higher than the HC group, though there was no significant difference between them. The concentration of MMP2 in the HT group was, however, lower than the HC group ($p = 0.000$).

MMP9 concentrations in both the diabetic groups were more than the HT and HC groups. The DM group had higher concentration of MMP9 when compared to the DM + HT, HT and HC groups ($p = 0.011$, 0.000 and 0.001, respectively). The DM + HT group also had increased levels of MMP9 when compared to the HT group ($p = 0.012$).

Tribbles 3 concentrations in the DM + HT, DM and HT groups were higher than the HC group, although there was no statistical difference in the concentrations of Trb3 across our study groups ($p = 0.163$).

PeriScope™ parameters for arterial stiffness were found to be influenced by the state of diabetes and hypertension. C-F PWV was increased in the DM + HT group when compared to the HC group ($p = 0.006$).

The average Brachial pulse wave velocity (ba PWV) in the DM + HT group was more than the HC and the HT groups ($p =$ 0.006 and 0.033, respectively). The average Brachial ASI (ba ASI) was higher in the DM + HT and the HC groups as compared to the HC groups ($p = 0.002$ and 0.021, respectively).

The values of average Ankle ASI were increased in the DM + HT and the HT groups when compared to the HC group ($p = 0.000$ and 0.036, respectively). The DM + HT and HT groups also had higher values of average Ankle ASI when compared to the DM group ($p = 0.000$ and 0.011, respectively).

Average ABI showed no significant difference across and within the groups.

Augmentation Index (AIx) value was increased in the DM + HT and the HT groups when compared with HC group ($p = 0.001$ and 0.036, respectively). The DM + HT group also had AIx value greater than the DM group ($p = 0.016$).

Results of correlation and multiple regression analyses conducted for these observations are listed in Table 3.

MMP2 had a positive correlation with average ba ASI and average ABI, indicating that with increase in these two parameters, the MMP2 levels in the serum increased. Average Ankle ASI exhibited a positive correlation with Trb3, but a negative correlation with MMP9.

Multiple regression models using MMP2, MMP9 and Trb3 for various arterial stiffness indicators resulted as follows:

Table 2 Results of serum biomolecules and arterial stiffness parameters in our study subjects

Parameter	Group A1 DM + HT $N = 55$	Group A2 DM $N = 28$	Group B1 HT $N = 31$	Group B2 HC $N = 30$	p value at 5% level of significance
MMP2 (ng/mL)	275.50 ± 46.25	243.17 ± 54.84	224.07 ± 44.71	263.06 ± 24.95	0.005[c] 0.012[d] 0.000[e]
MMP9 (ng/mL)	426.0 (270.0–519.0)	562.0 (382.25–661.75)	295.0 (184.0–415.0)	364.0 (242.75–456.50)	0.001[b] 0.011[d] 0.012[e] 0.000[f]
TRB 3 (ng/mL)	1.4720 (0.84–2.74)	0.9920 (0.35–2.11)	1.3820 (0.78–2.12)	1.2955 (1.09–2.38)	(0.163) (NS)
CF-PWV (Cm/s)	1105.5 (910.7–1345.3)	953.05 (816.68–1209.28)	1019.0 (839.0–1202.1)	857.15 (742.03–1192.6)	0.006[a]
Average baPWV (Cm/s)	1588.45 (1346.7–1860.4)	1336.70 (1156.1–1643.6)	1491.75 (1273.6–1678.3)	1299.45 (1150.5–1639.5)	0.006[a] 0.033[d]
Average Brachial ASI (mmHg)	31.2 (24.8–40.0)	28.1 (23.4–34.6)	29.5 (24.0–39.2)	26.4 (22.28–29.23)	0.002[a] 0.021[c]
Average Ankle ASI (mmHg)	46.2 (35.9–54.2)	31.55 (27.85–41.33)	40.2 (34.6–46.0)	35.65 (31.35–41.88)	0.000[a, d] 0.036[c] 0.011[f]
Average ABI	1.12 (1.04–1.16)	1.11 (1.03–1.17)	1.11 (1.06–1.14)	1.09 (1.05–1.17)	(0.979) NS
AIx	28.0 (22.0–34.0)	22.0 (15.0–29.0)	25.0 (19.0–30.0)	17.5 (10.75–29.0)	0.001[a] 0.036[c] 0.016[d]

Values above are expressed as mean ± SD or median and inter quartile range. Significance at 0.05 level between groups given as superscripts: a = A1 and B2, b = A2 and B2, c = B1 and B2, d = A1 and A2, e = A1 and B1, f = A2 and B1

NS not significant

- Average ba ASI: Adjusted $R^2 = 0.038$, F (3, 140) = 2.86, $p = 0.039$. For this model, MMP2 had significant positive regression weight, indicating that increase in serum MMP2 could increase the average ba ASI values.
- Average Ankle ASI: Adjusted $R^2 = 0.058$, F (3, 140) = 3.91, $p = 0.01$. For this model, MMP9 had significant negative regression weight, indicating that decrease in serum MMP9 could increase average Ankle ASI.

Table 3 Correlations and regression analysis for arterial stiffness indices in our study group

Dependent variable	Independent variable	Pearson's r correlation coefficient with dependent variable	Multiple regression		p value at 5% level of significance
			B	β	
Average ba ASI	MMP2	0.235**	0.209	0.229	0.007
	MMP9	0.048	0.009	0.044	0.597
	Trib3	0.066	1.221	0.031	0.711
Average Ank ASI	MMP2	0.139	0.029	0.119	0.151
	MMP9	− 0.184*	− 0.009	− 0.178	0.031
	Trib3	0.184*	1.598	0.153	0.065
Average AB Index	MMP2	0.225**	0.004	0.221	0.009
	MMP9	0.055	0.000	0.051	0.540
	Trib3	0.052	0.016	0.019	0.824

Multivariate regression model with MMP2, MMP9 and Trb3 as independent variables for ba ASI, Ank ASI and ABI as dependent factors. MMP2 is an independent factor that influences average Brachial ASI and average ABI positively. MMP9 and Trb3 do not influence these parameters. Average Ankle ASI on the other hand is negatively influenced by MMP9. Significance is considered at 5% level

*Pearson's correlation significance at 0.05 level of significance

**Pearson's correlation significance at 0.01 level of significance

- Average ABI: Adjusted $R^2 = 0.033$, $F(3, 140) = 2.62$, $p = 0.052$. For this model, MMP2 had a significant positive regression weight, indicating that increase in serum MMP2 could increase average ABI values.

Figure 2 shows correlations between the PeriScope™ parameters and MMP2, MMP9 and Trb3, represented as scatter plots.

A positive correlation of MMP2 with average Brachial ASI and average ABI (Pearson's $r = 0.235$, $p = 0.005$ and Pearson's $r = 0.225$, $p = 0.007$, respectively).

Figure 3 shows a negative correlation of Average Ankle ASI with MMP9 ($r = -0.184$, $p = 0.027$) and a positive correlation of Average Ankle ASI with Trb3 ($r = 0.184$, $p = 0.028$).

Discussions

Many studies have reported ba PWV and CF-PWV (gold standards for measuring arterial stiffness) as important independent factors for the assessment of the risk of atherosclerosis. In our study, we found that there was an increase in these risk factors in our DM + HT and DM subjects. Similar results were reported by Loehr et al. [28], but for diabetic patients with duration > 10 years. Identifying an increase in these risk factors at an early stage (as early as 5 years) can prove to be beneficial for positive intervention and reducing the risk of atherosclerosis.

Average ba ASI, average Ank ASI and AIx values were also elevated in the DM + HT and HT subjects as well, highlighting the risk of peripheral arterial disease.

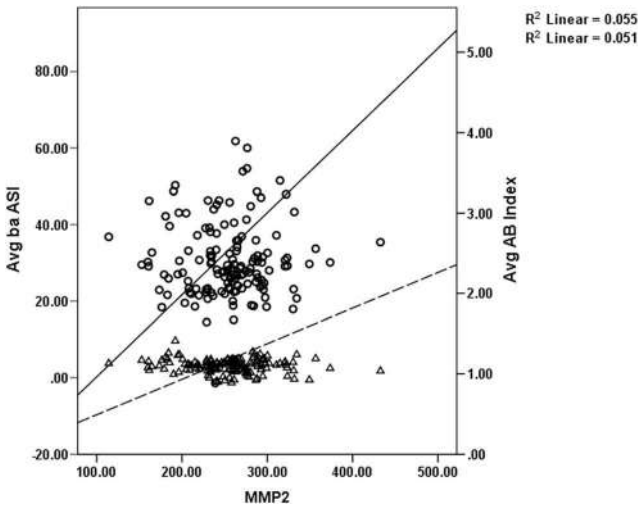

Fig. 2 Correlation between MMP2 and average ba ASI and average ABI for our data. The above figure depicts the correlation that is seen between MMP2 and arterial indices ba ASI and ABI. MMP2 is positively correlated with these two parameters. The influence of MMP2 over these indices is about 5%

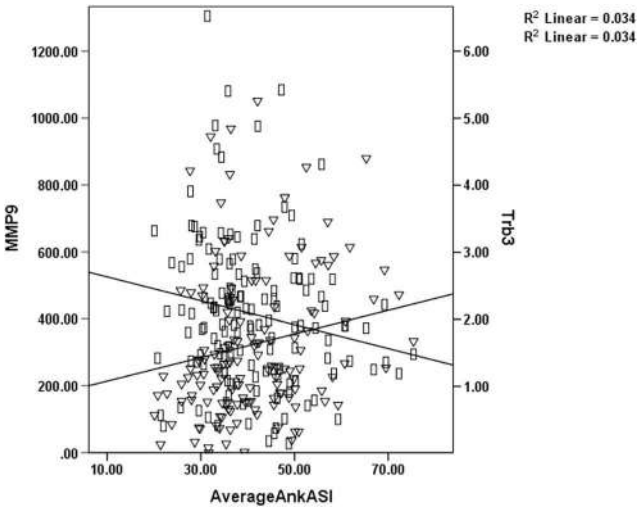

Fig. 3 Correlation between Average Ankle ASI, MMP9 and Trb3 for our data. The above figure depicts correlation seen between Ank ASI and serum biomolecules MMP9 and Trb3. MMP9 exhibits a negative correlation with Ank ASI, whereas Trb3 exhibits a positive correlation. Both these biomolecules explain up to 3% variability in Ank ASI

Statistical significance for these factors was seen particularly in the DM + HT subjects. Our results thus indicate that the risk of atherosclerosis in subjects with T2DM can be monitored from as early as 5 years from the onset of the disease using the above-mentioned indices. Zhang et al. [29], however, reported contrary to our findings that they did not find the association of AIx with diabetes, although they found carotid artery pulse wave velocity (CA PWV) strongly associated with diabetic subjects.

We found that circulating levels of MMP2 and MMP9 were increased in DM + HT and DM subjects, but there was no significant difference in the levels of Trb3.

MMP2 levels were significantly different between the DM + HT and the DM groups. Although there was an increase in the levels of MMP2 in DM + HT subjects, statistical significance was seen only between DM + HT and the DM and HT subjects. The DM group had lower values of MMP2, as similarly reported by Lewandowski et al. [30].

MMP9 levels were, however, increased in DM + HT as well as DM subjects. Greater differences were seen in the DM group (an observation contrasting to MMP2 of our study). The study of Lewandowski et al. [30], however, was contradictory to our findings.

MMP2 exhibited a linear correlation with average brachial ASI and average ABI, whereas MMP9 was negatively correlated with average Ankle ASI.

The role of Trb3 was, however, not clearly elucidated in our study, although we found that levels of Trb3 were increased in DM + HT group. We also found that Trb3 was positively correlated with average Ank ASI. Our study is

probably the first to report the association of Trb3 and arterial stiffness, although a similar study is reported by Ti et al. [31] by inducing diabetes in SD rats.

Strengths and limitations

In our study, we have attempted to correlate arterial stiffness indices with MMP's and a novel pseudokinase Trb3. Such studies will assist in elucidating the association between non-invasive indicators and circulating biomolecules at molecular levels. We could identify indicative changes in the levels of serum biomolecules and arterial stiffness indices for the assessment of risk of atherosclerosis in diabetic subjects with an onset period of as early as 2 years (DM group). Amidst conflicting data about the status of MMP (2 and 9) and their relation to arterial stiffness [17, 19], our study conforms that MMP (2 and 9) plays an important role in inducing arterial stiffness and ensuing atherosclerosis. Due to the linear association of MMP's with arterial stiffness, we also support the hypothesis that MMP (2 and 9) can be used as a therapeutic target for reducing the complications associated with arterial stiffness [8, 9, 15]. Our limitations are specially related to the sample size of our study (which may impair the resolution and interpretation of our results) the lack of data on tissue inhibitors of metalloproteinases (TIMP) within our study and the cross-sectional nature of our study.

Conclusions

Arterial stiffness induced by T2DM can be identified using oscillometric methods like PeriScope ™, as reported by Kulkarni et al. [32]. Arterial stiffness corroborates the changes in the serum concentrations of MMP2, MMP9 and Trb3, which are reported to play an important role in atherosclerosis. If these molecules are monitored in diabetic subjects, stiffness of the arteries can be reduced by targeting these molecules appropriately.

MMP's are implicated in a large number of diseases [33, 34], and thus correlation of MMP (2 and 9) specifically to atherosclerosis and cardiovascular diseases may be an illegitimate extension of the observation. There are many studies which corroborate that MMP2 and MMP9 levels in the serum rise in subjects who actually have undergone cardiovascular events [35, 36]. Studies also report that reduction in the myocardial activity of MMP2 and MMP9 results in accumulation of collagen, which induces LV hypertrophy [37–39]. This can be attributed to the changing levels of tissue inhibitors of metalloproteinases (TIMPs) [40].

As atherosclerosis is manifested by a variety of risk factors, identification of risk factors at an early stage can be beneficial in resorting to proper methods of management, particularly in diabetes. MMP2, MMP9 and Trb3 could be considered as an additional dimension for risk evaluation and risk management of cardiovascular events in diabetic subjects at an early stage, especially in a larger population. Therapies to control arterial stiffness and its associated factors (like Trb3, MMP2 and MMP9) would not only reverse the histological status of stiff arteries to normalcy but also reduce the risk of cardiovascular events in diabetic subjects.

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Authors' contributions PRASHANT SHIRISH RATNAPARKHI: Data acquisition for serum biomolecules, data analysis and drafting of manuscript.

NAMRATA BINDURAO KULKARNI: Data acquisition for anthropometry and biochemistry, and revision of the draft.

MEGHANA ULHAS GANU: Data acquisition for Periscope™ and its interpretation.

SANJAY GANESH GODBOLE: Designing the concept of the project, recruitment of subjects and defining intellectual content.

SUDHA SHRIKANT DEO: Designing the concept of the project, defining intellectual content, critically revising the draft and final approval for publication.

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Compliance with ethical standards

Ethical approval The study was approved by the Scientific Advisory Committee of the Sir H.N. Medical Research Society, Sir H.N. Hospital and Research Centre, Mumbai, India. All procedures followed the guidelines set by the Indian Council of Medical Research (ICMR) for conducting research on human volunteers, in accordance with the Helsinki declaration, 2008.

Informed consent Informed consent was obtained from these subjects, and their details were entered in the clinical proforma.

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ORIGINAL ARTICLE

Duration of type 2 diabetes mellitus and pulmonary function tests: a correlative study

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Abstract

Background Diabetes mellitus (DM) encompasses a group of metabolic disorders characterized by elevated levels of plasma glucose leading to macro- and micro-vascular damages to target organs. According to IDF, India had 73 million population of diabetes in 2017, and this figure may rise to 134 million adults by 2045 International Diabetes Federation, 2017. Since the alveolar capillary network in the lung is a large micro-vascular unit, microangiopathy may be targeting lungs along with other organs.

Objective To correlate pulmonary function tests with duration of type 2 diabetes mellitus.

Method The cross-sectional study was conducted on one hundred six patients of type 2 diabetes mellitus who met the inclusion and exclusion criteria. Diabetic cases were categorized on the basis of duration of disease. RMS Helios 401 computerized spirometer was used to assess pulmonary functions. Statistical tests employed include *t* test, ANOVA, and correlation coefficient analysis.

Result The mean duration of disease for patients with diabetes was 10.17 ± 5.16 year. There was a significant negative correlation between the parameters of lung function test (p value < 0.05) with duration of diabetes.

Conclusion We found that pulmonary functions have inverse relationship with increase in duration of type 2 diabetes mellitus.

Keywords Diabetes mellitus · Pulmonary function test · Forced vital capacity · Forced expiratory volume in first second

Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders characterized by elevated levels of blood glucose resulting from the ineffective utilization of insulin. These disorders include alterations in the bio-structures at nuclear, cellular, tissue, and organ level [1]. The etiology of type 2 DM is determined by interplay of genetic, environmental, and metabolic factors [2]. Globally it was estimated that 425 million people or 8.8% adults were living with diabetes in 2017[3]. There is evidence of alarming increase in the incidence and prevalence of DM in Asian Indians [4]. According to IDF, India had 73 million population of diabetes in 2017, and this figure may rise to 134 million adults by 2045 [3].

The injurious effects of hyperglycemia are broadly categorized into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). The alveolar capillary network in the lung is a large micro-vascular unit; hence, lungs also become targets for microangiopathy [5]. However, because of the huge pulmonary reserve in a healthy individual substantial loss of the microvascular bed can be tolerated without developing dyspnea or any other clinical symptom [6, 7]. As a result, pulmonary diabetic micro-angiopathy usually remains under-recognized clinically. Moreover assessment of pulmonary functions may also be a useful indicator of the progress of diabetic microangiopathy.

Several hypotheses that have been proposed as etiology of altered lung function in DM [8, 9] as non-enzymatic

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Table 1 Demographic profile of the subjects

Characteristics	Group a (23) (≤ 5 years)	Group b (30) (5–10 years)	Group c (28) (10–15 years)	Group d (20) (> 15 years)
Mean age (years)	41.65 ± 4.53	48.76 ± 6.27	55.96 ± 4.35	60.75 ± 3.61
Gender	Female—9	Female—17	Female—16	Female—7
	Male—14	Male—13	Male—12	Male—13
Mean duration of diabetes (years)	3.67 ± 1.10	7.81 ± 1.36	12.59 ± 1.18	17.9 ± 1.40
Mean BMI (kg/m^2)	25.88 ± 3.34	25.78 ± 3.74	26.27 ± 2.31	27.36 ± 2.66
Mean FBS (mg/dl)	124.39 ± 34.83	180 ± 90.67	180.44 ± 51.79	200.1 ± 55.33
Mean PPBS (mg/dl)	171.26± 26.63	244.4 ± 98.93	261.81 ± 67.35	276.3 ± 64.99
Mean HbA1c (%)	6.3 ± 0.80	7.39 ± 2.04	8.37 ± 1.60	8.55 ± 1.50

The groups were divided on the basis of duration of diabetes

glycosylation of connective tissue, especially collagen, diabetic myopathy, microvascular angiopathy, and increased levels of systemic inflammatory mediators and inflammatory markers.

Although a lot of research work is being carried out worldwide on the effects of DM on pulmonary parameters, the literature pertaining to this in Indian scenario is limited. Therefore, this study was undertaken to find out the influence of duration of type 2 DM on pulmonary function tests.

Material and method

The cross-sectional, prospective study was conducted in the Department of Physiology & Medicine of SMS&R, Sharda Hospital, Greater Noida, over a period of 1 year on patients of type 2 DM. One hundred one patients of type 2 DM of either sex in the age group of 35–65 years and having duration of diabetes > 1 year were randomly selected from the out-patient department (OPD) of Sharda Hospital. Subjects with a history of smoking, acute or chronic respiratory disease, occupational exposure to respiratory deterrents, neuromuscular or cardiovascular diseases, or any physical disability that may affect lung function like kyphoscoliosis and who were unable to perform pulmonary function test were excluded from the study.

After providing the relevant information of the study, the subjects were asked to fill the questionnaire that contained relevant personal, socio-demographic, and medical history. Their fasting plasma glucose, post-prandial glucose, and

Table 2 PFT parameters of diabetic subjects with different duration of diabetes

S. No.	PFT parameters	Duration of diabetes				*p* value
		< 5 years (n = 23) Group a	5–10 years (n = 30) Group b	10–15 years (n = 28) Group c	> 15 years (n = 20) Group d	ANOVA
1	FVC	2.89 ± 1.14	2.09 ± 0.69	1.4 ± 0.42	1.17 ± 0.414	0.0001
	% predicted	83.22	67.67	52.70	46.40	
2	FEV_1	2.3 ± 0.97	1.55 ± 0.56	1.02 ± 0.28	0.764 ± 0.28	0.0001
	% predicted	79.65	67.76	50.75	52.55	
3	FEV1/FVC	78.18 ± 10.55	79.07 ± 12.46	78.76 ± 15.9	80.68 ± 17.3	0.0001
	% predicted	96.96	97.60	92.81	100.85	
4	FEF (25-75%)	2.29 ± 1.2	1.63 ± 0.85	1.15 ± 0.71	1.60 ± 0.99	0.001
	% predicted	63.47	66.83	62.37	103.98	
5	SVC	3.05 ± 0.95	2.3 ± 0.65	1.99 ± 0.45	1.95 ± 0.45	0.0001
	% predicted	87.95	73.73	65.81	55.05	
6	MVV	107.61 ± 33.86	94.13 ± 28.30	69.78 ± 28.49	86.35 ± 29.16	0.0001
	% predicted	74.09	71.77	57.78	63.25	

Significance of variance of PFT parameters in different groups has been calculated by using the ANOVA test. The groups were adjusted for age, sex, and BMI

$p \leq 0.001$ highly significant

Subjects with a history of smoking, acute or chronic respiratory disease, occupational exposure to respiratory deterrents, neuromuscular or cardiovascular diseases or any physical disability were excluded

glycosylated hemoglobin levels were analyzed in biochemistry laboratory of Sharda Hospital by using an automated analyzer.

All the subjects underwent general examination, pertinent systemic examination, and anthropometric measurements. BMI was calculated using the formula: weight in kg/height in m^2.

Cases of type 2 DM were was further divided into four subgroups on the basis of duration of disease as group a (≤ 5 years), group b (5–10 years), group c (10–15 years), and group d (> 15 years).

Pulmonary function test of the subjects was performed by using RMS Helios 401 computerized spirometer. All the tests were conducted according to American Thoracic Society/European Respiratory Society (ATS/ERS guidelines) in a quiet room in sitting position between 10 a.m and 2 p.m at room temperature [10]. The cases performed the maneuver three times at the interval of 15 min, and the best of the three was used for the analysis. Parameters assessed were—forced vital capacity (FVC) in liters, forced expiratory volume in first second (FEV_1), FEV_1/FVC in percentage (%), forced expiratory flow during 25–75% of FVC ($FEF_{25–75}$), slow vital capacity (SVC), and the maximum ventilatory volume (MVV). For all these parameters, the percentage of predicted values for the respective age, height, and weight was taken into consideration.

Statistical analysis was done by using *t* test and one-way analysis of variance (ANOVA) test. Correlations were analyzed using Pearson's correlation coefficient (*r*). The *p* value < 0.05 was considered as statistically significant.

Result

The total number of one hundred one subjects has participated in this study. They were divided into four subcategories as detailed in material and method. Table 1 depicts the demographic characteristics of all the participants.

The mean duration of disease for patients with diabetes was 10.17 ± 5.16 years, ranging from 1.5 to 21 years. The association between pulmonary function parameters and duration of diabetes has been shown in Table 2, which revealed a significant (*p* value < 0.05) value. Pearson correlation analysis of different parameters of PFT with duration of disease revealed negative correlation for all the parameters studied (Table 3).

Figures 1–6 show moderate to weak negative correlation between different parameters of PFT and duration of disease in diabetic patients.

Table 3 Correlation of PFT with duration of diabetes

Parameters	Correlation coefficient (*r*)	*p* value
FVC	− 0.632	0.001
FEV1	− 0.677	0.001
FEF25–75%	− 0.293	0.002
FEV1/FVC	− 0.439	0.001
SVC	− 0.459	0.001
MVV	− 0.269	0.003

Correlation between duration of diabetes with different parameters of PFT has been studied using Pearson's test of co-relation. *p* value of < 0.05 has been considered significant

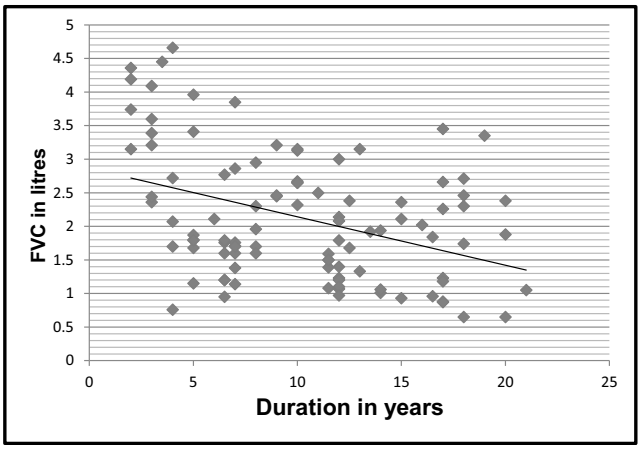

Fig. 1 Correlation between FVC and duration of diabetes

Discussion

Type 2 DM is a disease with profound multi-organ damage chiefly through its involvement of microvascular bed. Since microangiopathy is generalized, there is an increasing speculation that besides the commonly affected organs like kidney, heart, eyes, and nerves, a multitude of other organs like lungs may also be affected.

When all the pulmonary parameters were compared with respect to the increasing duration of disease, result analysis

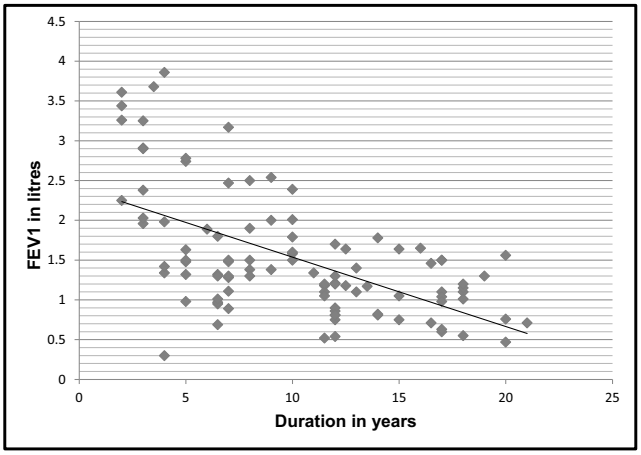

Fig. 2 Correlation between FEV1 and duration of diabetes

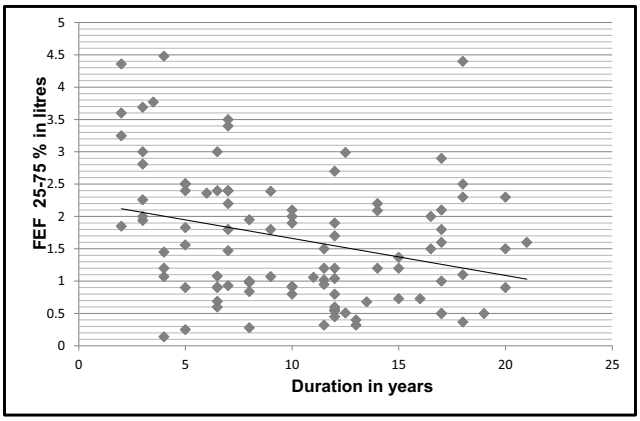

Fig. 3 Correlation between FEF 25-75% and duration of diabetes

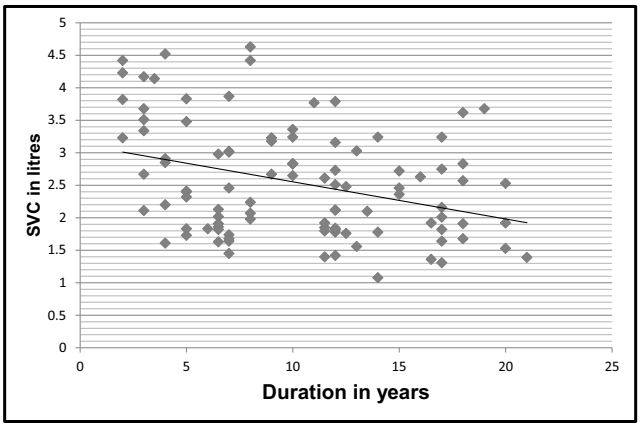

Fig. 5 Correlation between SVC and duration of diabetes

revealed that there was a propensity for all pulmonary function parameters to fall with increasing duration of diabetes.

The current study showed a significant ($p < 0.05$) decline in mean values of the FVC and FEV1 with a moderate negative correlation between them and increase in the duration of diabetes mellitus. In longitudinal studies conducted by Walter et al. [11] and Davis et al. [12], similar results were found, wherein Davis et al. [12] reported a mean 10% decrease of spirometric measure annually for FVC, FEV1, VC, and PEF and Walter et al. observed progressive decrease in mean FVC values by 68 ml/year and 109 ml/year respectively in a longitudinal study [11].

A significant association between duration of diabetes and FEV1, FVC, PEF ($p <$ or = 0.04) was also established by Hsin-Chieh et al. [13] and Davis et al. [14]. On the other hand, Mori et al. [15] and more recently, Shah et al. [16] found that pulmonary function tests were significantly decreased in diabetic patients compared with the healthy controls. However, no correlation was found between FVC, FEV_1, and duration of disease.

The present study revealed significantly higher actual value of FEV1/FVC% ($p < 0.05$) in comparison with the predicted values with the increase in duration of the diabetes mellitus. Pearson correlation test revealed moderate inverse correlation between FEV1/FVC and duration of diabetes. Parallel results were revealed by Ali et al. [17] and Sreeja et al. [18] in different diabetic group based on duration. However, Meo et al. [19] showed that subjects with > 10 years of disease have no significant difference in the values of FEV1/FVC% relative to controls.

Current study revealed lower values of FEF 25–75% with increasing duration of diabetes ($p < 0.05$). Meo et al. [20], Verma et al. [21], Ali et al. [22], and recently, Panpalia et al. [23] obtained similar results that there is significant reduction in FEF 25–75% with the increase in duration of diabetes. Contrarily, Yadav et al. [24] reported that PEER and FEF25–75% were not associated with duration of illness in Indian type 2 diabetic population.

In most of the previous studies, SVC and MVV are the less studied parameters. In this study, it was noted that values of SVC and MVV were significantly lower with the higher duration of diabetes mellitus. The findings were consistent with the results met by Guleria et al. [25]. They related this observation to the inflammatory process, the severity of which would increase with an increasing duration of diabetes. However, Yadav et al. [24] did not find any association between MVV and duration of type 2 DM.

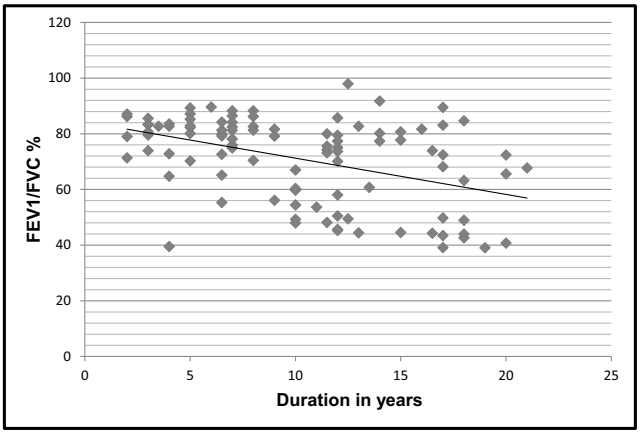

Fig. 4 Correlation between FEV1/FVC% and duration of diabetes

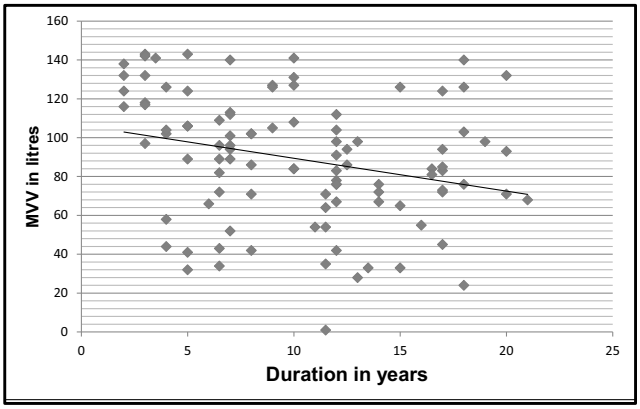

Fig. 6 Correlation between MVV and duration of diabetes

Probable pathophysiological explanations for decreasing pulmonary function associated with increase in duration of diabetes mellitus can be acceleration of aging process in the connective tissue of the lungs, interference with the connective tissue crosslinks, the presence of non-enzymatic glycosylation and modification of alveolar surfactant action leading to reduction in pulmonary function parameters [5].

Conclusion

Present study demonstrates a significant correlation of pulmonary functions with the duration of diabetes. It is important to increase awareness of potential damage to the lungs and is advisable to offer lung function as part of diabetes annual checks for early detection of lung abnormalities. Additional research can be planned with a larger study group in a larger geographical area to determine the significance of this association.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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ORIGINAL ARTICLE

Prediction of diabetes distress among adults with type 2 diabetes

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Abstract

Objective Diabetic patients will possibly be burdened with certain emotional distress level due to their health conditions. Emotional distress must be distinguished through observations on the general symptoms of depression and anxiety experienced by non-diabetic patients. Due to an insufficient amount of empirical works of research on the symptoms of diabetes distress in Iran, this research was conducted to investigate on diabetes distress and identify the factors which lead to the continuous distress among type 2 diabetic patients in the Iranian population.

Methods In order to appeal to the type 2 diabetic outpatients ($n = 109$), the Persian version of the Diabetes Distress Scale (DDS) was operated on a consecutive basis from May to August 2017 in all diabetes specialist hospitals in Kashan, Iran. With the application of full and trimmed methods on logistic regression, the comparison was made between the diabetic and the non-diabetic patients in terms of their behavioral (exercise) and biological variables (cholesterol level, blood pressure, and smoking status), including their demographic factors.

Results It was found that males, unmarried individuals, low-income employees, diabetic patients who have been diagnosed for over 5 years, those whose previous family members were not suffered from diabetes, and individuals who are diagnosed with high-level cholesterol and blood pressure are most likely to have diabetes distress.

Conclusion A list of important, interactive, direct, and independent factors of diabetes distress has been developed. This will be useful for the identification of high-risk patient cohort through patient screening. Furthermore, the benefits of the assessment of both disease-related and current life stressors in medical care have been found by identifying the effects of high diabetes distress and biological factors.

Keywords Diabetes type 2 · Diabetes distress · Adults · Prediction

Introduction

On the base of Lazarus theory of stress (1984), an individual has the capacity to evoke different responses to any given situation based on the meanings that are attributed to those situations [1]. Put differently, along with Lazarus, researchers focus on the interplay between cognition and stress placing a high value on individual interpretations and perceptions of events in order to better understand response styles and behavior [2]. According to World Health Organization (2017) and World Heart Federation (2017), diabetes mellitus (MD) is considered the cause of many diseases, and is one of the most established cardiovascular risk factors, affecting about 442 million people worldwide [3, 4]. For anyone diagnosed with MD, there may be many more cases that are undiagnosed [5].

The relationship between stress and diabetes is partly physiological and partly psychosocial. The increasing psychosocial problem among adult patients is the focal issue of type 2 diabetes mellitus (T2DM) [6, 7]. Furthermore, individuals' daily life and social-functioning [8, 9] will be affected by the highly demanding lifestyle in terms of health, this disease's recurrent symptoms, and regular medication-taking [10, 11]. T2DM patients will be burdened with their risky physical state [12, 13] and the communication they have to go through with health care professionals regarding their perception of health and illness [12, 13].

Moreover, diabetes-related distress (DRD) is the most prevalent psychological disorder to occur in T2DM adult patients [14]. As evidence, 30% of T2DM patients were proven

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by a research in the USA to have had been diagnosed with DRD for over an 18-month period [15, 16]. Similarly, a research in the UK revealed that T2DM patients had more tendency of being diagnosed with DRD after their age, gender, ethnicity, and socioeconomic status were assessed [17]. Meanwhile, the prevalence of DRD in Europe and the USA were reported to range between 15% and 20% [15]. However, the prevalence of DRD in Asian countries is not identified.

There has been a relationship between DRD and depression among T2DM adult patients with somatic symptoms [18, 19], smoking habits [20], and various psychological disorders [21]. Besides, there is an association between diabetes-related disease and deaths with DRD [22, 23]. However, DRD and depression across various geographical regions and cultural backgrounds possess different risk factors, and this can be seen from each of their socio-demographic-clinical characteristics [24, 25]. To illustrate, a relationship has been found between type 2 diabetes patients in Australia and Malaysia in terms of social support and self-efficacy, which affect medication, eating habits, and stress level [26–28]. Meanwhile, in the USA, the patients' young age, the absence of past time activities, frequent smoking, obesity, low household salary, low education level, and the presence of one or more micro- or macro-vascular diseases are related to their diabetes distress [29]. In China [30], there is a relationship between DRD and older age with diabetic depression.

However, type 2 diabetes was reported to occur in approximately 11.4% of adults in Iran, although limited number of studies worked on diabetes psychological complications [31].

This research aims to identify the relationship between the T2DM patients' socio-demographic clinical attributes and the occurrence of DRD in the Iranian population [32].

Research design and methodology

Participants and procedure

Outpatients who attended diabetes specialist centers in Kashan, a city in Iran, were selected for a cross-sectional study conducted from May to August 2017. The participants of this research consisted of (1) type 2 diabetes patients who were Persian speakers, (2) aged 30 years old and above, (3) were not diagnosed with several diabetes complications, and (4) there was no predecessor of this disease in their bloodline. Before the research commenced, the objective of the research was explained to all participants.

A number of 109 participants who fulfilled the inclusion and exclusion criteria, completed the Diabetes Distress Scale (DDS17) which was introduced by Polonsky (1995) [32]. There were 11 respondents who were excluded from analysis due to incomplete data. Two clinical psychology students explained the details of this research, and the demographic and clinical information were provided upon the questionnaire distribution.

Sample size

According to Charan and Biswas (2013), for small sample quantitative cross-sectional survey studies, the following formula was recommended [33].

$$SampleSize = \frac{Z_{1-a/2}{}^2 SD^2}{d^2}$$

where

$Z_{1-a/2}$	the standard normal variant (at 5% type 1 error ($p < 0.05$) is 1.96
SD	standard deviation of the main variable, which can be taken from the pilot study, which in our case was 26.65
d	absolute error or precision that has to be decided by the researcher. In our case, it was 5% based on the common practice of other studies

In substitution of the mentioned values in to the formula, an accurate sample size of 109 was obtained.

Procedures

The Diabetes Distress Scale (DDS), which was utilized in this study, consists of 17 items and is comprised of four domains: (a) emotional burden (EB), (b) physician distress (PD), (c) regimen distress (RD), and (d) interpersonal distress (ID). These domains lead to an overall score for Diabetes Distress (DD) including the four subscale scores. Each of the scores addressed different ranges of distress experienced over the last month, which were from 1 (no distress) to 6 (serious distress) [34].

Using a standard "forward-backward" translation method, the English version of the DDS was translated into Persian (Farsi). As a result, a positive reliability (Cronbach's alpha = 0.89) could be seen in the pilot test. In case of the cross-loading validity, which was performed in order to check the validity of the parameters, no cross component loading was found.

The methodology of this research was inspired and adapted by Fisher et al. [17] using five separate logistic regressions. The five dependent variables (DD, EB, PD, RD, and ID) were assessed through the overall scores from the aforementioned domains (DD, EB, PD, RD, and ID), with a mean item score of ≥ 3 (high scores of DD, EB, PD, RD, and ID obtained from the 1–5 response scale) used as the distress cut point. Furthermore, the participants would be classified as being diagnosed with high + Diabetes Distress (DD) provided if their DDS mean score was equal to 3 or exceeded that amount. Meanwhile, those with low DD score obtained less than 3 for

their DDS score. Moreover, a similar classification method was applied to the scores for EB, PD, RD, and ID. Three groups of variables were identified as the potential contributors of high DD. In performing the analysis on the patients, a set of general patient characteristics that was comprised of their age, gender, marital status, income, and employment was identified. These characteristics also included blood pressure, smoking status, cholesterol level, and the control variables related to diabetic stress due to their high prevalence among diabetes patients and their potentially remarkable effects on the primary variables of this research. As for the patients' behavioral variables, they consisted of a minimum of once a week exercise, the duration of diabetes diagnosis, and daily diet. Each respondent was asked to state whether they exercised on the week before this research was conducted and if they adhered to their diet.

Data analysis

Logistic regression analyses were conducted in order to develop a parsimonious model of diabetes distress over time. Referring to Hosmer et al. [35], a step-wise strategy was implemented in order to determine the significant factors of diabetes distress among the five models, which were small in amount. Two types of characteristics were made into several variables, specifically the general patient characteristics which were made into the independent variables and DD, EB, RD, RD, and ID which were made into the dependent variables [35]. Significant variables at $p < 0.10$ were retained within each of the five models. Moreover, in order to identify potential background factors, the general characteristics of the patients were involved in the logistic regression analyses. Following that, each of the five models was involved in an assessment that only identified the significant variables using a backward selection method. Finally, a combined model was developed and tested as it had emerged with the most significant predictors of diabetes distress from the final analysis of each of the 12 independent variables. At each stage of the analysis, the assessment was conducted on the non-linear effects of the continuous variables, multicollinearity, heteroscedasticity, the unusual changes occurring in the coefficients across analyses, and large standard errors [35].

Results

Table 1 summarizes the descriptive statistics of the participants of this research. Out of the 109 participants, more than 35% of them who had a high level of diabetes distress were 60 years old and older. Meanwhile, the rest of the participants (64%) were under 60 years old. Although the percentage of female participants was higher (61.5%) than the percentage of male participants, the percentage of male participants with a high total DD score (51.6%) was higher.

In terms of employment, 80% of the participants were unemployed while 20% of them were employed. When compared with the employed participants, the unemployed participants displayed the same proportion. Moreover, the factors of high total DD score were mainly emotional burden, regimen distress, and interpersonal distress. Nevertheless, employment and unemployment maintained the same proportion when compared to the proportion of physician distress.

High diabetes distress level could be seen in more than 67% of the participants. Not only that, it was found that they had been suffering from diabetes for five or more years. However, emotional burden had the highest factor percentage (85.1%) compared to the other factors, which would probably be the biggest contributor to the high score of total diabetes distress.

It was interesting to see that 56% of the participants had a predecessor of this disease in their families, compared to the remaining 44% who had a slight history of diabetes in their families. Despite the percentages, participants who had no history of diabetes in their families would have a higher tendency of diabetes distress (67.7%) compared to participants who had a history of diabetes in their families (32.7%).

In respect to emotional burden (53.2%), it was found to be higher among participants who had history of diabetes in their families, while those with no history of diabetes in their families had higher physician distress (75%), regimen distress (66.7%), and interpersonal distress (65.6%).

In terms of income, more than half of the participants (65.1%) fell under the low-income category, leaving only 35% from the high-income category. On the contrary, high-income participants had a higher tendency of having physician distress (60%), while the low-income participants had a lower tendency for it (40%). Nevertheless, the low-income participants sustained higher emotional burden (78.7%), regimen distress (59.3%), and interpersonal distress (56.3%) compared to the high-income participants.

As for the results of the logistic regression analyses, it was revealed that married participants had significantly lower probability of gaining high total DD scores. To be specific, the probability of a married individual to be diagnosed with high diabetes distress fell by 1.5%. However, when the analyses were performed separately on the participants' emotional burden, physician distress, regimen distress, and interpersonal distress as dependent variables, no significant p value was obtained for all marital status regression coefficients. The odds for married couples to have physician and interpersonal distress only reduce significantly by 1% and 9% respectively.

Employed participants were found to have a higher probability for high total DD scores compared to unemployed participants. After controlling all other dependent variables, it was found that only employed individuals had an increased probability of having a higher level of regimen and interpersonal distress.

Table 1 Percentage of type 2 DM patients with diabetic distress according to Diabetes Distress Scale and Subscales ($N = 109$)

Independent variable		Percentage of sample	Total DD (%)	EB (%)	PD (%)	RD (%)	ID (%)
Age	30–49 years old	32.9	32.0	10.6	50.0	40.7	37.5
	50–59	39.4	32.4	40.4	15.0	26.0	25.0
	60–more years old	36.7	35.6	49.0	35.0	33.3	37.5
Gender	Female	61.5	48.4	72.3	25.0	44.4	56.3
	Male	38.5	51.6	27.7	75.0	55.6	43.8
Marital status	Single	23.9	51.6	25.5	75.0	48.1	53.1
	Married	76.1	48.4	74.5	25.0	51.9	46.9
Employment status	Non-employee	79.8	61.3	91.5	50.0	55.6	59.4
	Employee	20.2	38.7	8.5	50.0	44.4	40.6
Blood pressure	Normal	14.7	32.3	2.1	50.0	33.3	28.1
	High	85.3	67.7	97.9	50.0	66.7	71.9
Smoking status	Non-smoker	87.2	71.0	95.7	55.0	63.0	71.9
	Smoker	12.8	29.0	4.3	45.0	37.0	28.1
Cholesterol status	Normal	17.4	32.3	2.1	50.0	33.3	28.1
	High	82.6	67.7	97.9	50.0	66.7	71.9
Exercise status	No exercise	44.0	48.4	59.6	35.0	40.7	50.0
	At least once a week	56.0	51.6	40.4	65.0	59.3	50.0
Diabetes duration	Less than 5 years	24.8	32.3	14.9	40.0	37.0	37.5
	Five or more years	75.2	67.7	85.1	60.0	63.0	62.5
Family history of diabetes	No	44.0	67.5	46.8	75.0	66.7	65.6
	Yes	56.0	32.5	53.2	25.0	33.3	34.4
Income per month	Less than 1000$	65.1	61.3	78.7	40.0	59.3	56.3
	More than 1000$	34.9	38.7	21.3	60.0	40.7	43.7
Sum of total		–	100.0	100.0	100.0	100.0	100.0

DD diabetes distress, *EB* emotional burden, *PD* physician-related distress, *RD* regimen-related distress, *ID* interpersonal distress

As for the participants' blood pressure level, high blood pressure increased the odds to have high total DD, EB, PD, RD, and ID scores. Similarly, the strongest coefficients obtained from the regression analyses were high blood pressure and high cholesterol. Furthermore, exercising decreased the probability of gaining a high total DD score. To be specific, the odds of an individual who exercised at least once a week would reduce the risk of diabetes distress by 2.4%. It would also significantly reduce the probability of sustaining emotional burden and interpersonal distress.

Apart from that, although having a history of diabetes in the family significantly decreased the probability of having a high total DD score, it did not decrease the probability of sustaining emotional burden, physician distress, regimen distress, and interpersonal distress.

In terms of their income, although participants with a high income had a lower probability of getting high total DD, ED, RD, and ID scores, this was not the case for their probability of getting physician distress (Tables 2 and 3).

Discussion

In this research, it was found that there was a 67% prevalence of high diabetes distress among patients who had been diagnosed with type 2 diabetes for over 5 years. Furthermore, among the dimensions of DDS, emotional burden was the most prevalent dimension with 85.1%, which would possibly contribute to a high total DD score. It was also found that non-employed participants, who owned the majority of this research sample, had high total DD score, emotional burden, and regimen and interpersonal distress.

A high percentage (67.7%) of participants with no history of diabetes in their family obtained a high DD score, while 53.2% of the participants who sustained high emotional burden were those with a history of diabetes in their family. It was stated by Liu et al. [36] that self-healthcare behaviors of individuals are highly influenced by emotional burden. Therefore, emotional burden among diabetes patients should be checked so that emotional support can be provided and the individual's ability to act the way they desire can be improved through better self-healthcare

Table 2 Results of coefficient in multiple logistic regression analysis showing the predictors of distress in type 2 DM patients ($N = 109$)

Variables		Total DD		EB		PD		RD		ID	
		Coefficient	RSE	Coefficient	RSE	Coefficient	RSE	Coefficient	RSE	Coefficient	RSE
Age	50–59	0.67	0.74	0.52	0.70	− 0.37	1.25	− 0.40	0.84	− 1.23	0.77
	60 years old and above	1.04	0.76	1.02	0.80	0.52	1.14	0.36	0.93	− 0.05	0.74
Male		0.60	0.72	− 0.03	0.59	3.68	2.02	− 0.09	0.69	− 0.97	0.75
Married		− 1.88**	0.73	− 1.13	0.65	− 4.40**	1.69	− 1.23	0.72	− 2.33**	0.78
Employee		1.52**	0.77	− 0.43	0.66	0.25	0.98	1.72**	0.73	2.56**	0.81
High blood pressure		5.3**	1.47	3.2**	1.43	3.8**	1.92	4.0**	1.29	4.0**	1.29
Smoker		0.81	0.78	0.00	0.94	− 0.28	1.06	2.04**	1.01	0.58	1.00
High cholesterol		4.4**	1.10	15.7**	1.01	2.8**	1.44	4.20**	1.00	4.9**	1.00
Exercise at least once a week		− 1.39**	0.67	− 0.90*	0.48	− 1.02	0.99	− 0.63	0.65	− 1.21**	0.61
Five or more years diabetes duration		− 0.41	0.56	0.55	0.57	− 0.34	0.77	− 0.68	0.66	− 1.24	0.76
With family history of diabetes		− 1.32**	0.59	− 0.62	0.46	− 0.58	0.81	− 0.97	0.64	− 1.00	0.62
More than 1000$ income per month		− 1.82**	0.72	− 1.48**	0.58	− 1.05	0.79	− 1.96**	0.68	− 1.17*	0.69
Constant		2.07	1.09	− 1.28	1.33	1.25	1.44	0.90	1.17	2.62	1.21

*Significant at 5% level; **significant at 1% level

RSE = robust standard error

Reference categories: age (30–49), female, single marital status, unemployed, not having blood pressure, non-smokers, respondents with normal and low cholesterol level, respondents who do not exercise, diabetes duration less than 5 years, respondents without family history of diabetes, respondents with less than 1000$ of income per month

DD diabetes distress, *EB* emotional burden, *PD* physician-related distress, *RD* regimen-related distress, *ID* interpersonal distress

behaviors [36]. Besides, it was proven by a research in Malaysia, that emotional burden is one of the most influencing dimensions for type 2 diabetes patients among the dimensions of DDS. Psychotherapy was utilized in this research to take better control of the patients' blood sugar so that their emotional burden could be reduced [37].

The participants who suffered from regimen (33.3%) and interpersonal distress (34.4%) consisted of those with a history of diabetes distress in their family. Generally, being born into a family with a history of diabetes distress decreases the probability of having a high total DD score. Based on this finding, it can be concluded that diabetes can be overcome by patients who are born into a family with a history of diabetes, while those who are born into a family with no history of this disease probably need to be educated and trained to get accustomed to a new lifestyle. With increased knowledge of this disease, individuals would have more preparations in the prevention of diabetes. In addition, diabetes complications, which could lead to negative thinking such as "I cannot enjoy eating," can be curbed [38].

Individuals who suffered from physician distress consisted of low-income individuals (60%), while the remaining 40% were not diagnosed with this distress. In this research, it was found that high-income participants had a lower chance of having a high total DD score. One of the controversial issues related to diabetes treatment is the provision of medication for free or a low price. Moreover, type 2 diabetes was found to occur 1.4 times more frequently among individuals who earn a USD of 15,000 of household salary or less [39, 40]. Therefore, people with lower education level, lower socioeconomic status, and poverty would face a higher risk of having type 2 diabetes due to the high costs that medical treatments sustain, such as daily blood sugar monitoring and regular medical check-ups [41]. Nevertheless, there are many alternatives to the reduction of the diabetes treatment costs, which come with equivalent impacts. To illustrate this point, governmental hospitals and clinics can offer expensive treatments at a low price.

Based on the review of the exploratory analyses of the interaction between the logistic regression variables, similar patterns were obtained, such as married participants being less likely to have high total DD score. Furthermore, works of research on diabetes management have proven the positive effects of social support on the factors of diabetes patients' healthy behaviors such as taking up physical activities and nutrition [42, 43].

Employed participants had a higher probability of getting a high total DD score and regimen and interpersonal distress. It can be concluded from the previous section that inadequate social support can result in interpersonal distress and high total DD score. This can occur in patients who do not have sufficient time to prepare healthy meals at home; therefore, they tend to eat out and consume fast food. This unhealthy food intake will increase their blood sugar level and bring more distress to their life.

Table 3 Results of odds ratios in multiple logistic regression analysis showing the predictors of distress in type 2 DM patients ($N = 109$)

Variables		Total DD			EB			PD			RD			ID		
		Odd	[95%	CI]	Odd	[95%	CI]	Odd	[95%	CI]	Odd	[95%	CI]	Odd	[95%	CI]
Age	50–59	1.95	0.33	11.79	1.69	0.42	6.86	0.68	0.05	9.46	1.69	0.13	3.42	0.29	0.06	1.51
	60 years old and above	2.85	0.47	17.24	2.77	0.69	11.18	1.69	0.16	17.59	2.77	0.27	7.77	0.95	0.18	4.91
Male		1.82	0.45	7.34	0.96	0.31	2.97	3.97	1.84	8.06	0.96	0.21	3.89	0.37	0.08	1.73
Married		0.15**	0.04	0.59	0.32	0.08	1.24	0.01**	0.00	0.21	0.21	0.07	1.16	0.09**	0.02	0.42
Employee		4.60**	0.96	22.06	0.64	0.15	2.84	1.28	0.16	10.49	0.64**	1.10	28.72	13.0**	2.43	69.67
High blood pressure		4.03	0.00	0.00	0.01**	0.00	0.00	3.88**	0.00	0.00	4.97**	0.00	0.00	0.00**	0.00	0.00
Smoker		2.26	0.29	17.38	1.00	0.12	8.16	0.75	0.05	10.85	1.00**	1.01	59.32	1.80	0.17	18.93
High cholesterol		4.50**	0.00	0.00	0.66**	0.00	0.00	4.08**	0.00	0.00	3.82**	0.00	0.00	1.02**	0.00	0.00
Exercise at least once a week		0.24**	0.07	0.83	0.40*	0.16	1.04	0.35	0.06	2.02	0.40	0.17	1.70	0.29**	0.09	0.99
Five or more years diabetes duration		0.66	0.18	2.45	1.75	0.53	5.83	0.70	0.13	3.97	1.75	0.14	1.83	0.28	0.07	1.13
With family history of diabetes		0.26**	0.08	0.85	0.53	0.21	1.41	0.56	0.11	2.97	0.53	0.12	1.20	0.36	0.11	1.17
More than 1000$ income per month		0.16**	0.03	0.79	0.22**	0.07	0.70	0.34	0.05	2.29	0.22**	0.03	0.77	0.30*	0.07	1.38
Constant		7.99	0.76	84.52	0.27	0.02	4.44	3.48	0.15	8.74	0.27	0.25	24.09	13.8	1.05	181.74

*Significant at 5% level; **significant at 1% level

Odd = odds ratio, 95% confident interval

Reference categories: age (30–49), female, single marital status, unemployed, not having blood pressure, non-smokers, respondents with normal and low cholesterol level, respondents who do not exercise, diabetes duration less than 5 years, respondents without family history of diabetes, respondents with less than 1000$ of income per month

DD diabetes distress, *EB* emotional burden, *PD* physician-related distress, *RD* regimen-related distress, *ID* interpersonal distress

High total DD score also occurs due to high blood pressure and cholesterol level, while exercising decreases the possibility of obtaining any DD score. More importantly, diabetes management is highly necessary, including self-management behaviors such as having a balanced diet and performing regular exercises. On the other hand, the level of diabetes distress will increase when the perceived coping resources gained by the researchers are exceeded [44]. Considering how the death of 65% of type 2 diabetes patients is due to cardiovascular disease [45], it is important that all health issues, such as diet, exercises, and diabetes complications are managed so that patients can obtain a positive healthy condition in both physical and psychological terms.

Through the results proposed in this research, a particular idea has been formulated. It mainly addresses that besides not contributing to any positive or negative progress to the diabetes disease, the issues revolving around a diabetic patient's life usually impose several factors which result in diabetes distress (e.g., diabetes complications). In fact, there are many elements in the disease of diabetes that can lead to a series of various impacts on the patient's life. Besides, clinicians spend a significant amount of time in order to identify the factors of this distress and their impacts on the patients' self-care. Even so, the amount of awareness given to the patient's broader life context, which is another prominent indicator of their health, is insufficient. Therefore, the presence of clinicians is important, especially during the time when healthcare programs for diabetic patients are organized. With this, the patients' family, life outside their homes, financial capability, and the factors of their life that can cause stress can be identified. Additionally, in order to reduce the negative impacts of stressors in a patient's life, more assistance is required from services that specialize in managing these factors, before the patient's diabetes management is affected.

It is important to note that there were limitations, which came with the results of this research. Initially, a large number of diabetes indicators were investigated on a large number of participants, which was an appropriate-sized sample although considering the inclusion and exclusion criteria in the present study, a smaller sample size was made. Furthermore, an assessment was conducted in detail on the potential issues related to diabetes distress by evaluating the odds ratios and conducting the multicollinearity tests. These tests were performed in order to evaluate the inconsistent research results. Even so, replication was required for the remaining findings, especially the interaction of the term data. The second limitation could be seen in the fact that investigation was not performed on the patient's social support although social support could improve the patients' health and curb diabetes distress.

In spite of the limitations of this research, identification has been made on the prominent and independent indicators of high diabetes distress. These will play an important role in diabetes patient's screening process which functions in determining the high patient cohort. Considering the significant impacts of diabetes distress on this disease biological and behavioral indicators, these results will be useful for a better identification of the elements of stress in a patient's life and disease when medical treatment is conducted on them.

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Compliance with ethical standards

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

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the national committee.

Informed consent A written informed consent was distributed to each participant of this research before proceeding to the data collection process.

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ORIGINAL ARTICLE

Knowledge, attitude, and practices of fasts in patients with type 2 diabetes among different religions in North India

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Abstract

Background and objectives There is no data regarding knowledge, attitude, and practices (KAP) of fasts in subjects with type 2 diabetes (T2DM) among different religions in India. Study was done to assess KAP regarding fasts among subjects with T2DM from various religions.

Material and methods A total of 300 consecutive participants with T2DM (age ≥ 25 years) from 3 diabetes clinics in North India after consent were subjected to predesigned study pro forma.

Result A total of 300 subjects participated in the study with 76.3% being Hindus, 11% Muslims, 4.3% Sikhs, and 5.7% from other religions (Christians and Buddhists). Knowledge and attitude regarding fasts in context of diabetes were poor in majority of subjects. 59.7% participants agreed that diabetic patients can fast. 36.3% participants believed that people with poor glycemic control (HbA1c ≥ 10%) can also observe fast. 40.7% believed medicines/insulins are not allowed during fasts because of religious reasons. 46.7% participants were aware of hypoglycemic symptoms. Among Hindus 2 most common fasts were Navratri (observed by 53%) and Karva Chauth (observed by 47.6%). Overall, 66.7% of Muslims observed Ramadan. Only 1.3% of participants discussed with their doctors before observing fast. 51.7% of participants who observed fasts had symptoms suggestive of hypoglycemia during fasts. 60.4% of subjects missed their medications during fasting. 28.8% altered their drug regimen by themselves without doctors' consultation. 78.5% of participants observed fasts because of social obligations. Hindus were more likely to consult doctors during fasts and experienced comparatively lesser hypoglycemia and hyperglycemia as compared with Muslims and other religions. Women had greater inhibition to disclose about diabetes during fasts, were more likely to consult doctor during fasts, and perceived greater changes in body weight, hypoglycemia, and hyperglycemia during fasts.

Conclusion The study showed significant gaps in knowledge and attitude regarding fasts among subjects with T2DM, in spite of fasting being common among all religions. The different nature and duration of different fasts and associated religious practices may contribute to these differences.

Keywords Diabetes · Fast · Religion · Hindu fast · Navratri · Karva Chauth · Ramadan · Diet · Knowledge

Introduction

There is an estimate of 82 million people suffering from type 2 diabetes (T2DM) in South East Asian Countries, and the number is expected to increase by 151 million by 2045 [1]. In India, estimates of 2017 have shown that there were 72,946.4 adults with T2DM in the age of 20–79 years with 997,803 diabetes-related deaths per year [1]. Diabetes onset in

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Indians is nearly 2 decades earlier as compared with that in the west [2]. Indians have the highest global rates of prediabetes progression to diabetes [3]. Diabetes occurs in Indians at a lower body mass index and is characterized by greater insulin resistance and beta cell dysfunction, all highlighting a more aggressive nature of the disease [2, 4].

India has a huge variety of cultures and religions following a huge number of festivals and rituals. In India, people celebrate numerous festivals irrespective of their caste, age, status, and creed with different rituals. India constitutes of a large variety of religions where 80% of the population is Hindu, while other major religions are Sikhism, Islam, Christianity, Jainism, and Buddhism [5].

In India, different festivals are celebrated with different patterns, customs, beliefs, and rituals by different individuals. Fasting or abstain from all or some kinds of food or drink and feasting are followed among people in India differently, on the basis of religion, rituals, and their beliefs [5]. Inappropriate fasting and feasting can significantly contribute to the glycemic morbidity in Indians. People do fasts from every community; fasting can be done on festivals, on any occasions, or on other beliefs. Each religion has their distinct belief and different patterns of fasting and feasting [6]. Fasting with diabetes requires careful diabetes management. People observe fast with various reasons that can vary in durations and the conditions of the fasting. For reasons of health, people with diabetes may often be exempt from having to observe the fasting [5, 6].

There are a number of studies in context of Ramadan fasting in Muslims. However, there is paucity of data in context of Hindu/other religions' fasting and diabetes. There is lack of data regarding knowledge, attitude, and practices (KAP) of persons with diabetes in context of fasting from India. Also, data is not available from India regarding impact of fasting on acute diabetes complications.

The current study was planned to understand the practices of fasts (frequency as well as patterns) being observed by persons with T2DM from different religions in India. We also planned to study KAP of persons with diabetes in context of fasts from different communities. Impact of fasting on acute diabetes complications was also planned to be ascertained. Also, the study was intended to explore treatment adherence during fasts and social and cultural factors involved in fasting in persons with T2DM.

Material and methods

This cross-sectional, non-interventional study was done in 3 diabetes centers of North India (2 from New Delhi and 1 from Bhopal) from 1 January 2018 to 31 March 2018. A total of 300 consecutive participants with T2DM (age ≥ 25 years) after verbal consent were subjected to predesigned study pro forma. Subjects were recruited while they were in waiting for their consultation in the outpatient department (OPD). All patients were explained regarding the study. Only those who gave informed written consent were recruited in the study. Demographic and disease-related information was collected followed by subjecting them to KAP questionnaire. Patients self-reported home monitoring of blood glucose as well as latest fasting and postprandial blood glucose (within last 1 week of visit) done as part of routine clinical practice was captured for data analysis. Similarly, last HbA1c% done (within last 1 month of visit) was also recorded for all subjects.

KAP questionnaire

The pro forma had multiple-choice questions. The average amount of time consumed per participant was 20–25 min. Initial section (knowledge and attitude) was basically to understand their knowledge regarding their diabetes in context of fasting. It had questions like participants' knowledge regarding acute complications of diabetes (i.e., symptoms of hyperglycemia and hypoglycemia), whether diabetic patients can observe fast, whether fasting can cause hyperglycemia and hypoglycemia, and whether fasting should be terminated if he/she develops severe hyperglycemia or hypoglycemia. Additional questions were whether fasting is safe in special situations with diabetes like pregnant women and subjects with very poor glycemic control (HbA1c ≥ 10%). Also, questions were designed to assess their knowledge whether dietary changes during fasting can affect blood glucose level and whether anti-diabetic or insulin should be taken during fasting, whether blood glucose monitoring is beneficial during fasting, patient can make alterations of medications during fasting, and regarding exercise during fasting. There were questions also to understand other family members' awareness related to symptoms of hyperglycemia and hypoglycemia.

Later part (practices) was about their practices like whether they observed fast in last 1 year, number of fasts they observed in last 1 year, and which fasts they observed which were categorized as per religion. Questions were there to reveal their other practices like whether they consult a doctor/dietician before observing fast, glucose monitoring during fasts, symptoms suggestive of hypoglycemia/hyperglycemia during fasting, and if yes their decisions to terminate the fast. They were also asked regarding quitting of medications during fasting day and altering the timings of medications during fast. There were few questions pertaining to social context like any pregnant ladies/elderly (> 60 years) with diabetes observing fast at home and primary reason for observing fasts.

Statistical analysis

The data was entered and consolidated on Microsoft Excel 2010 version. Categorical variables were presented in

numbers and percentages (%), and continuous variables were presented as mean ± standard deviation. The analysis was done using Statistical Package for Social Sciences (SPSS) (Chicago, IL, USA) version 21.0 software. Normality of the distribution of variables was checked using the Kolmogorov-Smirnov test. Chi-square tests were used for categorical variables. A p value < 0.05 was considered statistically significant.

Results

Among the 300 study participants, 48% participants were males and 52% participants were females. The demographic parameters of study subjects are shown in Table 1. The mean age of the participants was 54.23 ± 11.86 years. 34.3% participants had duration of diabetes between 1 and 5 years. Among the participants, 76.3% were Hindus, and 11% were Muslims while 4.3% were Sikhs. Regarding pharmacotherapy, 93.3% of the study subjects were taking metformin, 57% were on sulfonylureas, and 30.3% of subjects were on insulins along with oral anti-diabetic medications. All subjects were receiving at least 1 drug for management of hyperglycemia. The latest fasting blood glucose (FBG), postprandial blood glucose (PPBG), and HbA1c were 158.04 ± 65.01 mg/dl, 222.63 ± 98.66 mg/dl, and 8.83 ± 2.20%, respectively (Table 1).

Frequency of major diabetic complications among the study participants is shown in Table 2. 37.7% of subjects reported symptoms suggestive of hypoglycemia in last year. Regarding knowledge, 59.7% participants agreed that diabetic patient can observe fasts. 49.3% responded that fasting can cause hypoglycemia. Most of the participants (52.3%) agreed that fasting should be terminated if hypoglycemia/uncontrolled hyperglycemia occurs, and 69.0% were aware that fasting is unsafe for type 1 diabetes mellitus (T1DM) individuals, pregnant women, and elderly people with diabetes. 36.3% patients believed that persons with poor glycemic control (HbA1c ≥ 10%) can also observe fast (Table 3).

Regarding attitude, 34% agreed that self-monitoring of blood glucose (SMBG) is beneficial during fasting. 68.7% agreed that DM medications can be altered by patients themselves. 38.7% believed that their family members should be aware of symptoms and management of hypoglycemia. Majority of the patients (56.7%) agreed that exercise should be done during fasting. 40.7% believed that medicines/insulins are not allowed in fasts because of religious reasons (Table 3).

Regarding practices, 49.7% participants observed fasts in last year. 19.7%, 9.7%, and 4.7% participants observed 1–5 days fast, 5–10 days fast, and > 30 days fast in last 1 year, respectively. The most common Hindu fasts were Navratris (53%) and Karva Chauth (47.6%), and the most common Muslim fast was Ramadan (66.7%) and among Jains, Digambar Upvas, and Ekasana (1 each). Among Hindus, the duration for Navratri varied from 1 to 8 days, tentatively a month of Ramadan among Muslims while other fasts lasted for a single day at a particular time period and frequency of occurrence throughout the year varied. The pattern of fasting also varied in terms of disease condition, choice of fasting,

Table 1 General characteristics of the participants

General characteristics	n (%) or mean ± SD
Sociodemographic profile	
Male	144(48)
Female	156(52)
Age (years)[a]	54.23 ± 11.86
Height (cm)[a]	160.29 ± 9.57
Weight (kg)[a]	72.42 ± 14.26
Systolic blood pressure (mmHg)[a]	125.03 ± 4.49
Diastolic blood pressure (mmHg)[a]	75.88 ± 8.56
Education level	
Postgraduate	41 (13.7)
Graduate	99 (33)
Senior-secondary	40 (13.3)
Secondary	94 (31.3)
Non-educated	26 (8.7)
Religion	
Hindus	229 (76.3)
Muslims	33 (11)
Sikhs	13 (4.3)
Jains	8 (2.7)
Others (Buddhists and Parsis)	17 (5.7)
DM duration	
1–6 months	25 (8.3)
6 months–1 year	16 (5.3)
1–5 years	103 (34.3)
5 to 10 years	74 (24.7)
More than 10 years	82 (27.3)
Anti-diabetes medication therapy	
Metformin	280 (93.3)
Sulfonylureas	171 (57)
Pioglitazone	22 (7.3)
DPP4 inhibitor	154 (51.3)
SGLT2I	18 (6.0)
AGI	12 (4.0)
Repaglinide	3 (1)
Insulins + oral hypoglycemic agents	91 (30.3)
Latest blood glucose levels	
FBS (mg/dl)[a]	158.04 ± 65.01
PPBS (mg/dl)[a]	222.63 ± 98.66
HbA1c (mg/dl)[a]	8.83 ± 2.20

Numbers are n (%)

DPP4 inhibitor dipeptidyl peptidase-4 inhibitor, *SGLT2* sodium-glucose cotransporter-2, *AGI* alpha-glucosidase inhibitors

[a] Indicates mean (SD)

Table 2 Frequency of complications in study participants

Complications	Yes, *n* (%)
Symptoms of hypoglycemia in last year	113 (37.7)
Neuropathy	59 (19.7)
Nephropathy	40 (13.3)
Retinopathy	15 (5)
CAD	25 (8.3)

Neuropathy was diagnosed based on symptoms and/or objective evidence of neuropathy on testing; nephropathy was diagnosed based on presence of microalbuminuria/macroalbuminuria and/or glomerular filtration rate less than 90 m/min; retinopathy was diagnosed based on presence of defects noted on digital fundus photography; CAD was diagnosed based on history and/or those having objective evidence of disease

CAD coronary artery disease

frequency, dietary intake, and medical compliance within religion and among different religions.

Only 1.3% consulted their doctor for pre-assessment. Glucose monitoring was allowed for 57% participants while only 4.1% actually checked their blood glucose levels. Despite 51.7% reported the symptoms of hypoglycemia (< 70 mg/dl; documented and undocumented), only 8% terminated fast. 81.8% and 60.4% of participants, who observed fasts, respectively altered their medications/insulins and missed diabetes medications during fasting by themselves without a doctor's consultation. 78.5% of subjects who observed fasts reported that fasting was mandatory as a part of social obligation. Subgroup analysis based on the religion of the individual revealed that there were significant differences with regard to planning for fasts from diabetes point of view, adjustment of medications, experience of hypoglycemia and hyperglycemia, and family support during fasts (Table 4). Hindus were more likely to consult their doctors before or during fasts, less often missed medications, but were more likely to self-adjust their medications, and had a comparatively lesser experience of hypoglycemia and hyperglycemia during fasts as compared with Muslims (Table 4). Analysis based on the sex of the individual revealed that women had a greater inhibition to disclose about diabetes during fasts, were more likely to consult doctor during fasts, perceived greater changes in body weight, were more likely to self-alter medications, and perceived greater hypoglycemia and hyperglycemia episodes during fasts (Table 5).

Discussion

The present study was conducted among patients with T2DM to assess the KAP during fasting in North India. Our study has shown that there are significant gaps in knowledge and attitude regarding fasts among subjects with T2DM, in spite of fasting being common among all religions. Most persons do not discuss regarding their fasts with diabetes care team ending up doing faulty dietary practices as well as self-adjustment/missing anti-diabetes medications. A large number of subjects had hypoglycemic symptoms during fasts jeopardizing their health. To the best of our knowledge, this is the first study assessing KAP regarding fasts among subjects with T2DM among different religions from North India.

Most of the literature regarding fasts in context of diabetes is available from Ramadan fasting [7–10]. Regarding knowledge and attitude, our study showed that a very few patients were aware of hypoglycemic symptoms while 52.3% knew that they should terminate fasting if there is hypoglycemia (< 70 mg/dl)/uncontrolled hyperglycemia (≥ 300 mg/dl). Majority of the patients were aware that fasting is unsafe for T1DM individuals, pregnant women, and elderly people. 36.3% patients believed that persons with poor glycemic control

Table 3 Knowledge and attitude regarding fasts among study participants

	Agree, *n* (%)	Disagree, *n* (%)	Don't know, *n* (%)
Knowledge			
•Patient with diabetes can observe fast	179 (59.7)	121 (40.3)	0
•Aware of hypoglycemic symptoms	140 (46.7)	160 (53.3)	0
•Fasting can cause hypoglycemia	148 (49.3)	49 (16.3)	103 (34.3)
•Fasting should be terminated if hypoglycemia/uncontrolled hyperglycemia RBS ≥ 300	157 (52.3)	53 (17.7)	90 (30.0)
•Fasting is safe for T1DM individuals, pregnant women, and elderly people	64 (21.3)	207 (69.0)	29 (9.7)
•Persons with poor glycemic control (HbA1c ≥ 10%) can observe fast	109 (36.3)	169 (56.3)	22 (7.3)
•Dietary changes in fasts can affect glucose levels	200 (66.7)	37 (12.3)	63 (21.0)
Attitude			
•Blood glucose monitoring is beneficial during fasts	102 (34)	198 (66)	0
•DM medications can be altered by patients themselves during fasts	206 (68.7)	94 (31.3)	0
•Family members should be aware of symptoms and management of hypoglycemia	116 (38.7)	183 (61.0)	1 (0.3)
•Medicines/insulins are not allowed in fasts because of religious reasons	122 (40.7)	178 (59.3)	0

(HbA1c ≥ 10%) can also observe fast while 56.3% patients disagreed and 7.3% were unaware about it. 34% agreed that self-monitoring of blood glucose (SMBG) is beneficial during fasting. 68.7% agreed that DM medications can be altered by patients themselves. 40.7% believed that medicines/insulins are not allowed in fasts because of religious reasons.

Similarly, the literature on fasting shows that patients with diabetes have low awareness, eventually causing varying diabetic complications [11]. A study published by Zainudin et al. has shown that mean knowledge score was 75.9% for fasting knowledge, but it also reported more than half did not recognize the need to monitor glucose levels more frequently during illness [12]. The participants did not know about the increased risk of these complications during fasting and did not recognize the increased risk of dehydration and thrombosis [13–16]. Another study conducted by Yaacob et al. has shown relatively lower mean scores of knowledge with respect to symptoms of hypoglycemia, correct practice during fasting, and problems that may arise from fasting. Further, the study reported a positive attitude for physical activity, self-control of the disease, and the ability to fast. A few participants in this study were not sure whether fasting benefits their disease condition, and a still few believed that it did not improve their disease condition. Most of the patients in this study reported a reduced frequency of medication [13]. Beshyah et al. has shown that self-reported competence is not matched by actual knowledge and treatment practices among patients [15].

Regarding practices, among the study participants, only 1.3% consulted their doctor for pre-assessment. 51.7% reported the symptoms of hypoglycemia, but only 8% terminated fast after hypoglycemia or hyperglycemia occurred. Majority of the patients (81.8%) and (60.4%) altered their medications/insulins and missed routine medications during fasting, respectively. They believed either that they require less medicines or that medicines are not allowed in fasting. 36.9% participants also reported that pregnant women and elderly people with diabetes observed fast in their family.

Contextually, it is evidenced from the literature that people with diabetes fast against general practitioners. [12, 16–18]. The literature on fasting shows that fasting is responsible for up to sevenfold higher risk of hypoglycemia, especially among those on insulin and those who are physically active [12, 19–22]. Further, it is shown that stoppage of insulin is associated with religious fasting [23]. It is further believed among the patients that blood glucose testing by finger pricking leads to break of fast during Ramadan [24, 25]. As a result, many episodes of hypo and hyper go unnoticed/undocumented. It further shows that a very few people consult their practitioners. It has also shown that people avoid eating even during hypoglycemic events. Studies have shown that fasting is an obligatory cultural practice and even the lactating mother observes fast [6, 17, 21].

A study conducted by Zainudin et al. has reported that among a total of 92 patients in the study, 71.4% of the patients consulted their physicians, 37.3% did not monitor their blood glucose levels, 47.0% had hypoglycemic episodes, and 10.8% who had hypoglycemia did not treat the hypoglycemia but continued to fast [12]. Another study conducted by Pinelli et al. has shown that 25% and 17% decreased frequency of home blood glucose monitoring (HBGM) or had not tested at all, respectively. Majority of the participants observed fasts without education regarding medications, risks of fasting, indications to terminate fasting, meal plans, and exercise. Therapeutic changes were made in 50% and 46% of insulin and oral medication users, respectively. It reported the most common symptom of dehydration among participants. Contrary to the study results, it reported a lower frequency of hypoglycemia and hyperglycemia with only one patient

Table 4 Practices regarding fasts among study participants based on their religion

Practices	Hindus (n = 229)	Muslims (n = 33)	Jains (n = 13)	Sikhs (n = 8)	Other (n = 17)	p value
Consulted your doctor for pre-assessment	3	0	0	0	1	0.007
Consulted a doctor during fast cause of problems	85	19	1	1	6	0.007
Embarrassed to disclose about diabetes during fasts	105	11	1	3	7	0.001
Body weight changed during fasts	77	16	0	1	4	0.836
Noticed weight gain following fasts	30	8	2	5	5	0.011
Felt lack of family support during fasts	19	13	0	0	0	< 0.001
Missed medications during fasting	106	21	1	3	5	0.363
Self-altered medications/insulins during fasting	49	4	1	2	3	0.134
Glucose monitoring was allowed during fasting	46	15	0	1	1	0.040
Experienced ketoacidosis during fasts	9	2	0	0	1	0.943
Terminated fast after getting hypoglycemia	51	22	1	1	2	< 0.001
Terminated fast after getting hyperglycemia	70	19	1	1	2	0.030
Pregnant women and older people with diabetes observed fast in family	14	9	0	0	0	0.016

Table 5 Practices regarding fasts among study participants based on their religion

Practices	Males (n = 144)	Females (n = 156)	p value
Consulted your doctor for pre-assessment	2	0	0.094
Consulted a doctor during fast cause of problems	29	83	< 0.001
Embarrassed to disclose about diabetes during fasts	39	88	< 0.001
Body weight changed during fasts	35	63	< 0.001
Noticed weight gain following fasts	26	24	0.798
Felt lack of family support during fasts	17	15	0.872
Missed medications during fasting	7	6	0.061
Self-altered medications/insulins during fasting	26	64	< 0.001
Glucose monitoring was allowed during fasting	28	57	0.593
Experienced ketoacidosis during fasts	8	4	0.005
Terminated fast after getting hypoglycemia	30	47	0.035
Terminated fast after getting hyperglycemia	29	64	< 0.001
Pregnant women and older people with diabetes observed fast in family	10	14	0.211

terminating fasting due to uncontrolled hyperglycemia [26]. Further study conducted by Salti et al. has shown that among the study participants, 42.8% of patients with type 1 diabetes and 78.7% with type 2 diabetes fasted for at least 15 days. Less than 50% changed their treatment dose and were challenged by hypoglycemia (type 1 diabetes, 0.14 vs. 0.03 episode/month; $p = 0.0174$; type 2 diabetes, 0.03 vs. 0.004 episode/month; $p < 0.0001$) [21].

Our study highlighted for the first time that religion and sex of an individual have a significant bearing on KAP with regard to diabetes during fasts. Hindus were more likely to consult their doctors before or during fasts, less often missed medications, and experienced comparatively lesser hypoglycemia and hyperglycemia during fasts as compared with Muslims. Analysis based on the sex of the individual revealed that women had a greater inhibition to disclose about diabetes during fasts, were more likely to consult doctor during fasts, perceived greater changes in body weight, were more likely to self-alter medications, and perceived greater hypoglycemia and hyperglycemia episodes during fasts. The reasons for such heterogeneity based on religion and sex is likely multifactorial. The work profession, different nature and duration of different fasts, and associated religious practices may contribute to these differences. Fasting alternating with religious feasting may contribute to this difference and the related glycemic variability.

It is recommended for the patients to have adequate knowledge and positive attitude among patients with respect to SMBG and corrective doses of medication, symptoms of hypoglycemia and hyperglycemia, osmotic symptoms, and diet and lifestyle to prevent risk of complications. It must be highlighted that monitored intermittent fasting with appropriate modulation of treatment regimen has been documented to have a beneficial impact on glycemic control and body weight along with reduction of diabetes medications in people living with diabetes.

There were a few limitations. This study relied on retrospective data and involved volunteer participation. The problems of recall bias remain. Also, the blood parameters, symptoms of diabetic complications, and data on records of medication and blood glucose monitoring were relied on patient's response. Religion-based subgroup analysis was limited by a small number of patients in Jains, Sikhs, and other religious groups. Future prospective studies involving a large number of subjects are needed to clearly understand these issues in Indian context.

Conclusion

This study highlights significant gaps in knowledge and attitude of subjects with T2DM in context of fasting among different religions of North India. Faulty practices are followed during fasting including self-adjustment of or missing diabetes medications leading to diabetic complications. Diabetes care team should discuss regarding fasts among subjects with T2DM in India and provide appropriate counseling.

Compliance with ethical standards

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

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ORIGINAL ARTICLE

Effectiveness of a school-based pilot program on 'diabesity' knowledge scores among adolescents in Chennai, South India

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Abstract

Background India has the 2nd largest number of children and adolescents living with diabetes in the world. Hence, the present study aimed to evaluate the knowledge on diabetes and obesity among school going adolescents in Chennai, south India.

Methods Study participants were 3505 adolescents belonging to sixth and seventh grades of select private and government schools in Chennai. A school-based intervention consisting of five classroom activities were delivered to the intervention group participants. Teachers and peer leaders from intervention schools played a key role in program delivery. The control group received a one-time standard training program on diabesity. A self-administered questionnaire was used for the assessment of knowledge levels at baseline and follow-up. Overall, 3,263 students completed the program (response rate 93.1%).

Results Mean age of the study participants was 11.1±0.8 years. The intervention group had a significant improvement in knowledge score with the overall mean score improving from 11.51 at baseline to 12.25 at follow-up (p<0.001). The mean composite score was higher in boys, compared to girls in both intervention (12.38 vs 12.08) and control (11.30 vs 10.99) groups. Participants in the intervention group were 1.31 (95%CI: 1.12-1.52 p value: <0.001) times more likely to have a good knowledge score about diabesity, compared to the control group.

Conclusions School-based interventions are a successful way of reaching out to a large number of adolescents in order to increase awareness about diabetes and obesity.

Keywords Knowledge · Diabetes · Obesity · Adolescents · School-based interventionOriginal Article

Introduction

Obesity and diabetes have become global burdens on our healthcare systems. According to the 2017 statistics, India has the 2nd largest number of children and adolescents living with diabetes in the world with about 11,800 newly diagnosed cases being added every year [1]. In a study conducted among children and adolescents in Chennai, the prevalence of overweight or obesity was found to be 20.7% in boys and 21.3% in girls according to Khadilkar's criteria [2]. The term 'diabesity' is often used to refer to the occurrence of diabetes and obesity in the same individual [3–5].

Increasing availability of energy-dense, high-fat, high-carbohydrate foods and sedentary lifestyles are two of the most important factors influencing childhood obesity. This in turn leads to type-2 diabetes, hypertension, polycystic ovary syndrome, and cardiovascular disease later in adulthood [6]. Poor knowledge about a healthy lifestyle could be one of the important factors contributing to the development of childhood obesity in Indian school children [7]. School children lack awareness about obesity and diabetes and its causes [8]. Therefore, raising awareness about these conditions should become a priority in India.

The Centers for Disease Control and Prevention (CDC) recommends that more knowledge regarding nutrition and nutritional-related disease should be incorporated into school curricula as a method of effectively encouraging

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lifestyle changes as this has been shown to reduce the risk of type 2 diabetes and cardiovascular disease [9, 10]. According to the World Health Organization (WHO), schools should form the basis for implementation of healthy lifestyle habits in children as part of obesity prevention programs [11]. Schools and the media are the two most common sources of knowledge for children and adolescents, and by utilising school-based educational interventions, there have been improvements in knowledge about nutrition and health [12]. However, there are only a few childhood obesity intervention programs implemented in India [13–17].

Indeed, systematic reviews of multi-model interventions show that most child- and adolescent-targeted programmes were effective in reducing body mass index (BMI) and sedentary lifestyle while increasing physical activity [18]. Improving knowledge about obesity can translate into adopting dietary changes and healthier lifestyles.

The present study is part of the Obesity Reduction and Awareness of Non-Communicable diseases through Group Education (ORANGE) school programme conducted in the year 2008–2011 where the prevalence of overweight/obesity was found to be higher in private schools (International Obesity Task Force (IOTF) criteria, 21.4%; Khadilkar criteria, 26.4%) and in the adolescent age group (IOTF, 18.1%; Khadilkar, 21.2%) [2]. Briefly, in phase I of the ORANGE school component, nearly 20,000 children and adolescents in the age group of 6–17 years were screened for overweight and obesity across 51 schools from Chennai, South India. The phase II of ORANGE is a school-based interventional program aimed at adolescents studying in the sixth and seventh grade from select private schools and government school in Chennai [19]. This intervention focused on health and nutrition education, led by both teachers and peers, allowing children and adolescents to take an active role in health education.

The aim of this paper was to evaluate (i) knowledge about diabetes and obesity among students in Chennai, (ii) changes in students' knowledge after a school-based intervention, and (iii) factors associated with intervention on the knowledge level given to adolescents aged 10 to 13 years from 6th and 7th grades in select government and private schools in Chennai city in South India.

Materials and methods

Participants

Study participants belonging to the sixth and seventh grades were recruited from private and government schools by purposive sampling. The private schools had children from middle to upper socioeconomic class families, while the government school children were from lower socio-economic backgrounds. Ten schools (7 private and 3 government) were chosen to be part of the intervention group, while 7 schools (4 private and 3 government) were chosen as control group schools. The schools chosen were a representative sample of school-going adolescents from all socioeconomic strata within the 15 zones of Chennai corporation limits. Private schools had an average of 4 sections with an average of 118 students in each grade, while government schools had an average of 2 sections with an average of 76 students in each grade.

Figure 1 describes the recruitment and retention of participants. There were a total of 3505 participants, 2591 from private schools and 914 participants from government schools. Out of the 3505 participants, 2345 belonged to the intervention group and 1160 belonged to control group. Overall, 3263 students completed the programme (response rate 93.1%). The 6.9% 'non-response' was due to incomplete filling in of questionnaires or due to dropouts. The reasons for dropouts were because of prolonged absence of the participants from school and few others having obtained a transfer certificate (TC) to another school during the academic year.

Intervention

Intervention strategies rely on the key factors influencing a given behaviour, which can be identified through a range of models. If only a single model is to be adopted, it must first be differentiated to make it specific to the audience groups in question, as well as to the target behaviour. Since our intervention was multifactorial, we have used a combination of the Health Belief and Social Ecological Models as we believe health behaviours are shaped through a complex interplay of determinants at different levels. For example, physical activity is influenced by self-efficacy at the individual level, social support from family and friends at the interpersonal level, and perceptions of crime and safety at the community level. Ecological models suggest that these multiple levels interact with each other, whereas the health belief model suggests that people's beliefs about whether or not they are at risk for disease and their perceptions of the advantages of taking action to avoid the disease actually influence their readiness to take action [20].

The intervention was composed of five classroom activities delivered at monthly intervals in each school. After an initial meeting with the heads of all the selected schools, teachers and peer leaders from each school were

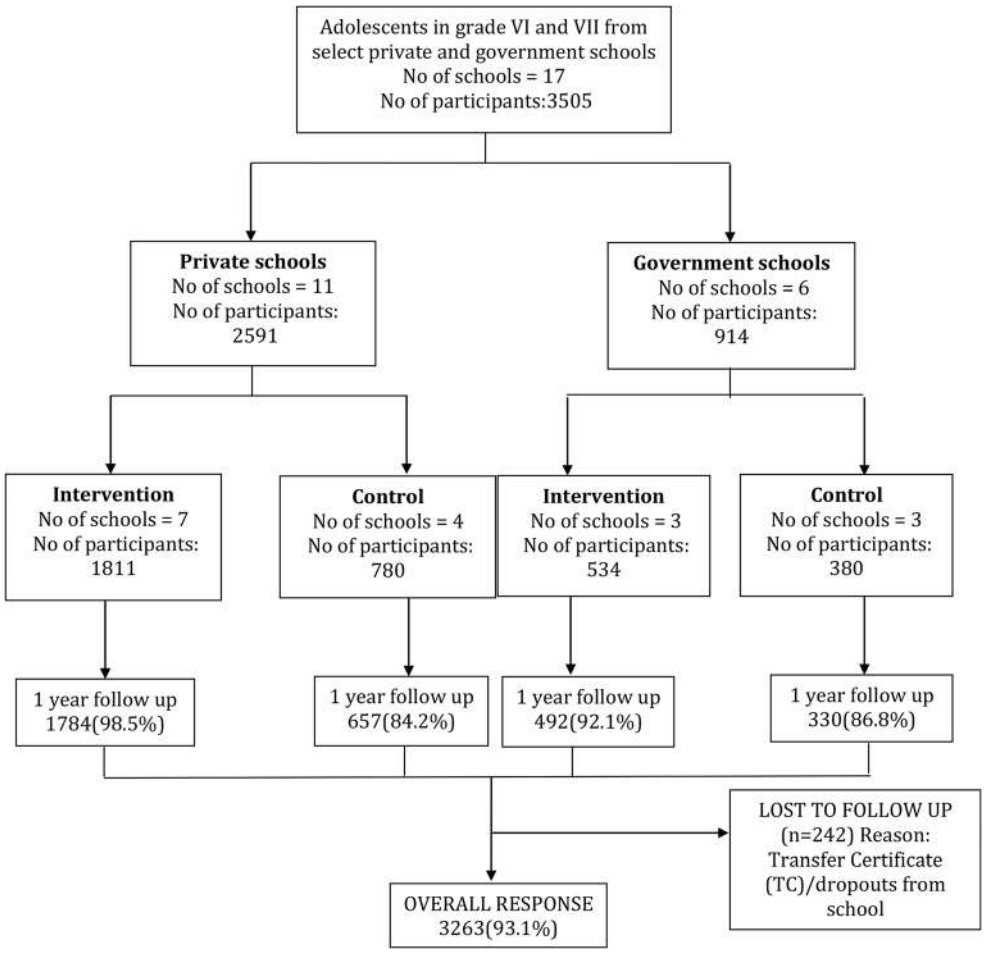

Fig. 1 Flow diagram of recruitment and retention of participants

trained in the intervention activities by the study team. One peer leader from each class was chosen by a teacher representative based on the leadership qualities and their cordiality with other class students. The teachers and peer leaders from intervention schools, were then invited for a day's training programme at the Madras Diabetes Research Foundation (MDRF) auditorium, who were coached to lead the intervention activities in their respective schools.

Each activity engaged students with a certain health topic of interest and was composed of two parts:

1) Active learning component: Teachers provided students with background information about the health topic through classroom discussions.
2) Application component: Peer leaders facilitated small-group and individual activities. Worksheets were collected from these activities and used in our analysis.

The peer leaders initiated the group activities in the classroom while the teachers facilitated discussion on the health topic. The education manuals developed for the teachers and peer leaders were culturally adapted and were readily available in English and Tamil for easier interpretation in private and government schools. Briefly, the activities involved diabetes awareness and prevention through fun-based learning and answering to creative worksheets after each activity. The topics covered under each activity were the following: (i) identification of healthy and unhealthy habits from a cartoon, (ii) climbing the health ladder through snakes and ladders game, (iii) designing healthy recipes by careful selection of ingredients and method of cooking, (iv) learning about physical activity using jumping race with backpacks, and (v) a case study regarding the lifestyle habits of an 11-year-old boy. Details on the intervention activities provided have been published previously [14]. The schools in the control group received a one-time standard training program on obesity and diabetes. These schools were not included in any specific activities which required teacher and peer leader involvement.

Knowledge questionnaire

A self-administered questionnaire, comprising of 17 questions was developed (Annexure 1). The questionnaire was first pilot tested and modified before starting the main study based on the feedback obtained from participants. Test-retest reliability was done to determine the stability of a tool in different situations and over a period of time [21]. This was done by randomly selecting 30 participants from private ($n = 15$) and government ($n = 15$) schools to complete the questionnaire twice, within a gap of 2 weeks. Internal consistency of the tool was measured using Cronbach's alpha = 0.77 (95% Confidence Interval, 0.71 to 0.83). The knowledge of students on diabesity was assessed using the same questionnaire before and after the intervention period. The change in knowledge was measured as percentage difference between pre- and post-test results.

Assessment of knowledge scores

A total of 17 questions were included in the assessment of knowledge level on diabesity. The questions were divided into 5 main sections, including knowledge on diet (2 questions), physical activity (4 questions), facts on diabesity (4 questions), risk factors for diabetes (5 questions) and diabetes complications and prevention (2 questions). The answer to each question had a scoring of 1 for the correct answers and was scored 0 for incorrect answers inclusive of 'not sure' option. The maximum possible score was 17 points. An improvement in knowledge scores from baseline to follow-up indicated a change in the knowledge level which was used to analyse the effectiveness of the intervention in school students.

Sociodemographic characteristics

Data was collected using a structured and pre-tested questionnaire which included details on demography, general awareness, non-communicable disease (NCD) awareness, physical activity and dietary patterns. However, only the demographic characteristics and awareness questions have been included in this paper.

Data analysis

Data from pre- and post-questionnaires was entered in IBM SPSS Statistics version 23 for analysis. The general characteristics of adolescents in intervention arm were compared with those in control arm. Data were summarised as mean–standard deviation for continuous variables and frequency (percentages) for categorical variables and data was compared using independent t test for continuous variables and chi-square test for categorical variables. The knowledge assessment scores between intervention and control group and also between private and government schools were compared using chi-square tests. Binary logistic regression analysis was done to see the independent effect of predictors on the dependent variables. Using group as dependent variable and change in knowledge level as an independent variable, the odds ratio and 95% confidence interval (CI) were calculated and $p < 0.001$ was considered statistically significant.

Results

Demographic characteristics

The mean age of the participants was 11.1 ± 0.8 years with mean body mass index (BMI) of 17.5 ± 3.7 kg/m^2 in boys and 17.9 ± 3.7 kg/m^2 in girls. There were more boys than girls in both the intervention (1294 boys and 982 girls) and control (664 boys and 323 girls) groups (Table 1).

Knowledge assessment in the two groups

Table 2 shows the change in knowledge in the intervention and control groups compared to baseline and follow-up. The knowledge score for facts regarding diabesity was found to have significantly improved from 2.62 at baseline to 2.92 at follow-up in the intervention group. There was also a significant improvement in the intervention group on knowledge regarding physical activity and risk factors for diabetes from 3.33 at baseline to 3.52 at follow-up and from 2.53 at baseline to 2.70 at follow-up respectively ($p < 0.001$). Knowledge regarding complications and prevention also significantly improved from 1.29 at baseline to 1.33 at follow-up ($p < 0.05$).

In the control group, the knowledge on physical activity had shown improvement from 3.15 at baseline to 3.39 at follow-up ($p < 0.001$). There was, however, a decline in the mean scores from 2.44 to 2.41 at baseline and follow-up for knowledge on risk factors of diabetes and from 1.13 to 1.09 for knowledge on complications and prevention in the control group. Overall, the intervention group had higher improvement in knowledge scores from 11.51 at baseline to 12.25 at follow-up ($p < 0.001$).

Mean composite score of diabesity (diabetes and obesity)

Table 3 represents the mean composite score of diabesity in the intervention and control group. The mean composite score

Table 1 General characteristics of participants at baseline

Variable	Control group ($n = 987$)	Intervention group ($n = 2276$)	p value
Age (years)	11.2 ± 0.9	11.1 ± 0.9	< 0.001
Height (cm)	143.0 ± 9.2	144.0 ± 8.2	0.003
Weight (kg)	36.6 ± 10.4	37.1 ± 10.3	0.212
Body mass index (kg/m^2)	17.6 ± 3.8	17.7 ± 3.6	0.651
Waist circumference (cm)	62.9 ± 11.0	62.6 ± 10.5	0.531
Blood pressure (mmHg)			
Systolic	112 ± 12	112 ± 23	0.947
Diastolic	66 ± 10	65 ± 10	0.002
Gender			
Boy	664 (67.3)	1294 (56.9)	< 0.001
Girl	323 (32.7)	982 (43.1)	< 0.001
Grade			
VI	461 (46.7)	1158 (50.9)	0.029
VII	526 (53.3)	1118 (49.1)	0.029

was found to be higher in boys than the girls in both intervention (12.38 vs 12.08) and control (11.30 vs 10.99) groups. The private schools (12.40 in intervention and 11.20 in control) outperformed the government schools (11.73 in intervention and 11.18 in control).

Factors associated with knowledge score

Binomial regression analysis was performed using group as the dependent variable and knowledge as the independent variable (Table 4). When compared to control group, those in the intervention group were 1.31 times more likely to have a good knowledge score regarding diabesity (95% CI, 1.12–1.52; p value < 0.001). When adjusted for confounding factors like class, gender and school type, the odds ratio was 1.32 (95% CI, 1.13–1.53; p value < 0.001).

Discussion

We have shown that a simple intervention carried out in schools in Chennai significantly improved the knowledge of students on the prevention and lifestyle management of diabetes and obesity. The salient findings from the study are the following: (i) The questions on risk factors for diabetes and its complications and prevention had a significant improvement in the intervention group while the control group had a decline in scores. (ii) The participants from private school and VI grade students from the intervention group performed better when compared to the control group. (iii) Using peer leaders and teachers as role models helps in the effective implementation of such intervention programs and also gives hope for sustainability.

Children need adequate knowledge to change their food habits and make healthy choices with the help of

Table 2 Changes in knowledge scores in control and intervention group

Knowledge questions	Max score	Control ($n = 987$)				Intervention ($n = 2276$)				p value*
		Baseline	Follow-up	Diff	p value (paired)	Baseline	Follow-up	Diff	p value (paired)	
Knowledge on diet	2	1.69	1.70	0.01	0.544	1.74	1.78	0.04	0.002	0.292
Knowledge on physical activity	4	3.15	3.39	0.24	< 0.001	3.33	3.52	0.19	< 0.001	0.153
Facts on diabesity	4	2.47	2.60	0.13	0.003	2.62	2.92	0.30	< 0.001	< 0.001
Risk factors for diabetes	5	2.44	2.41	− 0.03	0.565	2.53	2.70	0.17	< 0.001	< 0.001
Diabetes complications and prevention	2	1.13	1.09	− 0.04	0.167	1.29	1.33	0.04	0.039	0.025
Total score	17	10.88	11.20	0.32	< 0.001	11.51	12.25	0.74	< 0.001	< 0.001

*Comparison of difference between control and intervention group

Table 3 Mean composite score of diabesity

	Control			Intervention			*p* value*
	Baseline	Follow-up	*p* value	Baseline	Follow-up	*p* value	
Gender							
Boy	10.95 ± 2.67	11.30 ± 2.68	0.003	11.62 ± 2.76	12.38 ± 2.81	< 0.001	0.003
Girl	10.72 ± 3.06	10.99 ± 2.63	0.111	11.38 ± 2.83	12.08 ± 2.87	< 0.001	0.020
School type							
Private	10.89 ± 2.88	11.20 ± 2.75	0.008	11.67 ± 2.84	12.40 ± 2.84	< 0.001	0.002
Government	10.85 ± 2.66	11.18 ± 2.49	0.041	10.94 ± 2.54	11.73 ± 2.76	< 0.001	0.034
Grade							
VI	10.49 ± 2.92	10.89 ± 2.71	0.004	11.28 ± 2.78	12.25 ± 2.80	< 0.001	0.001
VII	11.22 ± 2.65	11.46 ± 2.60	0.056	11.75 ± 2.79	12.25 ± 2.88	< 0.001	0.090
Total	10.88 ± 2.80	11.20 ± 2.67	0.001	11.51 ± 2.79	12.25 ± 2.84	< 0.001	< 0.001

*Comparison of difference between control and intervention group

their teachers and parents who can strongly influence their behavioral and lifestyle patterns [22, 23]. In a study conducted at Kannur, Kerala, the need for a healthy lifestyle training in school children was emphasised as there was poor awareness on risk factors for NCDs among them [8]. Another study done at Sri Lanka indicated that there was poor knowledge regarding diabetes and other non-communicable diseases among adolescents in the age group of 13–17 years [24].

Notably, majority of the participants in both the intervention and control groups agreed with the statement that regular exercise keeps a person fit and healthy. This clearly shows that students attending regular formal physical education classes at school contributed to better knowledge on benefits of physical activity among them. According to Xu et al., knowledge regarding physical activity can bring about a positive behavioral change in adolescents [25]. Physical activity contributes to efficient glucose disposal and also prevents obesity in adolescents by increased energy expenditure [26].

This intervention program conducted in schools is similar to the computer-based 'Gustavo' web game programme designed to prevent diabesity among school-going adolescents in Italy [22]. There was a significant improvement in nutritional knowledge and dietary habits in these participants which is consistent with our findings too. In a study conducted mong Iranian adolescents aged 13–19 years, there were misconceptions noted about diabetes [27]. Such misconceptions were also common among Indian adults [28]. These misconceptions were noted in our study at baseline but then cleared to a large extent after the intervention, showing the benefit of the program. Through the baseline results, it is evident that there is clear-cut neglect of nutrition education in Indian schools which improved after the enthusiastic involvement of participants in the nutrition intervention which can in turn positively impact the food choices among adolescents.

The risk factors for diabetes along with its complications, as well as their prevention, had a higher score in the intervention group which is similar to a study done among secondary school students in Muscat, Oman. These results are promising in adopting a healthy lifestyle and also improve the quality of life in adolescents. About 99% of the

Table 4 Effect of intervention on knowledge level

Variables	Odds ratio (95% CI)		*p* value
	Control	Intervention	
Model 1: Knowledge level (Unadjusted)	1.00	1.31 (1.12–1.52)	< 0.001
Model 2: Knowledge level adjusted for grade	1.00	1.30 (1.12–1.51)	< 0.001
Knowledge level adjusted for grade and gender	1.00	1.31 (1.13–1.53)	< 0.001
Knowledge level adjusted for grade, gender and school type	1.00	1.32 (1.13–1.54)	< 0.001

students in this study were able to correctly identify at least one risk factor for diabetes and majority of the participants had a good knowledge on complications and prevention [29]. In a similar school-based intervention done in Delhi, Bassi et al. reported a significant improvement in knowledge regarding the risk factors of diabetes [30].

Findings from the MARG study revealed that the knowledge scores improved significantly in both the government and private schools [13]. Another study done at Lagos state revealed that the overall diabetes knowledge among adolescents in a government school was only 46% [31]. The findings in the current study showing better results in those belonging to the intervention group and in the private schools have also been shown by a previous study done in Delhi [30]. This could be attributed to the fact that the support system and monitoring were better in private schools when compared to the government schools and also the pilot school intervention proved to be successful in delivering an improved knowledge outcome.

Overall, the mean score of the intervention group improved indicating positive changes in the awareness levels. Also, peer-led intervention proved to be an attractive strategy for teaching health behaviors and improving health outcomes in adolescents. This shows that if knowledge regarding prevention of NCDs introduced early in schools, the results would be good. They also provide a cost-effective model of successful behavior change for the adolescent population which is at a high risk of developing diabetes and other obesity-related chronic diseases [32].

The strength of the study lies in the implementation of the programme through teachers and peer leaders who were effective mentors in delivering the intervention. The curriculum developed as part of the intervention was already pilot tested in Delhi schools [30], which made it easier for its implementation in Chennai schools. The uniqueness of the program lies in the way the intervention messages were delivered using pictures, games, developing healthy recipes, and a case study. The addition of a control group to assess the effectiveness of the implementation was another strength.

However, the study also has some limitations. The study design was a non-randomised controlled trial using a purposive design as this was primarily planned as a pilot study to evaluate the effectiveness of the intervention. The sample size was not evenly distributed in private and government schools. This could have resulted in some inherent bias in the type of school group comparisons which was mainly due to poor enrolment and high rates of dropout among government school students. The uneven distribution of school students in the intervention and control groups could have resulted in allocation bias and imbalance of confounding factors between the two groups. Also, some of the teachers lacked motivation in the implementation of the study due to their workload at schools which led the research team to intervene to run the program. Finally, there was no long-term follow-up evaluation done after the follow-up testing for the participants to assess the long-term retention of improved awareness levels. Nor was the effect of the improved knowledge tested with respect to retention of body weights or increased physical activity levels.

School-based interventions are a successful way of reaching out to a large number of adolescents. As adolescents spend most of their time in schools, more such programs could be implemented resulting in creating a healthier school environment. Also, as adolescents get easily influenced by their teachers, they could act as role models and may aid in encouraging students to implement healthy eating habits and improve physical activity through these intervention programs. We believe that the result with respect to increasing awareness about obesity is an important first step in bringing about behavioral change to prevent obesity and type 2 diabetes. The intervention can be easily scaled up to a larger number of schools throughout the country and these help in the primary prevention of NCD's like diabetes.

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Author's contributions T.S.M. analysed the data, wrote the first draft and carried out corrections in all the consecutive drafts of the manuscript. H.R conceived and coordinated the study and helped in data analyses and revisions of the manuscript. RMA and VM contributed to critical revisions of the manuscript for intellectual content. T.S.M., N.J. and D.P performed the data collection. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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

Informed consent Written informed consent from class teachers and assent from study participants were obtained prior to the study. This study was approved by the Institutional Ethics Committee of the Madras Diabetes Research Foundation (MDRF) with Registration no. ECR/194/Inst./TN/2013 with Clinical Trials Registry-India (CTRI) ID number CTRI/2016/09/007323.

ANNEXURE-1

A. GENERAL AWARENESS

QA1. Please *TICK* the option for each question, that you find correct.

S.No	QUESTIONS	YES	NO	NOT SURE
A.	It is essential to have a balanced diet (choosing a wide variety of foods from all the food groups like cereals, pulses, milk products, meat products, vegetables, fruits, etc.)			
B.	Children who miss breakfast (or do not eat breakfast) may get tired easily and therefore not be able to focus on studies			
C.	Being active and doing regular exercise keeps a person fit and healthy			
D.	Dancing, climbing stairs, doing household chores (like cleaning the house, gardening, etc.) are all forms of physical exercise			
E.	All types of physical activities (cycling, climbing stairs, dancing, cleaning the house, exercise, yoga, etc.) are beneficial (helpful) to you			
F.	Only overweight people should exercise			
G.	Eating too much of sugars/sweets causes diabetes			
H.	Diabetes affects only adults			
I.	Unhealthy eating habits can put one at risk for diabetes			
J.	Being overweight or obese in the present are not related to getting diabetes in the future			
K.	Being physically inactive or not doing regular exercise can put one at risk of diabetes			
L.	Having a family history of diabetes increases the chances of getting diabetes			
M.	People who use tobacco, have a higher risk of getting diabetes as compared to people who do not use tobacco			
N.	Exercise and healthy eating can prevent diabetes			
O.	People with diabetes are more likely to develop heart disease, stroke, kidney and eye problems			

B. NCD AWARENESS

QB1. What is obesity/overweight?

A	Too much body fat
B	Too little body fat
C	A cold or cough
D	A stomach ache
E	I do not know

QB2. What is Diabetes?

A	Stomach pain
B	Cold or cough
C	High blood sugar
D	Headache
E	I do not know

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ORIGINAL ARTICLE

Administration of coenzyme Q10 to a diabetic rat model: changes in biochemical, antioxidant, and histopathological indicators

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Abstract

Background Diabetes mellitus is a metabolic disorder caused by impaired glucose metabolism. Coenzyme Q10 is an endogenous vitamin with significant antioxidant properties.

Aims and objective The aim of our study is to investigate the protective effect of coenzyme Q10 against streptozotocin-induced diabetic rats.

Materials and methods Five groups of rats were used as follows: normal control (given normal saline), diabetic control (STZ 50 mg/kg b.w., i.p.), coenzyme Q10-treated diabetic rats (10 mg/kg b.w.), glibenclamide-treated diabetic rats (0.6 mg/kg b.w.) as standard group, and drug alone-treated group (coenzyme Q10 10 mg/kg b.w.). The rats were sacrificed after the study duration of 30 days. Biochemical and antioxidant parameters and histopathological evaluation were carried out in experimental rats.

Results and discussion The diabetic control group showed significant alterations in biochemical and histological parameters. Coenzyme Q10 was able to bring back the altered parameters to normal levels which were similar to that of the glibenclamide-treated group.

Conclusion Coenzyme Q10 could, therefore, be used as an adjunct in the management of diabetes.

Keywords Streptozotocin · Coenzyme Q10 · Diabetes mellitus · Antioxidant · Glibenclamide

Introduction

Streptozotocin (STZ) is a common agent for diabetes. It is derived from *Streptomyces achromogenes*. It induces type 1 diabetes, oxidative stress, and hyperglycemia. β Cell of the pancreas is destroyed by STZ. These induce DNA strand break and methylation in pancreatic islet cell. Several herbal drugs have been recognized to have clinical properties such as antibacterial, antiinflammatory, antiallergic, and antiviral activity [1]. There is no effective therapy in modern medicine for diabetes mellitus (DM), which results due to multiple factors that may be environmental, hereditary, and/or abnormal insulin secretion. This may affect various metabolisms like carbohydrates, protein, fat, and also damaging the β cells of the pancreas, kidney, and liver [2]. Diabetic nephropathy is the major cause of death in type 1 DM [3, 4], whereas liver disease is the major cause of death in type 2 DM [5]. Type 2 DM is a common disorder in developed countries which has affected 190 million people globally [6]. DM is due to high blood glucose level and abnormal metabolism in carbohydrate, protein, and fat which cause damages to many organs [7]. Long-term complications of DM can be grouped into vascular and non-vascular complications [8]. The vascular complications involve microvascular retinopathy, neuropathy, nephropathy, and macrovascular complications like coronary disease, cerebrovascular disease, and peripheral vascular disease [9]. Non-vascular complications involve sexual dysfunction, gastroparesis, and skin changes [8]. DM is a chronic disease associated with a reactive oxygen species (ROS) [10–12]. World Health Organization (WHO) has estimated that DM patients would double in number in 2025; it would rise from 150 million to 300 million [13]. Coenzyme Q10 is a vitamin-like substance which is found in every cell of the body. Coenzyme Q10 is endogenously synthesized antioxidant. It is a component of oxidative phosphorylation in mitochondria which produces ATP from the energy of carbohydrate and fatty acids. Coenzyme Q10 is rich in antioxidant

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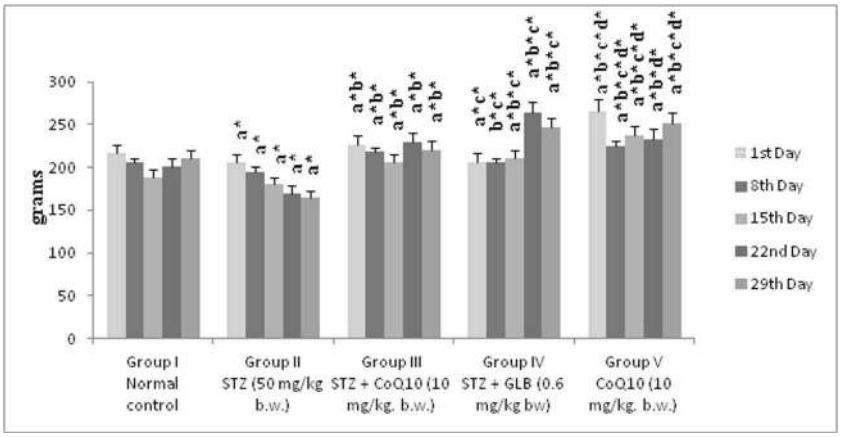

Fig. 1 Effect of CoQ10 on body weight of STZ-induced rats. Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

activity which is a potential lipophilic compound with long polyisoprene tail that is known to regenerate and recycle other antioxidants like tocopherol and ascorbate. It is known to degrade the free radical by suppressing the lipid peroxidation; preventing the injuries to DNA; and blocking oxidative injuries to protein, lipids, and all other compound essential for antioxidant. It is a cofactor that has the potential role in ATP production and mitochondrial respiratory chain [14]. It is also known as ubiquinone 50 that is known to have a crucial role in the functioning of heart muscles and muscle tissues. It is mainly produced in the body which decreases due to aging, cancer, and drugs [15].The aim of the research is to study the effectiveness of coenzyme Q10 in STZ-induced diabetes in rats. In this present scenario, scientist and pharmaceutical industries are focusing of herbal plants to discover a curative drug for DM.

General experimental procedures

Chemicals

Synthetic coenzyme Q10 and streptozotocin were purchased from Sigma-Aldrich, India, and the standard hepatoprotective drug silymarin from Quality Pharmaceuticals Ltd., India. Streptozotocin was dissolved in citrate buffer while silymarin was dissolved in sterile distilled water. Coenzyme Q10 was dissolved in 0.2 mL corn oil. All the other chemicals and reagents used in the current study were of analytical grade procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India.

Animals and treatment

Healthy female Wistar albino rats were purchased from Animal house, VIT University, Vellore, Tamil Nadu, India. The mean body weight of the rats ranged between 200 and 250 g. The rats were freely provided with water and lab rodent diet feed which was obtained from Hindustan Lever Ltd. (Mumbai, India). They were grown in a controlled temperature of 12 h light/dark cycle. The experiment was carried out under the guidelines of CPCSEA, and the ethical clearance number is VIT/IAEC/11th/October 10th/No. 26.

Experimental design

DM was induced to the rat by intraperitoneal injection of STZ after overnight fasting. The presence of diabetes was

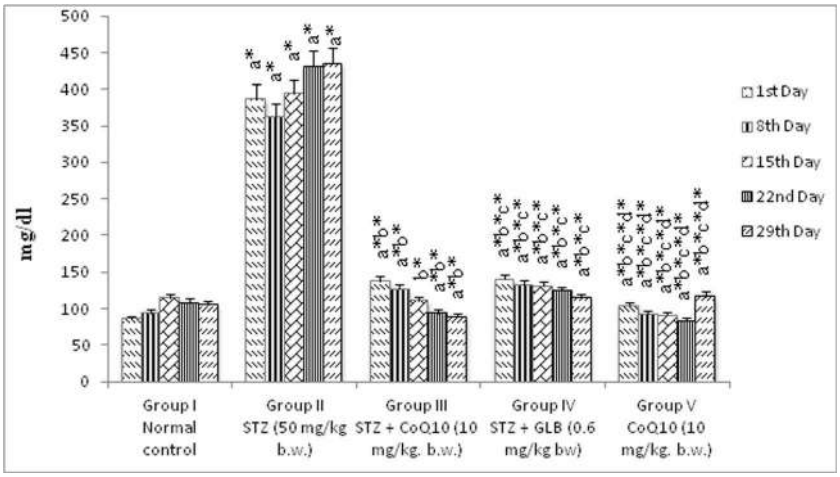

Fig. 2 Effect of CoQ10 on glucose of STZ-induced rats. Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

Table 1 Effect of CoQ10 on serum renal function markers of STZ-induced rats

Serum parameters	Group 1 Normal	Group 2 STZ (50 mg/kg b.w.)	Group 3 STZ + COQ10 (10 mg/kg. b.w.)	Group 4 STZ + GLB (0.6 mg/kg b.w.)	Group 5 COQ10 (10 mg/kg b.w.)
Urea (mg/dL)	25.11 ± 1.47	42.27 ± 1.19a*	30.55 ± 1.31a*b*	35.67 ± 1.37a*b*c*	26.57 ± 1.45b*c*d*
Creatinine (mg/dL)	0.600 ± 0.364	2.316 ± 0.289a*	0.548 ± 0.412b*	0.591 ± 0.335b*	0.484 ± 0.366b*
Uric acid (mg/dL)	0.748 ± 0.290	2.481 ± 0.300a*	0.450 ± 0.379b*	1.307 ± 0.288b*c*	0.567 ± 0.406b*d*

Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

*Statistical significance at $p < 0.05$

confirmed by measuring blood glucose after 24 h. Diabetes was induced to the rat by STZ (50 mg/kg b.w.). Glibenclamide (0.6 mg/kg b.w.) is used as the standard for this study [16]. The drug coenzyme Q10 (10 mg/kg b.w.) is used for studying its protective activities [17]. The rats were divided into five groups of six rats each. Group 1 includes normal control (given normal saline), group 2 includes diabetic control (STZ 50 mg/kg b.w., i.p.), group 3 includes coenzyme Q10 (10 mg/kg b.w.)–treated diabetic rats, group 4 includes glibenclamide (0.6 mg/kg b.w.)-treated diabetic rats as standard group, and group 5 includes drug alone–treated group (coenzyme Q10 10 mg/kg b.w.). This treatment was followed for a period of 28 days.

Assessment of body weight and blood glucose levels

The body weight and blood glucose levels were measured at regular intervals of 7 days. Blood glucose was measured using one-touch glucometer.

Serum sample preparation and tissue collections

At the end of the study, rats were killed by anesthesia (ketamine 60 mg/kg, xylazine 8 mg/kg, i.p.). Blood was collected from the trunk. The kidney, liver, and pancreas were dissected from the rat and washed free from blood in phosphate buffered saline solution. Later, they were stored in 10% formalin solution. The blood is collected in a centrifuge tube, and then, the serum is separated from the blood. The serum is used for analyzing various biochemical assays.

Renal enzyme markers and serum assays

The collected serum was used in analyzing the renal function markers like urea, creatinine, and uric acid and also to determine total cholesterol, high-density lipoprotein (HDL), triglyceride, HbA1C, and total protein levels [18]. Commercially available kits were used to analyze these parameters.

Histological and antioxidant analysis

Histological analysis of the liver, kidney, and pancreas in the study animals was carried out by routine histological methods. Portions of liver and kidney were homogenized in PBS, and the antioxidant parameters such as thiobarbituric acid reactive substances (TBARS) [19], activities of superoxide dismutase (SOD) [20], and catalase [21] were assayed and recorded.

Statistical analysis

The data obtained from each group were combined and its difference determined. They are statically expressed by mean ± SD. ANOVA was done. This was followed

Table 2 Effect of CoQ10 on serum total cholesterol, HDL, and triglyceride of STZ-induced rats

Serum parameters	Group 1 Normal	Group 2 STZ (50 mg/kg b.w.)	Group 3 STZ + COQ10 (10 mg/kg. b.w.)	Group 4 STZ + GLB (0.6 mg/kg b.w.)	Group 5 COQ10 (10 mg/kg. b.w.)
Total cholesterol (mg/dL)	78.63 ± 1.51	132.25 ± 1.74a*	90.37 ± 1.62a*b*	83.60 ± 1.42a*b*c*	81.70 ± 1.62b*c*
HDL (mg/dL)	30.55 ± 1.22	24.39 ± 1.34a*	29.42 ± 1.34a*b*	27.87 ± 1.57b*c*	31.65 ± 1.27b*c*d*
Triglyceride	63.94 ± 2.08	79.47 ± 1.29a*	69.99 ± 1.62a*b*	67.57 ± 1.43a*b*	61.64 ± 1.36b*c*d*

Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

*Statistical significance at $p < 0.05$

Table 3 Effect of CoQ10 on blood Hb A1C and serum total protein of STZ-induced rats

Blood parameters	Group 1 Normal	Group 2 STZ (50 mg/kg b.w.)	Group 3 STZ + COQ10 (10 mg/kg. b.w.)	Group 4 STZ + GLB (0.6 mg/kg b.w.)	Group 5 COQ10 (10 mg/kg. b.w.)
Hb A1C (%Hb)	4.50 ± 1.37	13.34 ± 1.49a*	6.59 ± 1.34b*	7.16 ± 1.44b*	5.45 ± 1.38b*
Total protein	7.05 ± 0.02	4.85 ± 0.08a*	6.44 ± 0.03a*b*	5.08 ± 0.05a*b*c*	7.54 ± 0.02a*b*c*d*

Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

*Statistical significance at $p < 0.05$

by Student Newman–Keul's test. $p < 0.05$ implied significance.

Results and discussion

Effect of CoQ10 on body weight of STZ-induced rats

The average body weight of the normal group was 200 g. The STZ-induced diabetic rats are observed to possess significant ($p < 0.05$) decrease in its body weight, which is gradually descending on each week (Fig. 1). CoQ10- and glibenclamide-treated diabetic rats are able to possess increase in the body weight. The CoQ10 alone group possessed the normal body weight.

Effect of CoQ10 on glucose level of STZ-induced rats

STZ-induced rats is observed to show a significant ($p < 0.05$) elevation in the level of glucose, which ranges from 350 to 450 mg/dL in the respective days (Fig. 2). The glucose level of group 1 ranges from 80 to 120 mg/dL. CoQ10 is observed to possess significant ($p < 0.05$) decrease in the glucose level of diabetic rats, which is descending respectively in the 1st day, 8th day, 15th day, 21st day, and 28th day. The recovering level of diabetes by CoQ10 is much greater than the recovering level of glibenclamide. The level of glucose in drug alone group is possessed to show normal level.

Effect of CoQ10 on renal enzyme markers of STZ-induced rats

Diabetic-induced group possessed a significant ($p < 0.05$) increase in urea, creatinine, and uric acid (Table 1). CoQ10-treated diabetic group was able to reduce significant ($p < 0.05$) level of the serum renal function markers, which is comparatively better than the glibenclamide-treated diabetic rats. CoQ10 alone–treated rats possessed the normal level of renal markers. Our result showed a significant ($p < 0.05$) elevation in the level of creatinine, urea, and uric acid (Table 1).

Effect of CoQ10 on serum assays of STZ-induced rats

Total cholesterol, triglyceride, and HbA1C possessed a significant ($p < 0.05$) elevation in the diabetic-induced group. HDL and total protein possessed decrease in the diabetic-induced group (Table 2 and Table 3). CoQ10- and glibenclamide-treated diabetic group were able to normalize the significant ($p < 0.05$) level of serum assay.

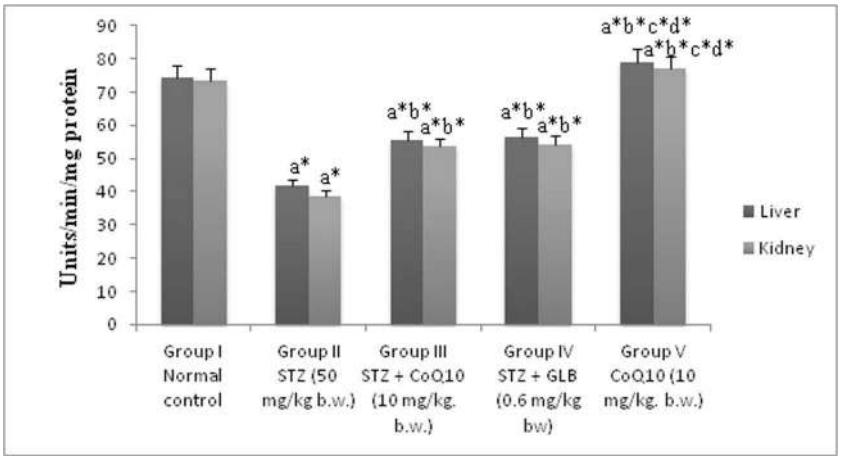

Fig. 3 Effect of CoQ10 on SOD in liver and kidney tissue homogenates of STZ-induced rats. Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. The symbols represent statistical significance at *$p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

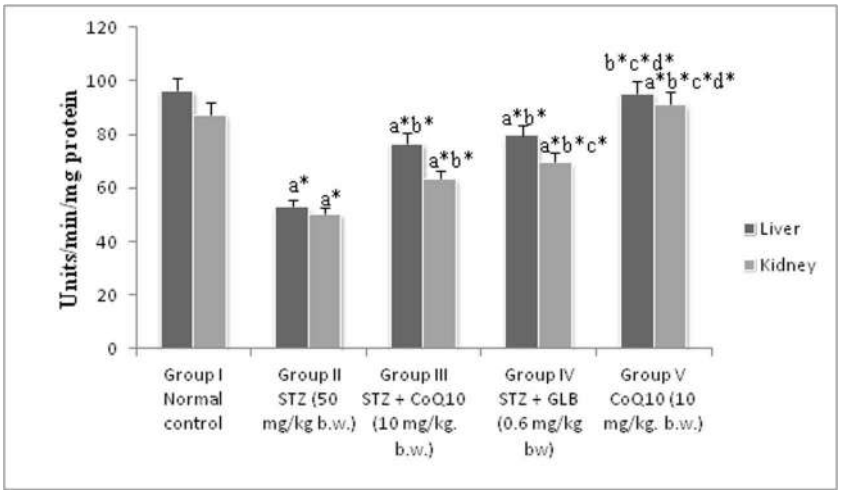

Fig. 4 Effect of CoQ10 on Catalase in liver and kidney tissue homogenates of STZ-induced rats. Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs groups 3, 4, 5; c—group 3 vs group 4, 5; d—group 4 vs group 5. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

Effect of CoQ10 on antioxidant of STZ-induced rats

The levels of antioxidants like SOD (Fig. 3), catalase (Fig. 4), and liver glycogen (Fig. 5) were measured to know the efficacy of CoQ10. Diabetic-induced group possessed significant ($p < 0.05$) decrease in the level of antioxidants. CoQ10 and glibenclamide were able to increase the levels significantly ($p < 0.05$) which are almost similar to the normal group. Group treated with CoQ10 alone has possessed normal antioxidant levels.

Effect of CoQ10 on liver histopathology of STZ-induced rats

Normal central vein and normal hepatocytes were observed in the group 1. Diabetic-induced rats were observed to possess periportal inflammation, periportal fatty infiltration, and pyknotic nuclei (Fig. 6).

Effect of CoQ10 on kidney histopathology of STZ-induced rats

Normal control group is observed to show normal glomerulus, which is also observed in the CoQ10 treatment group (Fig. 7), whereas diabetic rats possessed an inflammation in glomerulus with proteinuria, tubular damage, and spillage in Bowman 's capsule. Glibenclamide-treated diabetic group possessed normal glomerulus with mild inflammation and also mild proteinuria. Group treated with CoQ10 alone has showed normal tubules.

Effect of CoQ10 on pancreas histopathology of STZ-induced rats

STZ-induced diabetic rats were observed to possess damage in the islets (Fig. 8). The CoQ10-treated diabetic group possessed to recover the damaged islets, which is also similar in glibenclamide-treated group. Group treated with CoQ10 alone has shown normal islets.

Discussion

Our study reveals that the CoQ10 has effectiveness in recovering the STZ-induced rats. The complication in STZ-induced rats includes polydipsia (excess intake of water), hyperphagia (increased food intake), hyperglycemia, and severe weight loss [5]. Severe and continuous weight loss was observed in our study (Fig. 1).

Elevations in serum glucose, creatinine, urea, and uric acid are the major causes for diabetic nephropathy [22]. These elevations in creatinine and uric acid are due to the impaired function, and the significant ($p < 0.05$) elevation in urea is due to greater protein catabolism which shows the positive sign for

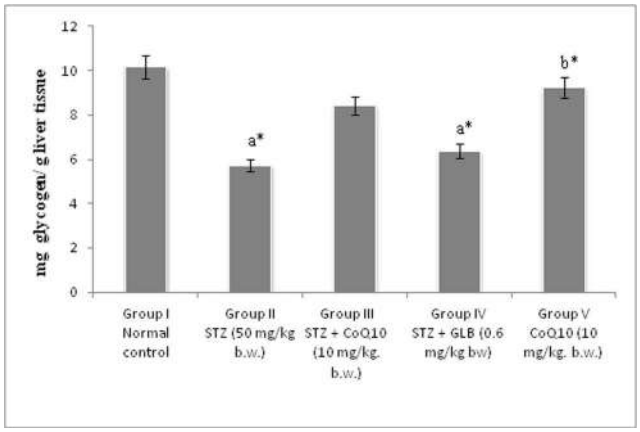

Fig. 5 Effect of CoQ10 on liver glycogen of STZ-induced rats. Each value represents the mean ± SD of six rats. Comparisons were made as follows: a—group 1 vs groups 2, 3, 4, 5; b—group 2 vs group 3, 4, 5; c—group 3 vs groups 4, 5; d—group 4 vs group 5. The symbols represent statistical significance at $*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test

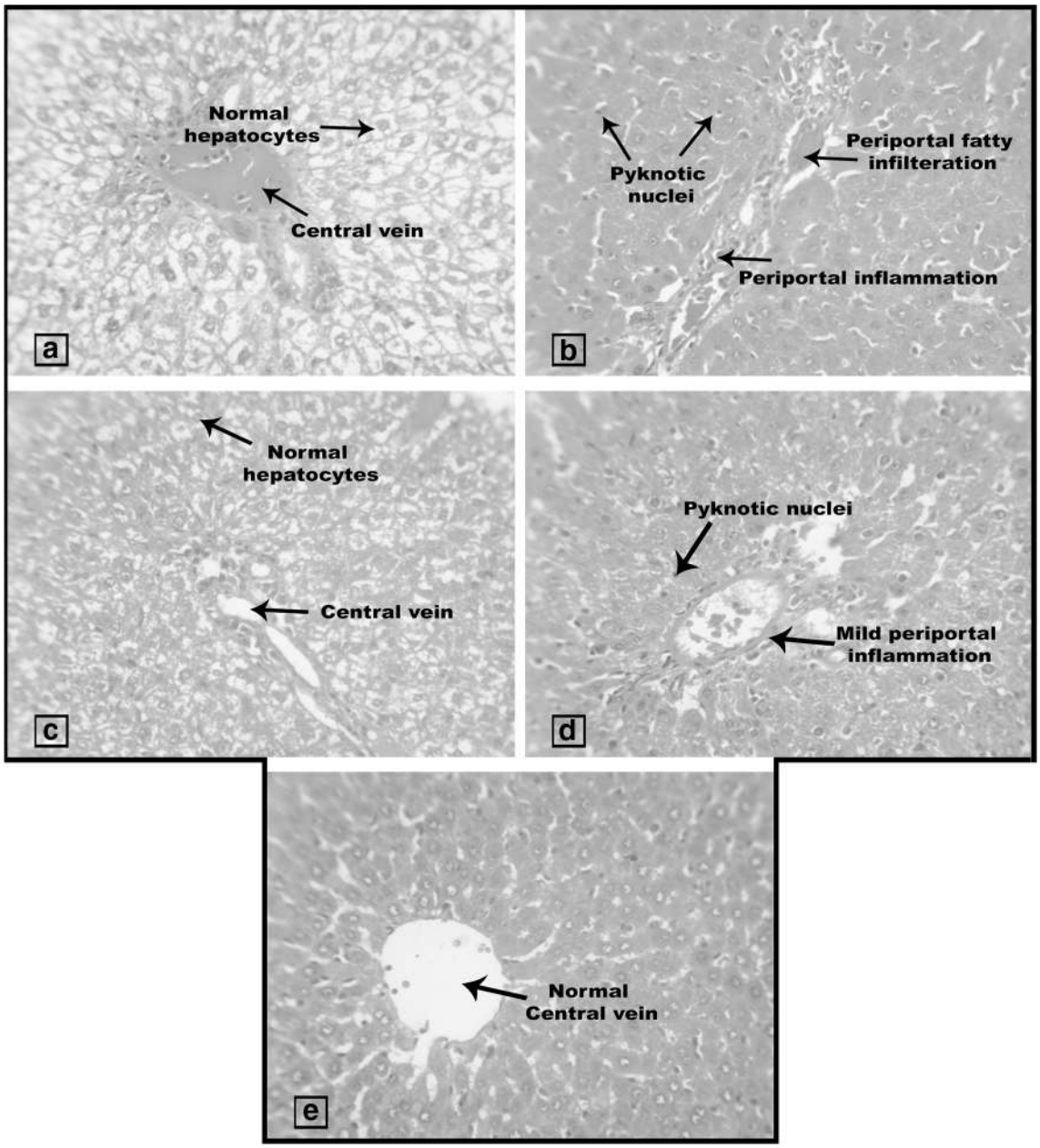

Fig. 6 Effect of CoQ10 on liver histopathology of STZ-induced rats. Liver histopathology (H&E staining). **a** Group 1 shows normal liver histology with normal central vein and normal hepatocytes. **b** Group 2 liver section shows periportal inflammation, periportal infiltration, and few pyknotic nuclei. The lobular architecture is maintained. **c** Group 3 shows normal central vein and normal hepatocyte morphology. **d** Group 4 shows some pyknotic nuclei and mild periportal inflammation. **e** Group 5 shows normal hepatocytes and central vein

hyperglycemia and nephropathy [23]. In our studies, CoQ10-treated diabetic rats were able to recover the renal marker enzyme due to the antioxidant property and its ability to regulate glycemia which possesses the better renal function [24–26]. The betterment of renal function is also due to the glycemic state by reduction of gluconeogenesis which reduces protein reduction [26]. STZ-induced rats also cause weight loss due to loss or degradation of the protein [13, 27].

In HbA1C, HDL and total protein CoQ10 are effective compared to glibenclamide, whereas in total, cholesterol and triglyceride glibenclamide possessed effectiveness compared to COQ10. In our studies, STZ-induced rats possessed a significant ($p < 0.05$) elevation in the level of total cholesterol, triglyceride, and decrease in the level of HDL (Table 2); this is due to dyslipidemia in untreated diabetic rats. This result was also observed in other studies on rat model [28].

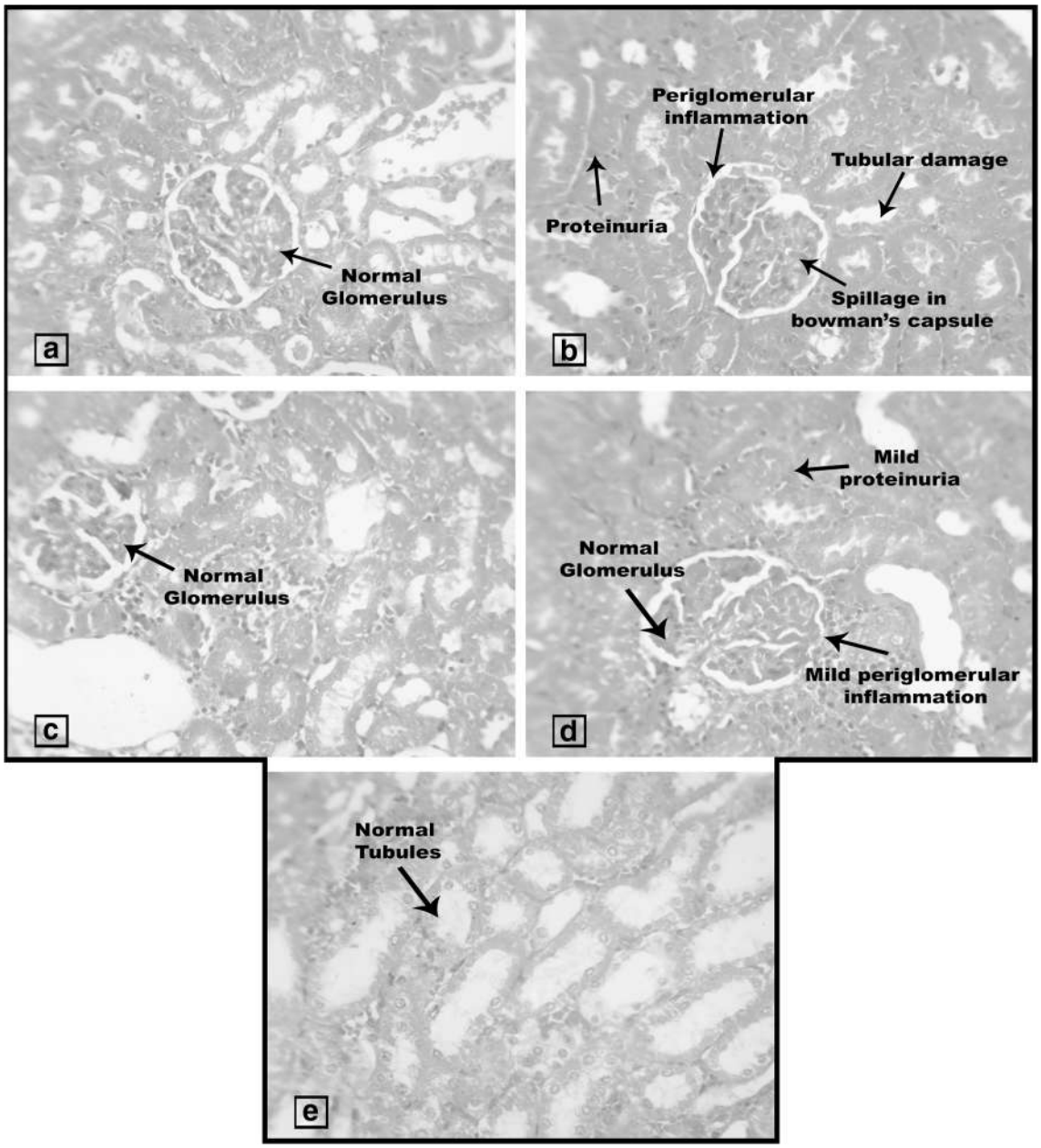

Fig. 7 Effect of CoQ10 on kidney histopathology of STZ-induced rats. Kidney histopathology (H&E staining). **a** Group 1 shows normal renal tissue morphology with normal glomerulus. **b** Group 2 shows tubular damage with proteinuria and periglomerular inflammation. **c** Group 3 shows normal renal tissue morphology. **d** Group 4 shows mild proteinuria and mild periglomerular inflammation. **e** Group 5 shows normal renal tissue morphology

SOD is used to eliminate the ROS by dismutation of superoxide radicals [29]. Excess formation of ROS is due to the depleted endogenous antioxidant; this will result in the reduction of SOD and the increase in lipid peroxidation [13]. Our studies showed a decrease in the level of SOD, catalase, and liver glycogen (Figs. 3, 4, and 5). The significant ($p < 0.05$) reduction in the level of antioxidant enzyme activity is due to the glycation of this enzyme, which occurs in elevated blood levels [13, 24, 25]. Decreases in the level of antioxidant level were also observed in various studies of STZ-induced rats [30]. The CoQ10-treated diabetic group possessed an increase in the level of antioxidant, which is due to the increased production of superoxide and H_2O_2 [31].

Diabetic group with the treatment of CoQ10 is possessed to normalize the changes caused by STZ. There is a mild periportal inflammation, and some pyknotic nuclei were observed in the standard group. CoQ10 alone

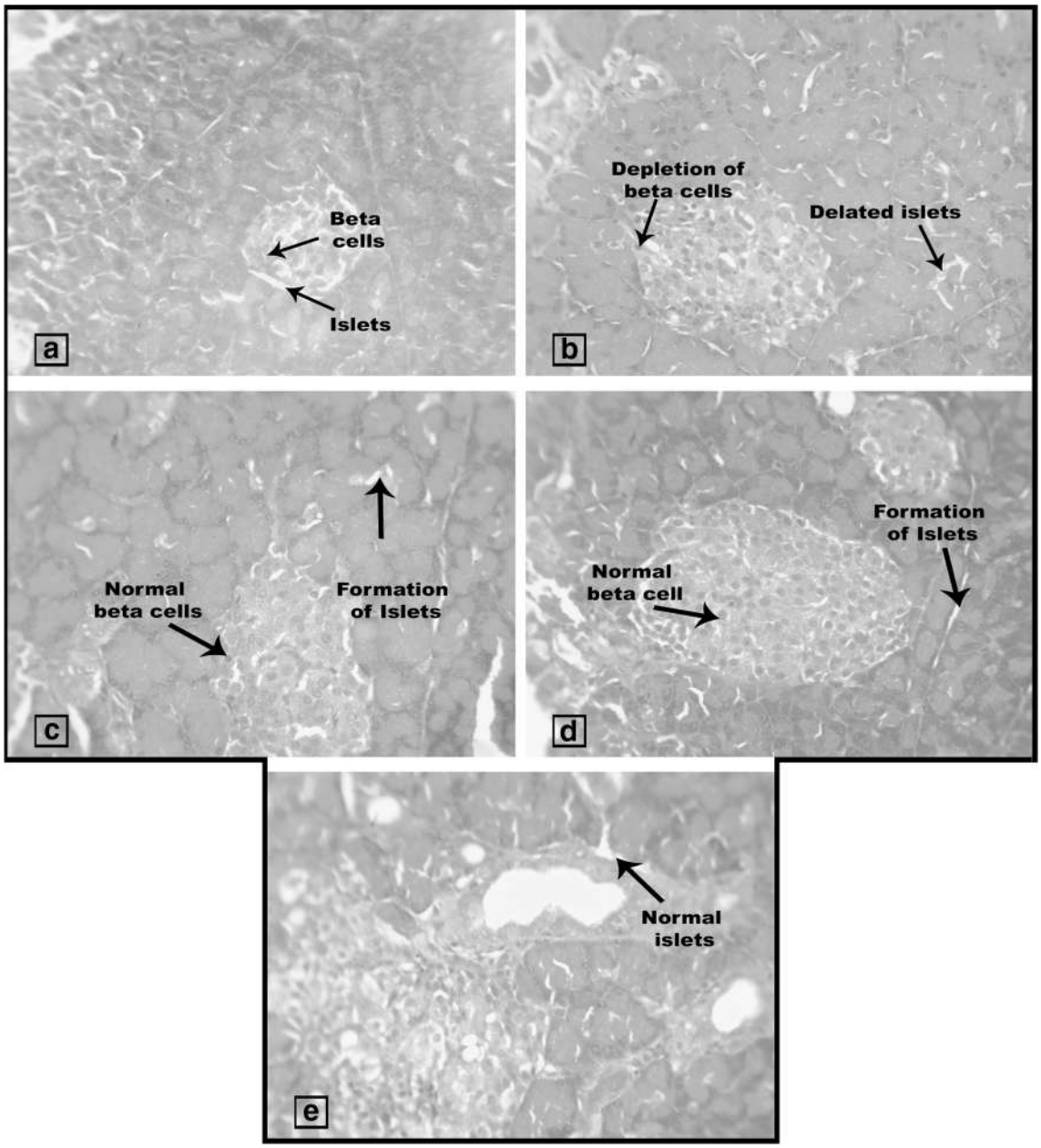

Fig. 8 Effect of CoQ10 on pancreas histopathology of STZ-induced rats. Pancreas histopathology (H&E staining). **a** Group 1 shows normal pancreas histology of beta cells and islets. **b** Group 2 damaged islets. **c** Group 3 shows the recovery of islets. **d** Group 4 shows the recovery of islets. **e** Group 5 shows normal islets. The lobular architecture is maintained

group has not shown any changes in its histopath. The alteration in kidney function would lead to proteinuria. Due to the glomerulus damages, kidney histopathology possessed tubular damages and Bowman's capsule in STZ-induced rats which are also observed in other studies [2]. The diabetic histopathology is known to cause the thickness in glomerulus basement membrane, which may lead to microalbuminuria, hyperfilteration, and increase in the extracellular matrix [32].

STZ is known to damage the pancreatic insulin secreting β cells. This also leads to the damages in the kidney [2, 7, 33]. This damage is able to be recovered by CoQ10 which is observed in histopath of the pancreas. Many herbal plants are reported to cause the increase in pancreatic β cells by regenerating the cells [34]. STZ-induced rats are known to cause increases in blood glucose level which is also known to cause depletion in pancreatic β cells [35–37].

Our study confirms the effectiveness of CoQ10, which is able to recover STZ-induced diabetic rats. CoQ10 has shown a beneficial effect when compared to the rat treated with the standard drug. CoQ10 alone–treated rats have not shown any adverse or side effect which demonstrates its potential efficacy. Some marketed drugs are reported to show physical weakness abdominal pain, muscle pain, diarrhea, gastrointestinal disorder [38, 39], low blood sugar level, and lactic acidosis [40]. The CoQ10 has not shown low blood sugar level, instead showed the normal level of blood sugar. Study on CoQ10 has reported not to have any adverse effect on long-term usage that was demonstrated in cardiovascular disorder, kidney disease, deficiency, diabetes, aging, inflammation, neurodegenerative disease, and fertility [41]. CoQ10 has a relation in both type 1 and type 2 as it is mediated by beta cells that could be effective in both type 1 and type 2 DM [42]. As a result of our study, we are concluding the antidiabetic activity of CoQ10 against STZ-induced diabetes in Wistar albino rats. This can be furthered studied in molecular level to know the molecular mechanism of CoQ10 against STZ-induced diabetes.

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Compliance with ethical standards

The experiment was carried out under the guidelines of CPCSEA, and the ethical clearance number is VIT/IAEC/11th/October 10th/No. 26.

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

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LETTER TO THE EDITOR

Diabetes mellitus in Pakistan: the past, present, and future

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Diabetes mellitus has reached epidemic proportions worldwide and it has emerged as a great socioeconomic burden for the developing world [1, 2]. It is predicted that between 2010 and 2030, there will be 67% surge in the prevalence of diabetes in developing countries [1]. Globally, in 2017, there were 451million people with diabetes. These numbers were estimated to reach 693 million by 2045 [3].It was also predicted that 49.7% of adult diabetics are undiagnosed; with a biblical proportion belongs to low income countries. Moreover, there was an estimated 374 million people with impaired glucose tolerance and it was projected that almost 21.3 million live births were affected by some form of hyperglycemia in pregnancy [3].

In Pakistan, the first survey [National Diabetes Survey of Pakistan (NDSP) 1994–98] revealed 8.7% prevalence [1].Since then, small-scale studies including survey of Pakistan Health Research Council (PHRC) reported prevalence between 13.1 and 26.9%. A latest survey [2nd NDSP 2016–17] estimated that approximately 26.3% of local population above 19 years age, is diabetic [Known diabetics; 19.2%, newly diagnosed diabetics; 7.1%]. The results show an increased prevalence compared to the 1st NDSP (Urban; 22.04% and rural; 17.15%). Overall, glycemic dysregulation (diabetes plus pre-diabetes) has doubled both in urban 43% and in rural dwellers 39% [1, 4]. The provincial pattern of prevalence is as follows: Punjab; 30.2%, Khyber PakhtunKhwa; 13.2%, Balochistan; 29.5% and Sindh; 32.3%. These figures equate to approximately 27.4 million people aged 20 and above, based on the 207.77 million total population [1, 6]. If the present situation continues, Pakistan is expected to achieve the highest prevalence of diabetes globally [2].The current status of diabetes presents a huge challenge for health care system, health professionals, and national health care policy makers. The economic burden, both in terms of morbidity and mortality associated with diabetes, will soon engulf a big portion of health budget in an already compromised health care setting with limited resources and funds. Poverty as a cause of complications of diabetes is posing an obstacle to the social and economic development. There is no operational policy or action plan yet for diabetes in the country [5].Similarly, the leading risk factors for diabetes, including unhealthy dietary habits, obesity, smoking, and sedentary lifestyle, are yet to be prioritized by the health policy makers. The country lacks consensus guidelines for diabetes management. Some support is available through the Advisory Board for the Care of Diabetes (ABCD) where guidelines, Recommendations for Optimal Management of Diabetes from Primary to Tertiary care level (PROMPT), are formed based on national data which should be implemented on priority basis [6]. However, there is no proper system organized for referral of diabetic patients with complications from primary to tertiary care level. At primary level, facilities for diagnoses and treatment are lacking. Similarly, diabetic registry does not exist so far [5].

In the light of the current country–wide statistics for diabetes both in terms of prevalence and overall existing healthcare focus on diabetes, it is highly emphasized that a multifaceted approach should be designed to curb diabetes and its complications. Operational policies and action plans to focus on primary prevention, delivering preventive services that target early detection of the disease and address the risk factors. Risk assessment of Pakistani individuals for diabetes

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(RAPID) tool has been developed with national prospective studies [6], a step ahead of primary prevention. Diabetic awareness programs including healthy lifestyle encouragement and education especially at school level, screening campaigns, nationwide networks for diabetes care, and management and prioritizing mother and child health for prevention of transgenerational obesity and diabetes will definitely help in saving millions from morbidity and mortality secondary to diabetes.

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Compliance with ethical standards

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

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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

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- For providing research grants, RSSDI invites proposals from Indian scientists, interested in conducting original research in the field of diabetes mellitus. Furthermore, limited grants are also available for the students of medical colleges for smaller projects.
- There is no deadline for submission of the proposals, which can be sent throughout the year. These proposals may fall into one of the following three categories:

1. Projects involving funding up to Rs 40,000 per project (preference will be given to young scientists <40 years).
2. Projects involving funding up to 10 lakhs.
3. We also invite proposals for more than 10 Lakhs as major projects but these have to be preferably multicentric.

- 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 and bibliography. Brief biodata of principal investigator and other co-investigators
  ◊ Importance of work in the context of national priorities. Detailed budget sought along with full justification/ proposed utilization, of funding sought from RSSDI
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- Applicant should apply 2 months in advance.
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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 carefully looked into all aspects of this course & has accredited & recognized 17 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.

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S.N.	Institute Name	Institute Location
1.	Diacon Hospital	Bengaluru, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
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6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	G D Hospitals and Diabetes Institute	Kolkata, West Bengal
10.	Aditya Diagnostics & Hospital	Dibrugarh, Assam
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12.	Marwari Hospital and Research Centre	Guwahati, Assam
13.	Down Town Hospital	Guwahati, Assam
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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 preference.

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)

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