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

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

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International Journal of Diabetes in Developing Countries

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EDITORIAL

Editorial: Advancements and insights into diabetic nephropathy

Rajeev Chawla[1] · **Abhishek Garg**[2]

Published online: 11 October 2024

Diabetic nephropathy (DN) remains a critical concern in the management of diabetes, significantly impacting patient health outcomes and healthcare systems. Recent research has illuminated several facets of DN, from inflammatory biomarkers and genetic factors to the effects of nutrition and new diagnostic methods. This editorial integrates findings from recent studies to provide an updated perspective on the understanding and management of diabetic nephropathy.

Inflammatory markers and genetic influences

Recent research underscores the importance of inflammation in diabetic nephropathy. A systematic review by Chastene Christopher Flake, Imoan Shallom Aguas, and Raphael Enrique Tiongco in this issue of the *International Journal of Diabetes in Developing Countries* highlights elevated levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in patients with DN. These inflammatory cytokines are consistently higher in DN patients compared to those with type 2 diabetes mellitus (T2DM) without renal complications and healthy controls. Notably, these markers correlate negatively with estimated glomerular filtration rate (eGFR) and positively with albumin-to-creatinine ratio (ACR), emphasizing their role in the inflammatory processes that aggravate renal damage [1].

Furthermore, a study by Brijesh K. Dabhi and Kinnari Nitin Mistry, also featured in this issue, explores the role of cytokine gene polymorphisms in DN susceptibility. Variations in genes related to inflammation and apoptosis are associated with increased risk of diabetic complications, suggesting that both genetic and inflammatory factors are crucial in the pathogenesis of DN [2].

Diagnostic challenges and innovations

Accurate glucose monitoring is vital for managing diabetic kidney disease (DKD). However, Yi Lu and colleagues have found that HbA1c-derived estimates of average glucose (AG) often fail to reflect true AG levels in patients with DKD before dialysis. Their study indicates the need for revised AG-HbA1c equations that account for diabetes type, body mass index (BMI), and chronic kidney disease (CKD) stage to improve glucose management in this patient group [3].

Additionally, novel biomarkers offer new opportunities for early diagnosis and prognosis of DN. Research by Negeem and colleagues identifies elevated levels of microRNA-192 (miR-192) and pentraxin-3 (PTX-3) as associated with lower eGFR in DN patients, suggesting these biomarkers could enhance diagnostic precision and disease monitoring [4].

Impact of nutrition and physical function

Nutrition and physical health play crucial roles in the management of DN, yet they are often overlooked. Lu Zhang and Sumei Zhang's study compares patients with and without diabetes undergoing maintenance hemodialysis (MHD), revealing that diabetic patients exhibit poorer nutritional outcomes, reduced muscle strength, and a lower quality of life. These findings highlight the need for targeted nutritional interventions and physical therapy to improve outcomes and quality of life for patients with diabetes undergoing MHD [5].

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Pathological lesions and clinical implications

A comprehensive study by Kurtipek and colleagues examines the relationship between diabetic retinopathy and renal lesions. The study reveals that diabetic retinopathy is associated with glomerular and arteriolar lesions but not with interstitial lesions. Additionally, proteinuria and hematuria are identified as independent predictors of glomerular lesions. These insights stress the importance of tailored monitoring and treatment strategies for managing specific types of renal lesions [6].

Further, research by Chawla and colleagues demonstrates a relationship between diabetic retinopathy, microalbuminuria, and modifiable risk factors, such as HbA1c and BMI, highlighting their cohesive impact on retinopathy severity [7]. Additionally, a study by Chawla and team underscores the positive correlation between cortical renal thickness and eGFR, suggesting that cortical renal thickness might serve as a better predictor of renal function compared to bipolar renal length [8].

Conclusion

The field of diabetic nephropathy is rapidly evolving with advancements in biomarkers, genetic research, and personalized care strategies. Integrating these new insights into clinical practice is essential for improving patient outcomes. Ongoing research will continue to deepen our understanding and refine strategies for early detection, management, and prevention of diabetic nephropathy. Clinicians must stay informed about these developments to provide the most effective and comprehensive care for their patients.

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REVIEW ARTICLE

Burden of non-communicable diseases in India: Findings from the ICMR-INDIAB study

Ranjit Mohan Anjana[1] · Wesley Hannah[1] · Mohan Deepa[1] · Rajendra Pradeepa[1]

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Abstract

Background The prevalence of type 2 diabetes is rising quickly in low- and middle-income nations like India. In order to develop preventive strategies and comprehend the effects of diabetes as a contributing factor to other non-communicable diseases (NCDs), it is essential to have robust population-level epidemiological data. It is challenging to generalize findings from regional studies given the diversity of India's population, cultural practices, and dietary patterns.

Objective The primary goal of the national Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study was to produce high-quality data on the prevalence of diabetes and other NCDs, such as dyslipidemia, hypertension, and obesity at the country and state levels.

Methods This is a door-door cross-sectional, multi-stage stratified sampled survey from 2008 to 2020; 31 Indian states and union territories were surveyed for the ICMR-INDIAB study.

Findings National projections for NCDs from this study include 101.3 million with diabetes, 136.0 million with prediabetes, 315.5 million with hypertension, 254.2 million with generalized obesity, 213.3 million with hypercholesterolemia, and 185.7 million with high HDL cholesterol. Knowledge and awareness of diabetes are still largely inadequate. Less than 8% of individuals met the treatment goals for glycemia, blood pressure, and lipid goals. Physical inactivity levels are high in India. Reducing carbohydrate intake and increasing protein intake could help in the prevention of progression of dysglycemia as well as reversal of diabetes.

Conclusion In India, the prevalence of diabetes, prediabetes, and other metabolic risk factors such as obesity, dysglycemia, blood pressure, and physical inactivity is rising.

Keywords Non-communicable diseases · Diabetes · Prediabetes · Obesity · Hypertension · Dyslipidemia · India

Introduction

Non-communicable diseases (NCDs) include disorders like diabetes, cancer, heart disease, chronic respiratory diseases, stroke, cerebrovascular disease, and mental health issues. Lately, NCDs have become a major public health concern [1]. Previously NCDs disproportionately affected individuals residing in wealthy and developed nations; however, currently, this has become a significant public health concern in India and other low- and middle-income countries (LMICs). Indeed, it is now true that a substantial amount of the worldwide NCD burden is borne by LIMCs [2]. As of 2021, there were over 537 million cases of diabetes worldwide, a number that is expected to increase to 783 million cases by 2045. According to International Diabetes Federation country-level figures, China has the greatest diabetes burden (140.9 million) in 2021, followed by India (74.2 million), whose burden is expected to rise to 124.9 million by 2045. Due to the nation's diverse range of lifestyle behaviors, the prevalence found in several regional studies, whether conducted in urban or rural areas, cannot be applied to the entire country. The increasing incidence of diabetes highlights the need for thorough, trustworthy, and useful national epidemiological research to calculate the burden and develop measures for prevention and control.

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Nation-wide implementation of ICMR-INDIAB study

The Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) project is a cross-sectional survey of the Indian population over 20 years old with the goal of estimating the national burden of diabetes and other metabolic NCDs, by determining the state-by-state prevalence of the same [3]. A multi-stage stratified sampling method akin to that of the National Family Health Survey-3 (NFHS-3) was employed in this study to choose 113,043 participants from 31 states and union territories (33,537 urban residents and 79,506 rural residents). To provide a representative sample of the population in each state, stratification at three levels—geographical location, population size, and socio-economic status (SES)—was employed. Census enumeration blocks were used as primary sampling units (PSU) in urban regions, whereas villages were used in rural districts. In both these areas, households were the final phase units with 24 urban households and 56 rural households selected from each PSU. Using the World Health Organization (WHO) Kish methodology, only one person was identified from each household. ICMR-INDIAB is the first, nationally representative, population-based study on metabolic NCDs in India and has now published on the burden of diabetes, hypertension, dyslipidemia, and obesity [4–11].

Due to logistical and operational challenges, the ICMR-INDIAB study was implemented in phases spanning from 2008 to 2020 as shown in Figs. 1 and 2. The study methodology was consistent across all states and UTs [3]. The questionnaire was administered by a trained interviewer to collect demographics, behaviors, and medical history data. The diagnosis of diabetes entailed the use of capillary blood to measure fasting and post-glucose load blood glucose values, which has been demonstrated to be feasible and reliable as an alternative to venous plasma for screening for diabetes/prediabetes in large epidemiological studies conducted in resource-limited environments of developing countries. However, venous blood samples in a fasting state were also collected from all participants with self-reported diabetes and from one in every 5 participants for estimation of HbA1c and lipid parameters (total cholesterol, triglycerides, and HDL cholesterol). All field staff of ICMR-INDIAB study received 2 weeks of intense training at the National Coordinating Centre (Madras Diabetes Research Foundation, Chennai). This training included print and digital versions of standard training modules covering all components

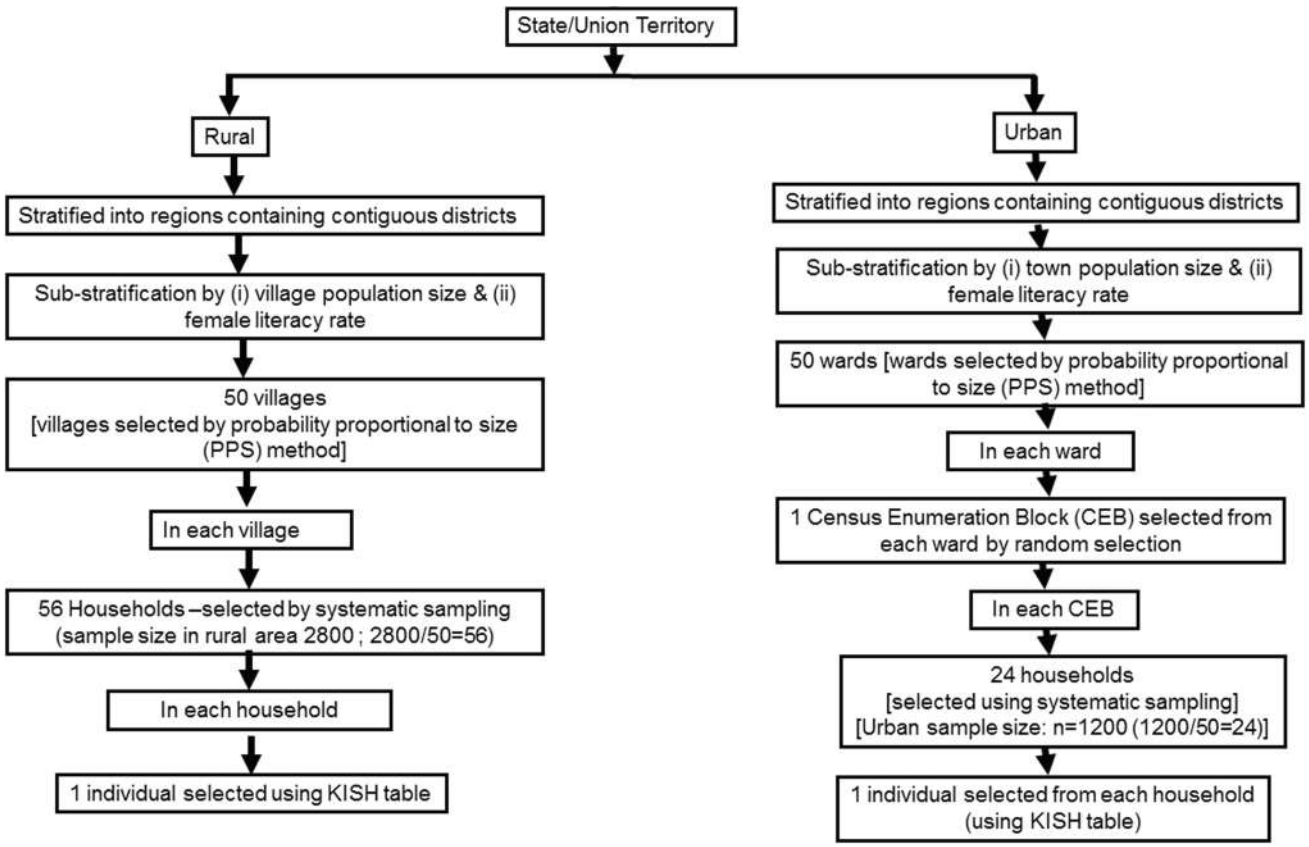

Fig. 1 ICMR INDIAB study sampling frame

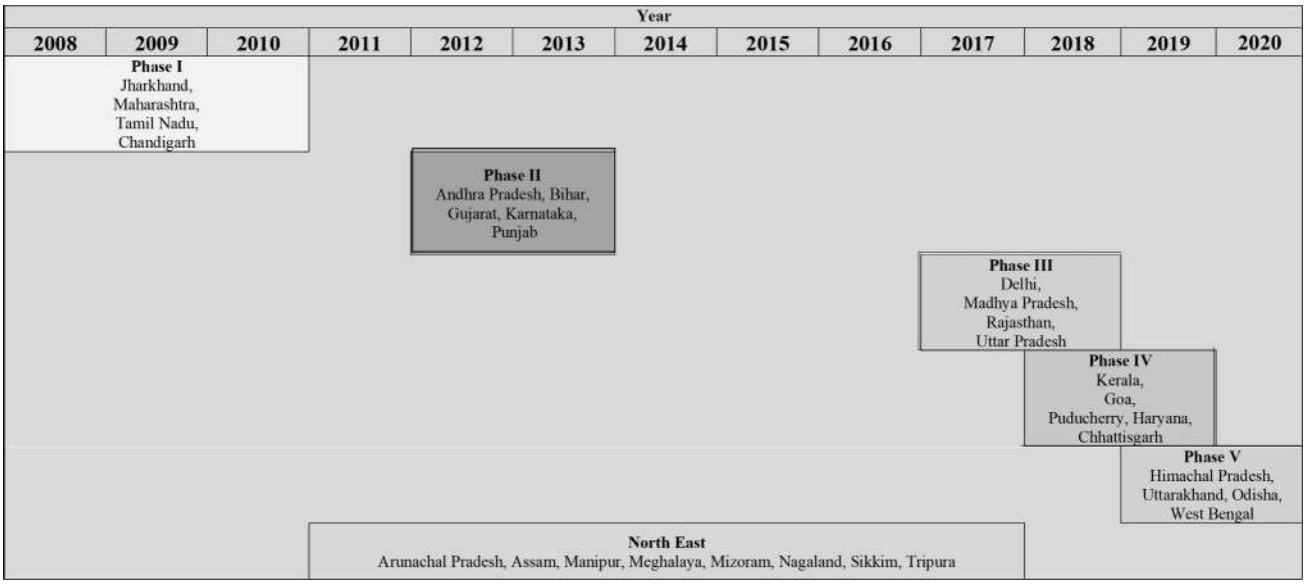

Fig. 2 ICMR INDIAB study implementation

of this study. In-field refresher training was also conducted at regular intervals. A three-tiered quality control process was followed to ensure the quality of the study according to the research protocols. The first tier was the quality assurance of data collection conducted by quality managers/quality supervisors. State investigators and the National Coordinating Centre (NCC) carried out regular quality monitoring visits to assess the quality of this study. The expert terms from ICMR also carried out quality monitoring visits to these states. The findings of the ICMR -INDIAB Study covering 31 States/Union Territories (UT) has recently been published [6]. This publication includes data from 1,13,003 individuals (urban inhabitants 33,547, rural inhabitants 79,506) who agreed to participate and submitted blood samples. The definitions used for the diagnosis of diabetes, prediabetes, generalized obesity, abdominal obesity, dyslipidemia, and hypertension are presented in Fig. 3 [12–16].

Prevalence of diabetes and prediabetes

The overall prevalence of diabetes (weighted by CBG values against OGTT diagnostic thresholds) was 11.4%, with a significantly higher prevalence in urban areas (16.4%) than in rural areas (8.9%) as given in Fig. 4 [6]. Males had a considerably higher prevalence of diabetes (12.1%) than females (10.7%). When HbA1c was used to diagnose diabetes, the prevalence was 13.3%; when combined with OGTT, the prevalence rose to 21.1%. Figure 5 shows the state-wise diabetes prevalence, which varied from 4.8% in Uttar Pradesh to 26.4% in Goa. In general, the prevalence of diabetes was higher among states with higher levels of socioeconomic development.

The weighted prevalence of IFG and IGT was 3.3% (95% CI: 2·6–4·0) and 10.1% (95% CI: 9·0–11·2), respectively. Figure 4 shows that the overall prevalence of prediabetes was 15.3% (95% CI: 13·9–16·6). IFG rates were higher in females and IGT rates in males. The prevalence of prediabetes by HbA1c was 21.0% (95% CI: 17·5–24·6), and the combined prevalence by OGTT and HbA1c was 26.6% (95% CI: 22·8–30·4). The prevalence of prediabetes did not significantly differ between rural (15.2%) and urban (15.4%) areas (Fig. 4). The prevalence of prediabetes was 15.0% in males and 15.5% in females ($p = 0.023$). The prevalence of prediabetes ranged from 8.1 (95% CI: 3.5–12.8) in Manipur to 31.3% (95% CI: 21.7–41.2) in Sikkim. Most of the less socioeconomically developed states had higher prevalence of prediabetes than diabetes, while the converse was true for the more developed states. This indicates that while the epidemic of diabetes has peaked in the more developed states, it is still to do so in the less developed ones.

Prevalence of obesity

The prevalence of abdominal obesity was 39.5% (95% CI: 37.7–41.4), and generalized obesity was 28.6% (95% CI: 26.9–30.3) [7]. In comparison to rural areas, the rates of both generalized and abdominal obesity were significantly higher in urban areas. Females had significantly higher rates of generalized obesity (females: 31.6% vs. males: 25.4%), and abdominal obesity rates (females: 49.6% vs. males: 28.8%) compared to males. High rates of generalized obesity

Fig. 3 Diagnostic protocol used in ICMR INDIAB study

Health condition	Definition
Diabetes	Diabetes is defined as any of the following: i. those on glucose-lowering medication after previous diagnosis by a physician (self-reported) ii. those with a fasting CBG value ≥126 mg/dL iii. those with a 2hr post load CBG value ≥220 mg/dL
Prediabetes	Prediabetes is isolated impaired fasting glucose (IFG) or isolated impaired glucose tolerance (IGT) or both i. Isolated IFG: a fasting CBG between 110 and 126 mg/dl or a 2-hour post-glucose CBG <160 mg/dl ii. Isolated IGT: a 2-hour post-glucose CBG between 160 and 220 mg/dl or a fasting CBG <110 mg/dl
Generalised obesity	Based on WHO Asia Pacific guidelines, obesity is defined as BMI of 25 kg/m² or higher
Abdominal obesity	Based on WHO Asia Pacific guidelines, abdominal obesity was defined as a waist circumference of 90 cm or higher for men and 80 cm or higher for women
Hypertension	According to the Eighth Joint National Committee criteria, hypertension was defined as, a systolic blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mm Hg or higher, or treatment with antihypertensive drugs
Dyslipidemia	According to National Cholesterol Education Programme - Adult Treatment Panel III guidelines, definitions for dyslipidaemia are as follows: i. Hypercholesterolaemia: serum cholesterol concentrations of 200 mg/dL (5·2 mmol/L) or higher ii. Hypertriglyceridaemia - serum triglyceride concentrations of 150 mg/dL (1·7 mmol/L)or higher iii. Low HDL cholesterol - HDL cholesterol concentrations of less than 40 mg/dL (1·04 mmol/L) for men and less than 50 mg/dL (1·3 mmol/L) for women iv. High LDL cholesterol—LDL cholesterol concentrations of 130 mg/dL (3·4 mmol/L) or higher as calculated using the Friedewald equation

were reported in Chandigarh, Delhi, Goa, Haryana, Kerala, Puducherry, Punjab, and Sikkim, while Puducherry, Haryana, Delhi, Punjab, and Kerala had high rates of abdominal obesity.

Prevalence of hypertension

According to the attributable burden of disease, high blood pressure is ranked third among risk factors in south Asia [17]. A growing number of Indians are developing hypertension. According to the diagnostic protocol described in Fig. 3, the prevalence of hypertension was 35.5% overall, with a significant difference between the urban and rural prevalence (40.7% vs. 33.0%) (Fig. 4) [8]. Males had a greater prevalence of hypertension (38.7%) than females (32.6%). The prevalence of hypertension, however, rose steeply to 66.3% with the application of the American College of Cardiology/American Heart Association (ACC/AHA) criteria [15]. The prevalence of hypertension in each state varied, with Meghalaya having a 24.3% (95% CI: 17.8–30.7) prevalence and Punjab having a 51.8% (41.3–62.2) prevalence. An earlier ICMR-INDIAB publication covering the states of Tamilnadu, Chandigarh, Maharashtra, and Jharkhand revealed a 3.8:1 ratio between newly diagnosed and self-reported cases of hypertension [4]. The prevalence of hypertension rose with age, however even individual in the age group 20–24 years had a prevalence that ranged from 5.4 to 13.9% in urban areas and 9 to 10% in rural areas. A daily intake of 6.5 g of salt (after correction for confounding variables) was associated with a 1.4-fold increased risk of hypertension.

Prevalence of dyslipidemia

Asian Indians have been found to exhibit a distinct dysglycemia pattern that is characterized by elevated triglyceride levels, decreased HDL cholesterol, and a high percentage of small dense LDL cholesterol [18]. In order to ascertain the pattern and prevalence of dysglycemia in the country,

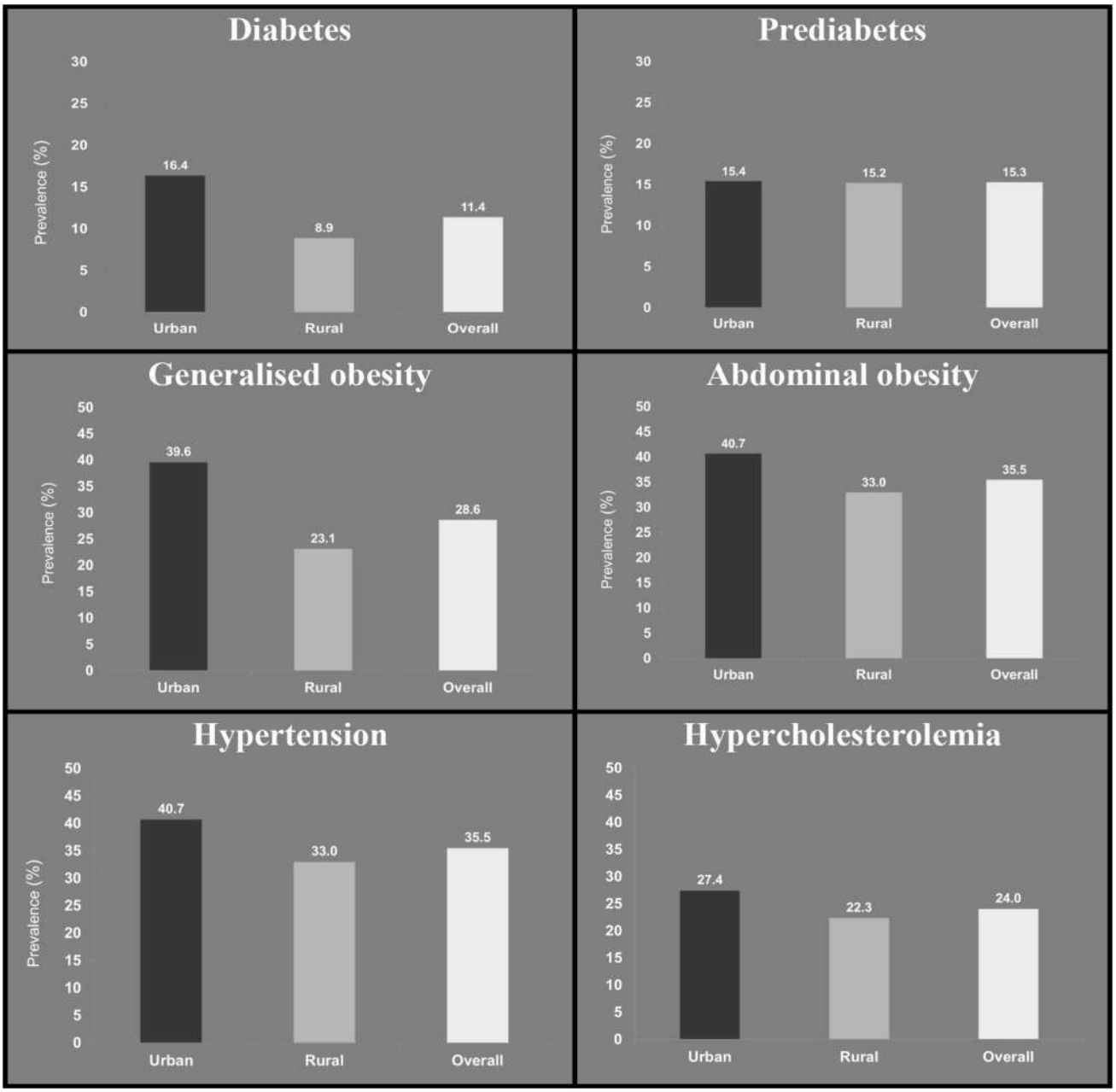

Fig. 4 Prevalence of metabolic disorders

ICMR-INDIAB evaluated lipid parameters in every fifth study participant as described in Fig. 3. Overall, the prevalence of dyslipidemia was 81.2% (24.0% for hypercholesterolemia, 32.1% for hypertriglyceridemia, 66.9% for low HDL cholesterol, and 20.9% for high LDL cholesterol) [9]. The prevalence of dyslipidemia in cities was noticeably greater than that in rural areas (Fig. 4). Males had significantly higher rates of hypertriglyceridemia than females, but females had higher rates of abnormal other lipid parameters.

The rates of hypercholesterolemia varied from 4.6% (95% CI: 0.2–10.8) in Jharkhand to 50.3% (95% CI: 30.3–70.3) in Kerala. High LDL cholesterol also showed a similar pattern, with Jharkhand having 3.2% prevalence and Kerala, 52.1%. The prevalence of hypertriglyceridemia was lowest in Chhattisgarh (21.2%, 95% CI: 8.4–34.4) and highest in Punjab (47.9%, 95% CI: 28.4–68.6). All states reported low HDL cholesterol levels above 50%, with Delhi having the lowest at 51.8% and Puducherry having the highest at 83.1%.

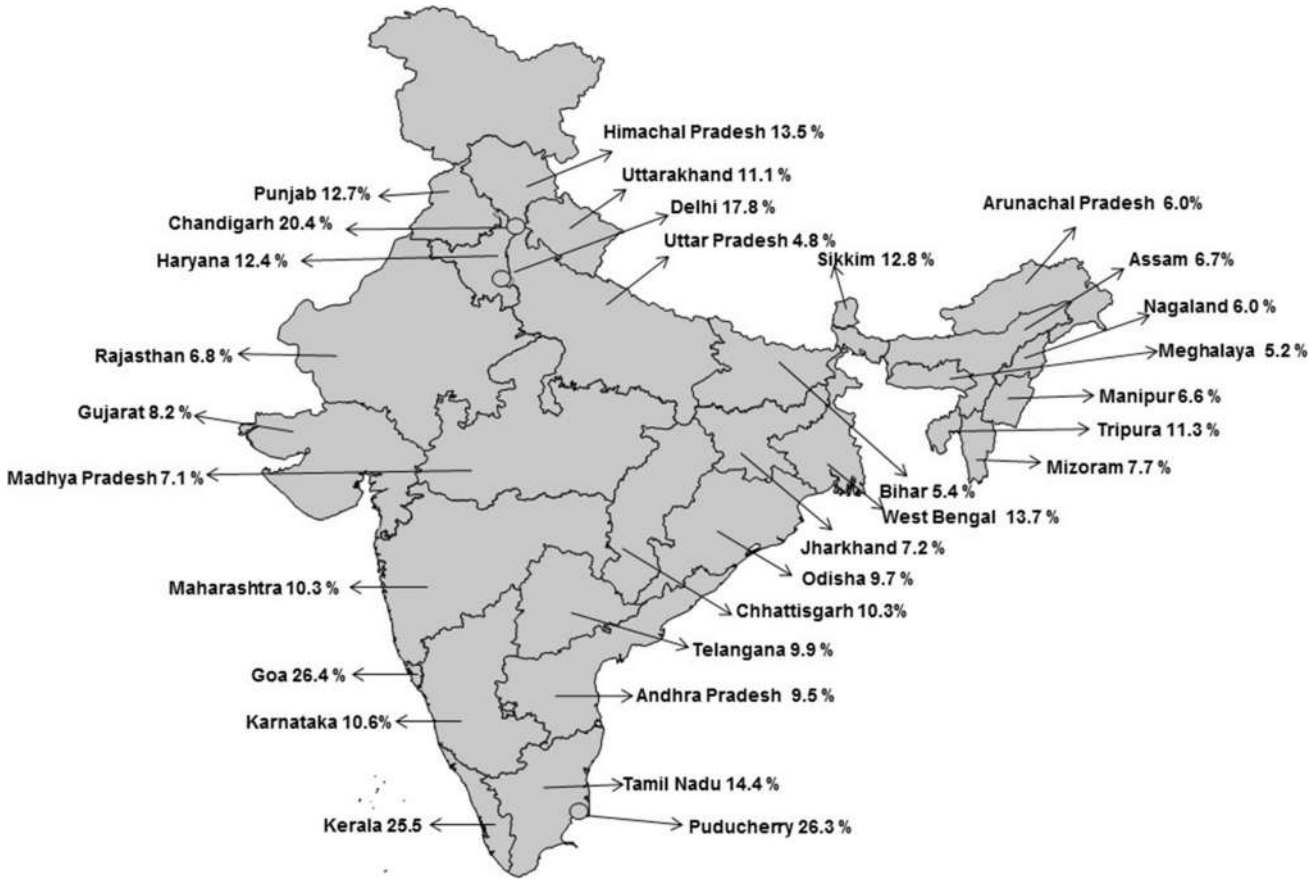

Fig. 5 State-wise prevalence of diabetes

Diabetes awareness and knowledge

Reducing diabetes-related morbidity and mortality can be achieved by increasing awareness of the disease and its complications [19]. Diabetes awareness is also linked to improved treatment compliance, a better prognosis that lowers healthcare costs, and a higher quality of life [20, 21]. Just 43.2% of participants surveyed in Phase I of the ICMR-INDIAB had ever heard of diabetes as a medical condition [22]. Compared to rural areas (36.8%), urban areas had higher levels of diabetes awareness (58.4%). Tamilnadu was found to have the highest awareness at the state level among all regions studied in Phase I. Men were more aware of diabetes than women, with the exception of Chandigarh. The general population ($n = 13,794$) and those who self-reported having diabetes ($n = 480$) were compared for diabetes awareness levels. Compared to the general population, those who had diabetes were more aware of the rising burden of the disease (93.3% vs. 80.7%), the possibility of preventing diabetes (63.4% vs. 56.3%), and the complications of diabetes (72.7% vs. 51.4%). Particularly, compared to the general population, persons with self-reported diabetes also showed a greater awareness of the following afflicted organs: the eyes, kidney, heart, feet, and nerves.

Management and treatment goals in diabetes

The objectives of diabetes management are to reduce mortality, manage symptoms, postpone the development of micro- and macrovascular diabetes-related complications, and guarantee a quality of life that is comparable to that of healthy people [23]. Diabetes management includes not only glycemic control but also blood pressure regulation and management of abnormal lipid levels [24]. The ICMR-INDIAB study evaluated the proportion of individuals with known diabetes meeting their treatment objectives. The targets studied were as follows: glycemic control (A) A1c < 7%, blood pressure control (B) < 140/90 mmHg, and cholesterol (C) LDL cholesterol < 100 mg/dl. This is the first nationally representative study conducted in India that details treatment goal achievement among people who self-report having diabetes. Just 7.7% of these 5789 individuals with self-reported diabetes met all three

treatment objectives. Only 41.5% had good control of LDL cholesterol, 48.8% had controlled blood pressure, and 36.6% had good glycemic control. Residents in rural areas had better blood pressure control than those in urban areas (50.9% vs. 46.4%). Compared to females, males had better control over LDL cholesterol (46.2% vs. 36.3%). Furthermore, only 16.7% of these people self-monitored their blood glucose, and only 36.9% of those on insulin did so. The amount of fruits and vegetables consumed (three servings or more per day) was low; not even 20% of these individuals with diabetes met this recommendation. Less than 25% exercised in a moderate-to-intense manner. The study's conclusions thus highlight the importance of encouraging Indians to lead healthy lifestyles. Additionally, the results of this study will guide policy to improve primary, secondary, and tertiary diabetes management in the Indian healthcare system.

Migration and diabetes

One of the main socioeconomic factors influencing health is migration status [25]. There is mounting evidence that urbanization, industrialization, and migration are environmental and social risk factors for NCDs. India is not an exception to the diaspora of rural-to-urban migration that has affected most LMICs [26]. Natural disasters, population growth that is outpacing the infrastructure, poverty, unemployment, and a lack of access to basic infrastructure, such as health care and education, can all be factors that lead to migration, with the lure of better opportunities on many fronts [27, 28]. Changes in diet and exercise patterns brought about by migration increase the risk of NCDs like diabetes [29]. Diabetes is more common in migrants, and there are also high rates of obesity, insulin resistance, physical inactivity, poor diet, and gene-environment interactions [30–32]. South Asian immigrants abroad have been shown to have higher rates of obesity and diabetes compared to native populations, but there is little data on rural-to-urban migration within India [33]. Therefore, the ICMR-INDIAB study examined the prevalence rates of diabetes and other metabolic disorders in migrants and non-migrants who had lived away from their birthplace for at least a year [34]. Rural–urban migrants were found to have a 1.9-fold increased risk of diabetes compared to non-migrant rural residents, and the prevalence of diabetes rose with the length of time after migration. These results highlight the necessity of strengthening the healthcare system capacity in Indian cities to manage the rising burden of NCDs particularly given that migration is not expected to decrease anytime soon and will likely continue to increase the burden of these metabolic disorders. To reduce the burden of diabetes and NCDs, strategies to promote health and prevent metabolic insults must be directed towards these migrants and their families.

Physical activity and diabetes

Most NCDs are caused by a combination of lifestyle factors, including poor diet, substance abuse, and physical inactivity. The Global Physical Activity Questionnaire (GPAQ) was utilized in the ICMR-INDIAB study to evaluate Indians' levels of physical activity [35]. The overall physical activity status shown by Phase I of the ICMR-INDIAB study was as follows: highly active—13.7%; active—31.9%; and inactive—54.4% [10]. Compared to rural residents, urban residents (65%) were more inactive (50%). There was a statistically significant difference in physical inactivity between males and females. Less than 20 min were spent on average in moderate to high intensity activity among the 8.1% of participants who participated in recreational physical activity.

Role of diet in the prevention and remission of diabetes

Data-driven optimization was used to present macronutrient recommendations for diabetes prevention and remission based on pooled data from 31 states in the ICMR-INDIAB study [36]. A macronutrient recommendation was created for each of the three categories [1594 newly diagnosed diabetes (NDD), 7336 prediabetes, and 9160 normal glucose tolerance (NGT)]. Four macronutrient recommendations were developed through the application of a constrained quadratic programming problem (QPP). To bring about diabetes remission in individuals with NDD, the optimal macronutrient recommendations were 19–20%E protein, 49–54%E carbs, 21–26%E fat, and 5–6%E dietary fiber. Among those with prediabetes, the optimal macronutrient recommendations for remission were 50–56%E carbohydrates, 18–20%E protein, 21–27%E fat, and 3–5% dietary fiber. Similar recommendations have also been published for prevention of progression of dysglycemia in those with NGT and prediabetes.

Conclusion

The estimated numbers for the prevalence of diabetes and prediabetes in India in 2021 are 101.3 million and 136.0 million, respectively. The national diabetes prevalence in the thirty states and union territories included in the

ICMR-INDIAB study was 11.4%; it ranged from 4.8% in Uttar Pradesh to 26.4% in Goa. Different states in India are at different stages of the diabetes epidemic with some of them having reached the peak while the others are yet to do so. Generalized obesity was found in 28.6%, abdominal obesity in 39.5%, hypertension in 35.5%, dyslipidemia in 81.2%, hypercholesterolemia in 24.0%, hypertriglyceridemia in 32.1%, high LDL cholesterol in 20.9%, low HDL cholesterol in 66.9%, and physical inactivity in 54.4% of the population studied. The prevalence of most of the metabolic risk factors, including obesity, dyslipidemia, and blood pressure, was high nationwide, and in urban areas specifically. A significant amount of physical inactivity contributes to the obesity and diabetes twin epidemics. Migration increases the risk of metabolic disorders; therefore, specific interventions are needed in migrant populations within the nation. A suboptimal number of Indians meet glycemic, lipid, and blood pressure goals. Controlling one's diet, reducing carbohydrates, and increasing proteins help prevent the development of prediabetes and diabetes and helps remission of diabetes.

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Declarations

Ethical approval The study was approved by the Institutional Ethics Committee of the Madras Diabetes Research Foundation and individual states.

Consent to participate A written informed consent was obtained from all study participants.

Conflict of interests The authors declare no competing interests.

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REVIEW ARTICLE

Serum and urinary levels of tumor necrosis factor-alpha and interferon-gamma in diabetic nephropathy patients: a systematic review

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Abstract

Objective In this systematic review, we determined the association of selected inflammatory cytokines in blood and urine with diabetic nephropathy (DN).

Methods A literature search using the key terms "tumor necrosis factor-alpha," "interferon-gamma," and "diabetic nephropathy" was conducted in PubMed from December 2020 to January 2021. Relevant findings from these studies were collated for qualitative synthesis.

Results Based on the synthesis of results, TNF-α and IFN-γ levels were consistently higher among DN patients than T2DM patients with no renal complications and healthy individuals. A negative correlation with the estimated glomerular filtration rate (eGFR) and a positive correlation with the albumin-to-creatinine ratio (ACR) were also noted by most studies which assessed their relationship with renal markers.

Conclusion The findings of this systematic review suggest the association of serum and urinary TNF-α and IFN-γ levels with diabetic nephropathy. However, further studies must be conducted to confirm these findings.

Keywords Tumor necrosis factor-alpha · Interferon-gamma · Inflammatory cytokines · Diabetic nephropathy · Urine · Systematic review

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease that is currently among the leading causes of morbidity and mortality worldwide. Last 2019, approximately 463 million individuals aged 20–79 years were diagnosed with DM, and a continuous increase in both its incidence and prevalence is still expected to occur [1, 2]. Majority of these cases are classified as type 2 DM (T2DM), characterized by hyperglycemia due to an impaired response to insulin. If left unmanaged, this defect may cause damage to other organs, resulting in different microvascular complications, including diabetic nephropathy (DN), a serious glomerular condition affecting about 20 to 30% of T2DM patients [1, 3, 4].

The pathogenesis of DN is multifactorial, and among the potential factors implicated in its progression is immune-mediated inflammation [5].This may contribute to renal damage through different mechanisms, including monocyte migration and complement activations [6]. Studies have also noted that pro-inflammatory systems are involved in the activation and infiltration of inflammatory cells, which indirectly contribute to renal damage [7]. Pro-inflammatory cytokines produced by either renal or infiltrating cells are also involved in the process. These may directly damage renal architecture, promote extracellular matrix accumulation, and further activate inflammatory cells in the diabetic kidney [8, 9].

Tumor necrosis factor-alpha (TNF-α) is among the pro-inflammatory cytokines that may induce and magnify the inflammatory process in the affected kidney by promoting the release of chemokines and adhesion molecules involved in the process as well [7]. It may also contribute

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to the reduction of intraglomerular blood flow, increase of glomerular basement membrane permeability, increase in sodium retention and renal hypertrophy, which may all contribute to DN development [3, 9, 10]. Another cytokine that may be involved is interferon-gamma (IFN-γ). Data regarding its exact effect on renal abnormalities are limited. Still, some studies have noted its potential involvement in the modification of glomerular permeability and renal structure and the alteration of renal hemodynamics together with other cytokines [11]. It may also be involved in stimulating chemokine synthesis among resident renal cells such as mesangial cells and podocytes [12]. These effects emphasize the role of the said cytokines as pathogenic inflammatory mediators which contribute to organ damage and to the subsequent progression of renal diseases such as DN [11, 13].

Due to the role of inflammation in the DN, inflammatory mediators such as TNF-α and IFN-γ have been focused on by previous studies to elucidate their potential role as clinical biomarkers [14, 15]. These new markers may be useful since there are some issues with the current use of traditional markers such as the albumin excretion rate (AER) and glomerular filtration rate (GFR). One concern is that microalbuminuria may be reversible, and there are patients with renal damage who are in a non-albuminuric state. Another is that GFR reflects late functional changes, and it is not very useful in determining early structural damage in the kidneys [7, 16, 17]. This highlights the need for novel markers such as inflammatory cytokines, whose role in DN development and clinical detection has been focused on by previous studies. To provide a better understanding of this topic, a systematic review was conducted to assess the association of the serum and urinary levels of TNF-α and IFN-γ with DN and its conventional renal markers.

Methods

Search strategy and study selection

The search strategy that was employed for this study is illustrated in Fig. 1. The key terms "Tumor necrosis factor-alpha and diabetic nephropathy" and "Interferon-gamma and diabetic nephropathy" were used in the electronic search for potential studies on PubMed, which was conducted from December 2020 until January 2021. The studies that were included in this systematic review were those which fulfilled the following inclusion criteria: (1) written in English, (2) published in the last 10 years from the conduct of this study, (3) included participants with diabetic nephropathy or type 2 diabetes mellitus exhibiting macroalbuminuria, microalbuminuria or eGFR of < 60 mL/min/1.73 m^2, (4) included participants with T2DM without renal disease or healthy

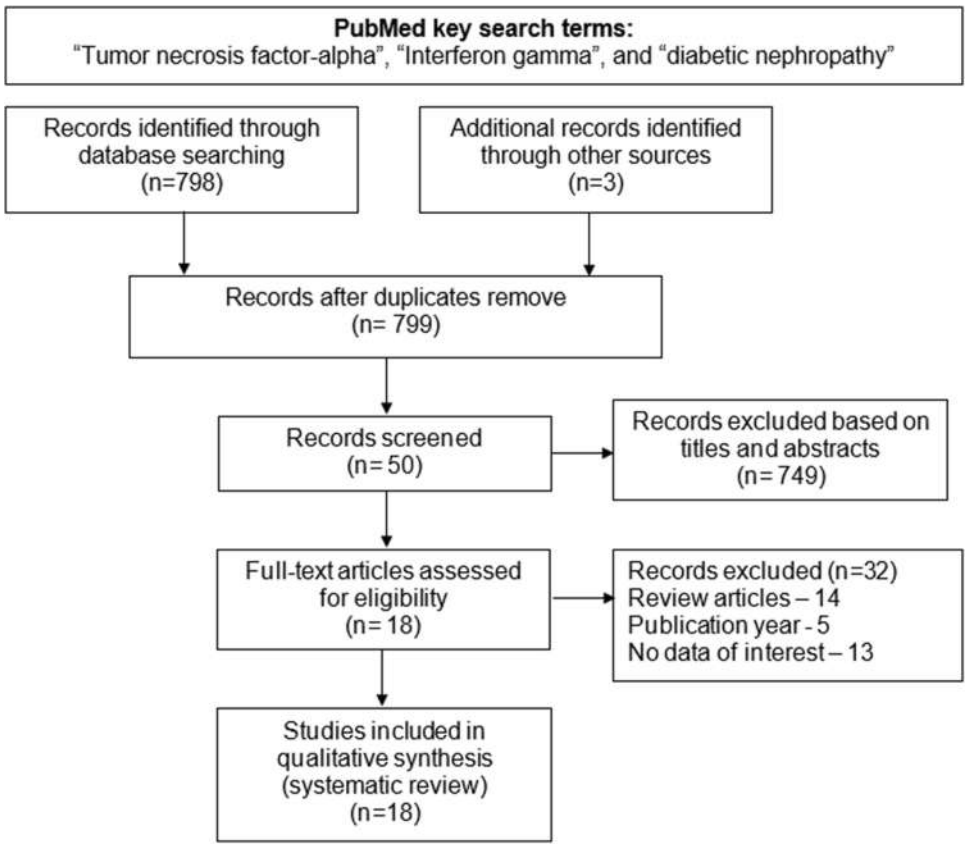

Fig. 1 Summary of the literature search. The figure illustrates the electronic search for studies which fulfilled the inclusion criteria and were included in this systematic review

controls, (5) used serum/plasma and/or urine as the samples for cytokine level determination, and (6) contained data on TNF-α and/or IFN-γ levels. No restrictions were set on the year the study was published.

The titles and abstracts of the resulting studies were then screened, and those which were not fit for the objective of this systematic review were excluded. The full papers of the remaining studies were evaluated to determine if they were to be included based on the set inclusion criteria. The references of these studies were also checked to see if additional studies may be included.

Data extraction

The data needed for this systematic review were extracted from the studies included in this paper. From each study, the following information was extracted: (1) the first author's last name, (2) publication year, (3) country where the study was conducted, (4) the total number of participants, (5) samples used for testing, (6) TNF-α and IFN-γ levels, and (7) other significant findings relevant to cytokine levels and diabetic nephropathy.

Quality assessment of the included studies

The quality of the included studies was assessed using the updated Standards for Reporting of Diagnostic Accuracy Studies (STARD) checklist published in 2005. This is focused primarily on the completeness and transparency of the reports from diagnostic accuracy studies. It includes 30 items focused on each study's content, from their title and abstract up to their results and discussion, with additional items concerning access to the full study protocol and sources of funding. These items ensure that the conducted studies contain sufficiently informative reports regarding the topic of interest [18].

Each item was rated as adequately reported (score = 1), partially reported (score = 0.5), or not reported (score = 0) to generate a semi-quantitative numeric total score, with the maximum score equivalent to 30 points. The total score obtained from each study may be interpreted using the following: a score of ≥ 15 would indicate that the study has an excellent quality of reporting; scores between 10 and 14 are considered to be of fair quality and those that are < 10 are considered to be of poor quality [18–20].

Qualitative synthesis of the results

The results from each included study were obtained and tabulated. Among the findings that were obtained were the TNF-α and IFN-γ levels of the DN, T2DM, and healthy participants, including the comparison of the results among the mentioned groups. This determines the potential association of the said cytokines with DN that may or may not have been established in the studies. Findings regarding the association of cytokine levels with other conventional renal markers were also summarized.

Results

Search result and characteristics of the included studies

The result of the literature search is summarized in Fig. 1. Seven hundred ninety-nine studies were obtained from PubMed using the key search terms. After screening, the resulting studies and excluding those that did not meet the objective and inclusion criteria, a total of 18 studies were included in this systematic review.

The characteristics of the included studies are summarized in Table 1. The studies included in the systematic review were published between 2010 and 2020, and most were conducted in different Asian and European countries. They varied in terms of sample size, with one study only having 24 participants which is the least among all studies [15], and another including a total of 594, which is the largest sample size [21]. The majority tested for the serum/plasma levels of TNF-α, although there were some which also used urine samples for testing [5, 6, 10, 11, 15, 22, 23]. IFN-γ levels were also determined in four of the included studies [8, 11, 12, 15].

Quality of the included studies

As reflected in Table 1, the included studies were subjected to further assessment using the STARD checklist. Upon evaluation by two of the researchers, 13 out of 18 studies received an excellent rating (score of 15 and above) for their quality of reporting while the remaining were of fair quality (score of 10–14). This critique reveals that the results of the current study are reliable since the included studies were found to contain sufficient information on the topic at hand.

Levels of TNF-α and IFN-γ

Although the studies were conducted in different countries with different sample sizes and different biological samples for testing, most had arrived with similar findings in terms of the levels of TNF-α and IFN-γ among DN or T2DM patients with kidney diseases, T2DM patients with no kidney diseases, and healthy individuals. The majority of the studies have reported that TNF-α and IFN-γ levels were significantly higher in DN than in T2DM and healthy participants, as shown in Tables 2 and 3.

Table 1 Characteristics of the included studies

Author	Publication year	Country	Ethnicity	Sample size	Cytokine tested	Sample	STARD result
Avci et al	2014	Turkey	West Asian	60	TNF-α	Serum	12
Cao et al	2019	USA	North American	79	TNF-α	Plasma, urine	21.5
Fathy et al	2018	Kuwait	West Asian	159	TNF-α, IFN-γ	Plasma	21
Gohda et al	2018	Japan	East Asian	314	TNF-α	Serum	22.5
Kamei et al	2018	Japan	East Asian	594	TNF-α	Serum	22
Karadag et al	2016	Turkey	West Asian	106	TNF-α	Serum	15.5
Kung et al	2010	Taiwan	East Asian	72	TNF-α	Plasma	13.5
Lampropoulou et al	2014	Greece	Southeast European	82	TNF-α	Serum, urine	18.5
Lampropoulou et al	2020	Greece	Southeast European	82	TNF-α	Serum, urine	19
Li et al	2016	China	East Asian	205	TNF-α	Serum	19
Lu et al	2011	Canada	North American	113	TNF-α	Plasma	14.5
Neolofar et al	2018	India	South Asian	150	TNF-α	Serum	16.5
Sangoi et al	2016	Brazil	South American	125	TNF-α, IFN-γ	Urine	21.5
Sil Yeo et al	2020	Korea	East Asian	543	TNF-α	Serum	22.5
Wu et al	2010	Taiwan	East Asian	24	TNF-α, IFN-γ	Serum, urine	16.5
Wu et al	2013	China	East Asian	264	TNF-α	Urine	14
Zhang et al	2019	China	East Asian	287	TNF-α	Urine	19
Aly et al	2020	Egypt	Northeast African	80	IFN-γ	Serum	13.5

TNF-α, tumor necrosis factor-alpha; *IFN-γ*, interferon-gamma

Some studies also noted that the TNF-α levels increased as one progressed from normoalbuminuria to microalbuminuria and macroalbuminuria [22, 23], with the latter being associated with the highest levels among the three. However, two studies obtained conflicting results. Cao et al. concluded that there was no significant difference between the plasma TNF-α levels of healthy participants and T2DM patients with normoalbuminuria [10], while the study of Lampropoulou et al. indicated that plasma levels were similar among T2DM patients with normoalbuminuria and microalbuminuria [6].

Three studies that used different samples for testing also determined the association of serum/plasma TNF-α levels with their urinary levels. The study of Lampropoulou et al. in 2014 established no association between the cytokine levels of these two samples [6]. However, the study conducted by the same authors in 2020 indicated that serum and urinary levels showed a borderline positive correlation [5]. Wu et al. on the other hand, stated that higher levels were observed in urine than in serum [15]. As observed, contradicting findings were obtained by these researchers.

Correlation of TNF-α with renal markers

Ten of the included studies determined the correlation of TNF-α with conventional renal markers such as the estimated glomerular filtration rate (eGFR), albumin-to-creatinine ratio (ACR), and the urinary albumin excretion (UAE) as summarized in Table 4. The studies which assessed serum and/or urine TNF-α levels and eGFR all stated that it was negatively correlated with the said renal marker [5, 9, 15, 24], except for the study of Lampropoulou, et al. in 2014 which did not observe any correlation between these two regardless of the sample used [6]. On the other hand, most of the studies which evaluated the correlation of TNF-α with either ACR or UAE had observed a positive correlation [21–23, 25]. However, two studies indicated that the correlation was only observed among urinary TNF-α levels and not in serum [5, 6]. Most of these findings suggest that TNF-α levels may also increase as kidney problems progress, similar to conventional renal markers.

Discussion

Summary and interpretation of findings

This systematic review was performed to investigate the association of serum and urinary TNF-α and IFN-γ with diabetic nephropathy and with conventional renal markers. The review included 18 studies that explored the role of these cytokines in DN patients across different population groups.

This review noted that there were higher levels of TNF-α and IFN-γ in DN patients compared to T2DM patients and healthy individuals. This finding suggests that the levels of these cytokines increase along with the deterioration of renal function. Moreover, it was also observed that TNF-α levels were negatively correlated with eGFR and positively

Table 2 Summary of findings on TNF-α

Author, year	Cytokine levels		
	Serum/plasma	Urine	Other findings
Avci et al. 2014	Significantly higher in DN than healthy control	-	-
Cao et al. 2018	Significantly higher in DKD than in T2DM with normo-and microalbuminuria, and in healthy control	-	No significant difference between DM patients with normo- and nondiabetic controls; levels of TNF-α increased steadily with progression from normo- to macroalbuminuria
Fathy et al. 2018	Higher in DKD than T2DM	-	-
Gohda et al. 2018	Significantly higher in normoalbuminuric-DKD than T2DM	-	-
Kamei et al. 2018	Significantly higher in T2DM patients with lower eGFR (< 60 mL/min/1.73 m^2)	-	-
Karadag et al. 2016	Significantly higher in group 3 patients (lowest eGFR, highest albuminuria)	-	-
Kung et al. 2010	Significantly higher in DN than T2DM and healthy control	-	-
Lampropoulou et al. 2014	Similar levels in T2DM with micro and normoalbuminuria	Significantly higher in micro- than in normo-	No association between serum and urinary TNF-α levels
Lampropoulou et al. 2020	-	Significantly higher in T2DM with moderate and severe albuminuria than those with no albuminuria	Serum and urinary TNF-α levels showed a borderline positive correlation
Li et al. 2016	Significantly higher in DN than healthy control	-	-
Lu et al. 2011	Significantly higher in DN and T2DM than healthy control; significantly higher in DN than T2DM	-	-
Neolofar et al. 2018	Significantly higher in DN than T2DM and healthy control	-	-
Sangoi et al. 2016	-	Significantly higher in DKD than T2DM	-
Sil Yeo et al. 2020	Significantly higher in DN than T2DM	-	-
Wu et al. 2010	Significantly higher in DN than T2DM	Significantly higher in DN than T2DM	Higher levels in urine than serum
Wu et al. 2013	-	Significantly higher in macro and micro than in normo- and healthy control	-
Zhang et al. 2019	-	Significantly higher in macro- and micro- than in normo- and healthy control	-

TNF-α, tumor necrosis factor-alpha; *DN*, diabetic nephropathy; *DKD*, diabetic kidney disease; *T2DM*, type 2 diabetes mellitus

Table 3 Summary of findings on IFN-γ

Author	Publication year	Cytokine levels		
		Serum/plasma	Urine	Other findings
Aly et al	2020	Significantly higher in DKD than T2DM and healthy control	-	Higher in T2DM than healthy control
Fathy et al	2018	Higher in DKD than T2DM and healthy control	-	Higher in T2DM than healthy control
Sangoi et al	2016	-	Significantly higher in DKD than T2DM	-
Wu et al	2010	Significantly higher in DN than T2DM	-	-

IFN-γ, interferon-gamma; *DN*, diabetic nephropathy; *DKD*, diabetic kidney disease; *T2DM*, type 2 diabetes mellitus

Table 4 Association of TNF-α with renal markers

Author	Publication year	Association with renal markers	
		eGFR/creatinine clearance	ACR/UAE
Gohda et al	2018	Serum TNF-a was negatively correlated with eGFR	-
Kamei et al	2018	-	Serum TNF-a was positively correlated with ACR
Kung et al	2010	Plasma TNF-a was negatively correlated with creatinine clearance	-
Lampropoulou et al	2014	No correlation for serum and urinary TNF-a	Urinary TNF-a has a positive correlation with ACR (no correlation for serum TNF-a)
Lampropoulou et al	2020	Urinary TNF-a has a negative correlation with eGFR	Urinary TNF-a has a positive correlation with ACR (no correlation for serum TNF-a)
Li et al	2016	-	Serum TNF-a has a positive correlation with ACR
Wu et al	2010	Plasma TNF-α was negatively correlated with creatinine clearance	-
Wu et al	2013	-	u-TCR was significantly correlated with u-ACR
Zhang et al	2019	-	Urinary TNF-α was significantly related with ACR

TNF-α, tumor necrosis factor-alpha; *eGFR*, estimated glomerular filtration rate; *ACR*, albumin-creatinine ratio

correlated with ACR and UAE. This strengthens the hypothesis that inflammatory cytokines, specifically TNF-α, reflect the same manifestations as that of traditional renal markers. In summary, the findings of this review highlight the potential of TNF-α and IFN-γ as clinical biomarkers for renal diseases such as diabetic nephropathy.

Association of inflammatory cytokines with DN

Cytokines are immune system effectors with beneficial regulatory roles in the body. However, to our knowledge, there are no published data relating these cytokines with different conditions, including DM and its complications. According to initial studies, peritoneal macrophages cultured within the glomerular basement membranes of diabetic rats produced significantly higher amounts of TNF-α and IL-1 than normal rats. Further studies demonstrated that their involvement was not limited to the pathogenesis of DM since these were also found to be involved in the progression of DN [26, 27]. This may be explained by their role in neutrophil infiltration, thickening of the basement membrane, activation of apoptosis, and altered glomerular hemodynamics [28]. Another study noted that their formation occurs via the advanced glycation end products (AGE) pathway and protein kinase C (PKC) pathway, which also eventually leads to DN development [29]. Recent studies have also noted that serum inflammatory and immune mediators are elevated in DN compared with healthy controls [8, 30]. These findings further support the potential association between inflammatory cytokines and diabetic nephropathy.

Association of TNF-α with DN

TNF-α is a multifunctional cytokine associated with cell-mediated immune response [31]. Gupta et al. stated that TNF-α is linked with DN's clinical development and progression due to its increased synthesis during inflammation [32]. This was supported by the study of Real et al. wherein

it was demonstrated that the upregulation of TNF-α plays a role in structural renal injury and nephrotoxicity in a diabetic state [33]. The researches above are aligned with the present study wherein it was determined that TNF-α levels of DN patients were significantly increased in comparison to T2DM and healthy participants. Various renal effects of this cytokine contributory to the development of nephrotic injury include hemodynamic alterations, impairment of glomerular permeability, procoagulant effects, recruitment of polymorphonuclear leukocytes, and cell death [34].

Association of IFN-γ with DN

IFN-γ, together with other proinflammatory cytokines, is produced by activated immune cells, including T helper cells [12]. This specific cytokine has a pivotal role in host defense due to its antiproliferative and immunomodulatory effects on cells [35]. However, its pro-inflammatory nature also enables it to become a pathogenic mediator that may contribute to organ damage through the activation of macrophages, modulation of effector T-cell responses, and upregulation of chemokines, increasing immune cell infiltration [11, 36]. This may explain its potential role in renal diseases. Aside from the aforementioned effects, it may also cause modifications in the renal structure and the permeability of the glomerular endothelium, and these may play a part in the advancement of kidney diseases such as DN [11]. These mechanisms may elucidate the findings of previous studies that stated that IFN-γ plasma/serum levels were significantly higher in diabetic patients with kidney disease compared to diabetic patients without kidney disease and healthy individuals [8, 12].

Difference from other studies

Based on our knowledge, this is the first systematic review that focused on the association of IFN-γ with diabetic nephropathy. With regards to TNF-α, this paper collated the findings of more studies from different countries in order to investigate its association with DN development further.

Limitations of the study

This systematic review is subject to certain limitations due to the included studies' coverage. First, the participants from the different studies are not homogenous since ethnicity was not considered. The diagnostic criteria used by the researchers for DN detection may also differ from country to country. Lastly, the duration of the DN patients' condition was not considered in this study.

Conclusion

Most studies have established that cytokine levels, particularly TNF-α and IFN-γ, were higher among DN patients than T2DM patients with no renal complications and healthy individuals. Others have also determined the correlation of TNF-α with certain renal markers such as eGFR, ACR, and UAE. However, data regarding their diagnostic accuracy are still limited. The association of serum cytokine levels to their urinary counterparts is yet to be established as well since previous studies obtained conflicting results. Thus, future studies may focus on these so as to definitively determine their potential use in diagnosing DN.

Author contribution All authors have contributed substantially to collecting and analyzing the data and in writing and critically revising the manuscript.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

Human and animal rights This article does not contain any studies with human or animal subjects.

Informed consent Not applicable.

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REVIEW ARTICLE

Effect of aerobic training on baroreflex sensitivity, heart rate recovery, and heart rate variability in type 2 diabetes: a systematic review

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Abstract

Objective Dysfunction of cardiac autonomic control (CAC) in people with type 2 diabetes mellitus (T2DM) is a predictor of cardiovascular disease mortality. The purpose of this study was to systematically review the literature on the effects of aerobic exercise training on CAC in people with T2DM.

Methods Electronic databases (MEDLINE, Scopus, and Web of Science) were systematically searched to retrieve relevant evidence from inception to April 2021. Studies investigating the effect of aerobic training on CAC assessed by heart rate variability, baroreflex sensitivity, and heart rate recovery were included in the review. Two authors independently assessed trial quality using the PEDro scale & extracted the following data: study design and participant characteristics, intervention protocol, outcome measures, & major findings of the study.

Results Ten studies enrolling 651 subjects met the eligibility criteria. Out of the 10 studies, only 4 were randomized controlled trials (RCTs). Eight out of ten studies showed a positive change in cardiac autonomic function. PEDro was used for quality and characteristic assessment which revealed fair-quality evidence. Six studies showed a high risk of bias according to the Cochrane risk of bias tool.

Conclusion Evidence from this systematic review suggests that aerobic training may positively influence CAC in people with T2DM. However, further large-scale, robust RCTs are required.

Keywords Heart rate variability · Baroreflex sensitivity · Cardiac autonomic control · Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is associated with a number of problems, the most common of which is cardiac autonomic neuropathy (CAN). The dysfunction of autonomic nerve fibers that supply the heart, causes aberrant control of heart rate and cardiovascular dynamics [1]. There is an overall increase in cardiovascular disease burden due to the high prevalence of CAN and its association with cardiovascular events in T2DM [2]. The prevalence of CAN ranges from 25 to 75% in T2DM [3, 4].

The regulation of heart function by the sympathetic & parasympathetic parts of the autonomic nervous system (ANS) is identified as cardiac autonomic control (CAC) [5]. Dysfunction of CAC is a predictor of cardiovascular disease mortality [6]. It can be diagnosed clinically by assessing heart rate variability (HRV), baroreflex sensitivity (BRS), and postexercise heart rate recovery (HRR) [7, 8]. All these outcome measures reflect both sympathetic & parasympathetic activity. HRV designates the oscillations in the interval between consecutive heartbeats [9] and is based on the interactions between the sympathetic & parasympathetic nervous systems [10] whereas baroreflex is an integrated sympathetic and parasympathetic autonomic reflex [11]. Abnormal HRR is related to decreased vagal activity [12, 13] and reflects the balance of reactivation of the parasympathetic nervous system and withdrawal of the sympathetic nervous system [14]. HRV, BRS, & HRR can be employed to identify subclinical CAN even before the appearance of any signs & symptoms [8, 15].

In those with T2DM, lifestyle changes enhance metabolic control, anthropometric measures, and blood pressure, especially in those with poorly controlled diabetes [16, 17]. Exercise is advised as one of the primary management measures for T2DM people, and it is an important

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part of all T2DM and obesity prevention programs, along with diet and behavior change [18]. Exercise training reduces HbA1c levels [19], and a structured exercise intervention program was effective in reducing insulin resistance [20].

Among different types of training, there are a lot of studies that support the beneficial role of aerobic training (AT) on endothelial function [21], blood pressure [22], nerve function [23], systemic inflammation [24], cardiorespiratory fitness [25], glycemic control [25, 26], visceral fat in obese T2DM patients [27]. Exercise is responsible for the modulation of the autonomic control of the heart [28]. In the case of T2DM, a systematic review reported enhancement in cardiac autonomic function following exercise training [29]. As reported in a systematic review and meta-analysis that exercise intervention enhanced HRV which is an indicator of enhancement in the autonomic function in people with T2DM [30]. Despite the fact that various studies have looked at the role of AT on CAC in human volunteers [31–33], no consensus has been reached, highlighting the urgent need to critically analyze the available research. As a result, this review aimed to assess studies to evaluate the effects of AT on CAC in T2DM in a thorough and systematic manner.

Methods

The eligibility criteria and methods of analysis were prespecified and registered (CRD42021270541) on PROSPERO (International Prospective Register of Systematic Reviews). This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [34].

Eligibility criteria

Full-text studies were included in this review if they fulfilled the following eligibility criteria: Participants*:* Individuals older than 18 years and have had a diagnosis of T2DM for more than 1 year. Intervention*:* We included studies administering aerobic exercise for at least 4 weeks. Studies comprising other forms of exercise training (resistance exercise, yoga, tai chi, breathing exercises) was excluded. Comparison: No restrictions were made on the comparison or control group. Outcome measures*:* Studies examining CAC either through HRV, HRR, and BRS. Types of study: Randomized controlled trials (RCTs), non-randomized controlled trials (nRCTs), and cross-over controlled trials were included in the study. Trials published in languages other than English were not considered in this review. Studies on animals and type 1 diabetes mellitus were excluded.

Search strategy

The following electronic databases: MEDLINE (accessed by PubMed), Web of Science (Web of Science Core Collection), and Scopus were used to perform a systematic search. The searches were carried out from inception till April 2021. Random search terms used were a combination of keywords, "aerobic exercise, aerobic training, autonomic function, heart rate recovery, baroreflex sensitivity, heart rate variability, cardiac autonomic control, type 2 diabetes mellitus". Boolean operators 'OR and 'AND' were used. The search was carried out from June 2021 to July 2021 (Fig. 1).

Selection process

The review process (screening the abstracts & titles of the retrieved records & full-text reading) was performed by two authors independently (SP & MA). Any conflict in the selection process was resolved by the third reviewer (MN).

Data extraction

Study characteristics were extracted from each of the trials including trial characteristics, the subjects, the intervention, control treatment, and main outcome measures by two authors (SP and MA). One author (SP) searched and extracted all data. Any study that did not fulfill the selection criteria was excluded. Any conflicts between the two reviewers (SP & MA) were resolved by consensus or in consultation with a third reviewer (MN).

Quality assessment of trials

Two authors (SP & MA) used PEDro scale to evaluate the methodological quality of the included clinical trials [35]. Any conflict over any criterion of PEDro was evaluated again by the two authors (SP & MA). Unresolved disagreements were taken to the third author (MN), and a final consensus was reached. Each criterion of PEDro was rated either yes (score = 1) or no (score = 0). A total score was obtained by summing the responses. Studies scoring ˃8 were classified as excellent quality trials, trials scoring between 6–8 were considered as good quality studies, trials that scored between 4–5 were considered as fair quality trials, and studies that scored < 4 falls into the category of poor quality trials [36]. The authors have experience of more than 5 years in using PEDro scale for the methodological quality assessment of the trials. The tutorials available at https://pedro.org.au/english/learn/tutorial/ were used as a resource [37].

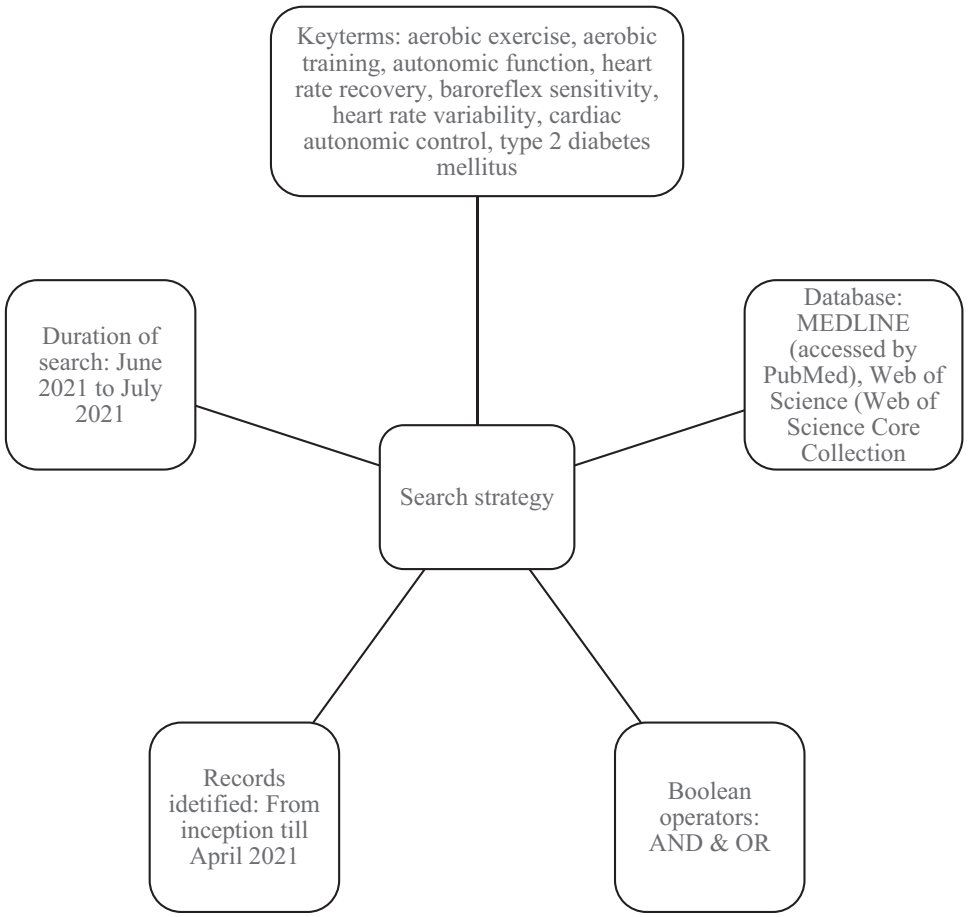

Fig. 1 A schematic presentation of search strategy

Risk of bias assessment

Cochrane risk of bias assessment tool was used to evaluate the risk of bias. Two authors (SP & MA) applied the risk of bias tool independently on the included full-text trials. Any conflict between the two authors (SP & MA) was resolved by discussing with the third author (MN). Every single domain of the tool was graded as either low, unclear, or high. Each study was categorized into three criteria which are low, moderate, and high risk of bias. When all the domains score low, they fulfill the criteria of low risk of bias, when one domain scores high or two domains score unclear, the study falls into the category of moderate risk of bias, and high risk of bias is considered when more than one domain score high or more than two domains score unclear [38].

Results

Study selection

Out of the total 1178 records identified, 20 duplicates were removed manually, and the remaining records were screened. Two authors (SP & MA) independently evaluated 10 articles (4 RCTs, & 6 nRCTs) that met the eligibility criteria for characteristics of the trials and examination of methodological quality. The selection of the studies was shown in the PRISMA 2020 flow diagram (Fig. 2).

Study characteristics

Included studies were published from 2006 to 2018. Four RCTs [39–42] and six nRCTs [31–33, 43–45] were included in this review. The control groups in the included trials either received other type of exercise or no treatment. The details regarding the study characteristics, study design, and intervention were shown in Tables 1 and 2.

10 included trials in this review consisted of 651 participants, with a sample size ranging from 22 [39] to 200 [44]. The majority of the studies examined middle-aged adults (age 40–60 years) [31–33, 45]. None of the trials had young adults as participants. One study recruited only women [43], while 9 trials had both males & females.

One of the groups in each included trials employed AT as an intervention. Treadmills were used for AT in four of the studies [32, 33, 39, 45] while only one study reported the use of a cycle ergometer [40]. Two studies used a combination of walking & treadmill for AT [31, 43]. The length of the

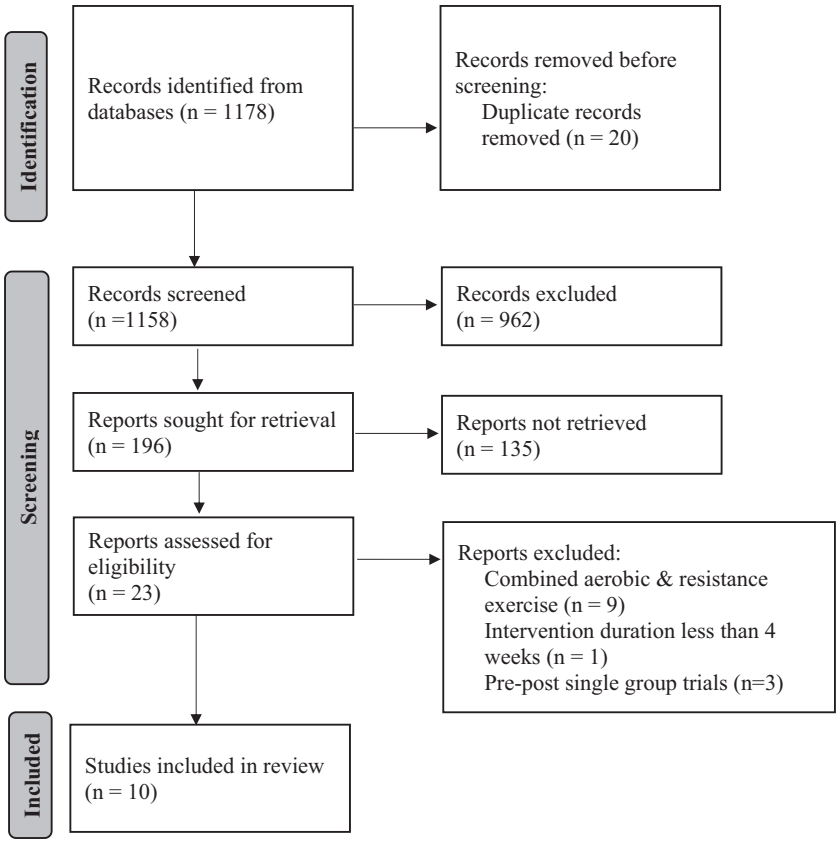

Fig. 2 Flowchart of search strategy, retrieval of articles, exclusion, inclusion, and evidence synthesis

exercise session/day ranged from 30 to 60 min, 2 to 5 times per week, and the duration of the intervention falls between 6 weeks to 8 months.

Four trials assessed & reported HRV [40, 42, 44, 45], two studies assessed BRS [32, 41], and four studies have reported both HRV & BRS [31, 33, 39, 43]. All trials reported linear HRV indices. Almost all studies measure HRV in resting conditions [31, 39, 42, 43, 45], while one trial measured HRV on lying to standing, & during the Valsalva maneuver [40], and one study did not report the condition in which HRV was measured [44].

Assessment of methodological quality of included studies

Eight trials were of fair quality and two were of good quality [41, 44]. The average PEDro score of the included trials was 5.4. Two studies included the blinding of the assessor [41, 44]. The majority of the trials on the other hand did not blind either of the subjects, the therapist, or the assessor. Seven trials applied intention to treat analysis [31–33, 41, 43–45]. Six out of ten studies reported dropouts [31, 39–41, 43, 44]. All the studies reported between-group differences and point estimates & variability. None of the included trials provided any information on power and sample size calculation (Table 3).

Risk of bias assessment of trials

According to the Cochrane risk of bias tool, one study had a low risk of bias [40], while three studies were judged to have had a moderate risk of bias [39, 41, 42]. Six studies showed a high risk of bias for not describing the randomization & allocation process, participants, assessors, & personnel blinding [31–33, 43–45] (Table 4) (Fig. 3).

Effect size calculation

The within-trial effect size (ES) was calculated. ES was not calculated for those studies where the data reported were incomplete, unclear, or not presented descriptively. An ES of 2.14 was obtained when the effect of AT on HRV in T2DM was evaluated [42]. Another study showed an ES of -0.55 [39]. The majority of the studies examining the effect of AT on obese individuals with and without T2DM showed an ES ranging from small to moderate effect [33, 43, 45]. In two studies [32, 44], the ES obtained was less than 0.1 when the effect of AT on different outcome measures of HRV (Tables 1 and 2). Due to insufficient data from the included trials, confidence interval (CI) was not calculated.

Table 1 Characteristics of included RCTs ($n=4$)

Author, year	Participants	Intervention	Outcome measures	Main findings	Effect size
Bellia et al. [39]	22 sedentary over-weight T2D patients Age: 57 ± 7 M/F: 16/6	Aerobic interval training for 12 weeks (4 min walk on a treadmill at 70–80% of HRmax, 2–4 repetitions/session interspersed with 3 min recovery to 40–50% of HRmax, along with 10 min of warm-up & cool-down at 40–60% of HRmax) Control: 10,000 steps/day or 70,000 steps/week of non-supervised brisk walking	BRS Resting HRV: Mean R-R interval	Significant positive changes in BRS & HRV	BRS: -0.55
Sridhar et al. [42]	105 T2D patients Age: 49.45 ± 2.75 (non-exercised group); 61.78 ± 3.10)	Intervention: 12 months of aerobic training for 30 min per session along with 5 min of warm-up & 10 min of cool-down Control: No supervised training was provided to patients in the control group	Resting HRV: R-R interval	↑ in HRV	HRV: 2.14
Bellavere et al. [40]	30 subjects with T2D Age: 57.1 ± 1.6 (aerobic group); 53.4 ± 2.0 (resistance group)	4 months of aerobic resistance training, 3 times/week AT: exercise on a cardiovascular ergometer for 60 min/session, progressed to 60–65% of HRR; RT: nine different exercises of major muscles for 60 min/session, progressed to 3 sets of 10 repetitions at 70–80% of 1RM with 1 min of recovery between set 4 months of aerobic + resistance training, 3 times/week	HRV in deep breathing, lying to standing, Valsalva maneuver, BP changes on 2 min standing, & after 5 min lying: Total PSA, LF component, HF component, LF/HF ratio	↑ in HRV	*
Madden et al. [41]	39 older adults with T2D Age: 71.5 ± 0.7	Patients exercised for 60 min (10 min of warm-up, 20 min of treadmill session, 20 min of cycle ergometer, & 10 min of cooldown) for 12 weeks, 3 times/week at 50% to 60^ of maximal HR for the first 2 weeks. Progression was made by exercising at 80%—85% of HR max Patients exercised non-strenuous core & non-strength training 3 times/week for 12 weeks	BRS	↑ in BRS	*

T2D Type 2 diabetes, *M/F* Male/female, *BRS* Baroreflex sensitivity, *HRV* Heart rate variability, *min* minutes, *HRmax* Maximal heart rate, *HRR* Heart rate reserve, *BP* Blood pressure, *PSA* Power spectral analysis, *LF* Low frequency, *HF* High frequency, *HR* heart rate; * the data reported were incomplete, unclear, or not presented descriptively

Table 2 Characteristics of included nRCTs ($n=6$)

Author, year	Participants	Intervention	Outcome measures	Main findings	Effect size
Kanaley et al. [31]	64 obese patients with & without T2D Age: 50.0 ± 1.6 (Obese with T2D); 49.0 ± 0.9 (Obese without T2D)	Participants trained at 65% of VO_2 peak for 16 weeks For the first 8 weeks, participants walked for 30 min/day for 4 days/week, duration was increased to 45 min by 8 weeks	BRS HRV: RR interval, LF, HF, LF/HF ratio	No change in LF/HF ratio & BRS after training	*
Kanaley et al. [32]	22 patients with T2D Age: 48.1 ± 1.04 (women); 49.9 ± 1.6 (men)	Patients walk on a treadmill for 30 min/day, 4 days/week at 65% of VO_2 peak. Progression was made by increasing the exercise duration from 30 min/day to 45 min/day at 8th week	BRS, HF_{RRI} (nu), $ln LF_{RRI}/HF_{RRI}$	No change in BRS	HF_{RRI} (nu): 0.08 $ln LF_{RRI}/HF_{RRI}$: -0.07 BRS: -0.2
Figueroa et al. [43]	28 obese patients with & without T2D Age: 50 ± 1 (T2D); 48 ± 2 (without T2D)	16 weeks of aerobic training, 3 times/week at 65% of VO2max. Progression was made by increasing the walking duration from 30 to 45 min/day at the 8th week	BRS HRV at rest & after 20 min of exercise (65% of VO2 peak): HF power, LF power, LF/HF ratio	↑ in HRV & BRS	HF power: 0.33 LF power: 0.25 LF/HF (ratio): 0.04 BRS: -1
Pagkalos et al. [44]	200 patients with T2D Age: 56.2 ± 5.8 (group A); 55.8 ± 5.6 (group B)	Participants performed aerobic exercise 3 times/week for 6 months at 70% to 85% of the HRR, where is session was 45 min with a 10–15 min warm-up/cool-down	HRV: SDNN, rMSSD, pNN50, HF power, LF power	↑ in SDNN, rMSSD, pNN50 in both the group; ↑ in HF power in group A	LF: -0.33HF: 0.21 LF:HF ratio: -0.03 SDNN: -1.76 rMSSd: -0.88
Goulopoulou et al. [33]	62 obese patients with & without T2D Age: 50 ± 1 (with T2D); 49 ± 1 (without T2D)	Patients exercised for 16 weeks, 4 days/week at 65% of VO2 peak. For the first 8 weeks, patients exercised for 30 min, after that exercise duration was gradually increased so that for the last 6 weeks, the exercise duration was 45 min	BRS Resting HRV: RR interval, LF power, HF power	↑ in LF power, LF/HF ratio	BRS: 1.91 LF_{RRI}/HF_{RRI}: -0.5 TP_{RRI}: 3.20
Baynard et al. [45]	59 obese subjects with & without T2D Age: 40–60 years old	Subjects performed 30 min of aerobic exercise at 65% of VO2 peak for 16 weeks, 4 days/week Exercise duration was increased over 2 weeks	Resting HRV: RR interval, LF power, HF power	No change in LF, ↑ in HF	LF_{ln} (LCVM): 0.43 HF_{ln} (LCVM): 0.28 LF_{ln} (HCVM): 0.56 HF_{ln}(HCVM): 1.03

T2D Type 2 Diabetes, *AIT* Aerobic Interval Training, *BRS* Baroreflex Sensitivity, *HRV* Heart Rate Variability, *HRmax* maximum heart rate, *VO_2 max* maximum rate of oxygen consumption, *HF* high frequency, *LF* low frequency, *HF_{RRI} (nu)* High frequency power using the R-R interval (normalized units), *ln*LF_{RRI} Low frequency R-R interval, *HRR* Heart rate reserve, *B.P* Blood Pressure, *PSA* Power spectral analysis, *1RM* 1 Repetition Maximum, *SDNN* Standard Deviation of normal to normal NN interval, *rMSSD* root mean square of successive differences between adjacent inter-beat (R-R), *pNN50* percentage of the difference between adjacent filtered RR intervals which were greater than 50 ms for the entire analysis.; natural log transformation; *LCVM* Low cardiovagal modulation, *HCVM* High cardiovagal modulation; *: the data reported were incomplete, unclear, or not presented descriptively

Table 3 Quality scoring of included trials using PEDro scale ($n = 10$)

Trials	Random Allocation	Concealed allocation	Group similarity at baseline	Participant blinding	Therapist blinding	Assessor blinding	< 15% Drop-outs	Intention to treat analysis	Between-group differences reported	Point estimates and variability reported	Total score	Quality
Bellia et al. [39]	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5/10	Fair
Figueroa et al. [43]	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	5/10	Fair
Sridhar et al. [42]	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5/10	Fair
Bellavere et al. [40]	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5/10	Fair
Kanaley et al. [31]	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	5/10	Fair
Kanaley et al. [32]	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	5/10	Fair
Madden et al. [41]	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	7/10	Good
Pagkalos et al. [44]	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	6/10	Good
Goulopoulou et al. [33]	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	5/10	Fair
Baynard et al. [45]	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	5/10	Fair

Table 4 Risk of bias assessment of included trials using Cochrane risk of bias tool ($n = 10$)

Study	Random sequence generation	Allocation concealment	Selective reporting	Blinding (performance bias)	Blinding (Detection bias)	Incomplete outcome data	Other sources of bias	Overall
Bellia et al. [39]	Low	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Moderate
Figueroa et al. [43]	High	High	Low	High	High	Low	Low	High
Sridhar et al. [42]	Low	Unclear	Low	Unclear	Unclear	Low	Low	Moderate
Bellavere et al. [40]	Low	Low	Low	Low	Unclear	Low	Low	Low
Kanaley et al. [31]	High	High	Low	High	High	Low	Low	High
Kanaley et al. [32]	High	High	Low	High	High	Low	Low	High
Madden et al. [41]	Low	Unclear	Low	Unclear	Low	Low	Low	Moderate
Pagkalos et al. [44]	High	High	Low	High	High	Low	Low	High
Goulopoulou et al. [33]	High	High	Low	High	High	Low	Low	High
Baynard et al. [45]	Unclear	High	Low	High	High	Low	Low	High

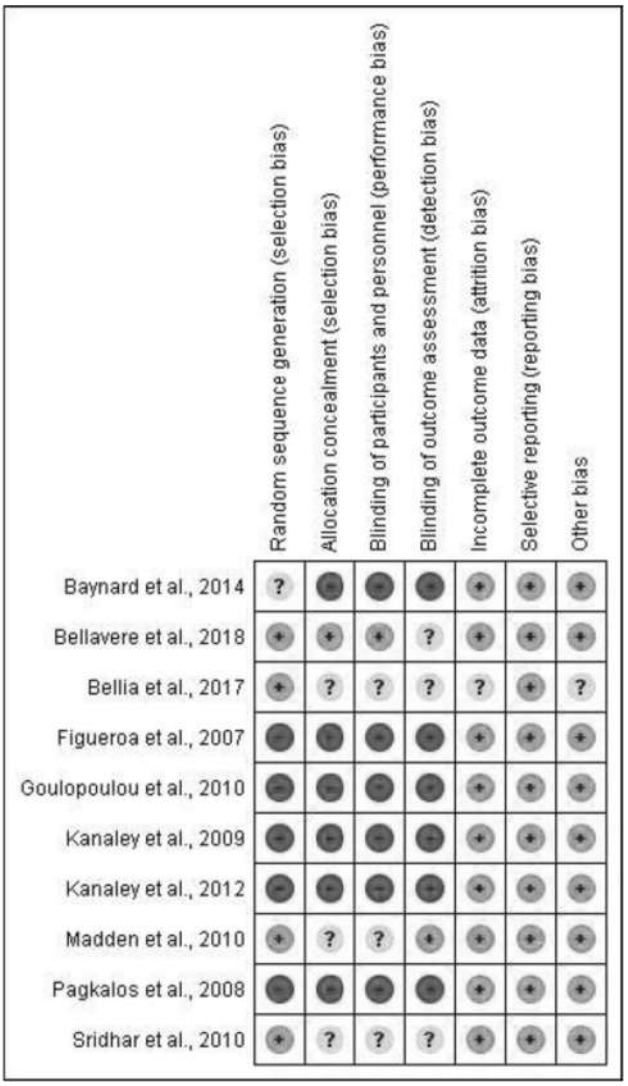

Fig. 3 Summary of the risk of bias for included trials where green indicates low risk of bias, yellow indicates unclear, and red indicates high risk of bias

Discussion

This systematic review used a rigorous methodology to deliver information regarding the effectiveness of AT in modulating CAC in people with T2DM. The review included 10 studies that met the eligibility criteria. Evidence from included studies indicated that AT results in positive adaptations in CAC in people with T2DM.

This review comprised four RCTs, in which three of the studies, AT was compared with no exercise [39, 41, 42], & only one study compared aerobic training with resistance training [40]. All four studies showed a positive change in cardiac autonomic function following AT. Bellia & colleagues in their study used 12 weeks of aerobic training, 2–3 times/week. They reported an increase in HRV & BRS [39]. Similarly, an improvement in BRS was found in the aerobic group, where T2DM patients use a treadmill & cycle ergometer for AT for 12 weeks, 3 times/week [41]. Two studies used HRV only as a measure of CAC, & reported an improvement in HRV following 4 months [40] & 12 months [42] of AT respectively.

Six clinical trials were identified nRCT [31–33, 43–45]. Out of these 6 nRCTs, 4 studies compared the effect of AT on obese individuals with and without T2D [31, 33, 43, 45], one study compared the AT effect between men & women [32], and one study compared T2DM with and without definite CAN [44]. Of the 6 nRCTs, four showed a positive change in CAC following AT [31, 43–45]. A study done by Pagkalos and colleagues suggested that AT for 6 months at 70 to 80% of heart rate reserve (HRR) for 45 min and progressing to 75 min has an effect on modulating the HRV (time & frequency domain indices) [44]. One study evaluating only obese females with and without T2DM indicated that 16 weeks of AT, where subjects were walking at 65% of VO_2 peak, 3 days/week, was able to produce positive changes in CAC, which was evident by improvement

in frequency domain indices of HRV [43]. Another nRCT on obese individuals with and without T2DM also showed enhancement in frequency domain indices of HRV following 16 weeks of AT, where participants were instructed to walk at 65% of VO_2 peak for 30 min which was progressed to 45 min during 11 to 16 weeks of training [45]. The same intensity and duration were used by Kanaley & colleagues and produced positive changes in BRS [32]. Two studies reported no improvement in BRS [33] & HRV [31] after 16 weeks of AT in obese T2DM patients. This can be explained by the decreased response of ANS in people with T2DM indicating that if obese people develop T2DM, then the dysfunction in ANS is either irreversible or there is a need for a more aggressive exercise training program [31].

The exact mechanism behind the enhancement in autonomic function is unknown. Some mediators were supposed to play a role in enhancing cardiac vagal tone in response to exercise training. Exercise training increases nitric oxide bioavailability which is known to affect cardiac vagal tone and sympathetic influence [46]. Exercise training has been shown to decrease angiotensin II levels which has a role in inhibiting cardiac vagal activity [47]. In addition, exercise training has anti-inflammatory effects which help in maintaining autonomic balance [28] disturbed in cases of chronic inflammation [48]. In people with T2DM, a decrease in parasympathetic activity combined with an increase in sympathetic activity results in an imbalance of the sympathetic/parasympathetic tone. Regular exercise reduces mortality and may protect against adverse cardiovascular events in people with T2DM [49]. In individuals with T2DM, variations in structure and function of arterial baroreflex occur, and only functional changes respond to exercise [50]. The central resetting of the baroreflex causes deterioration of functioning of the arterial baroreflex associated with diabetes [51]. Release of endothelial factors [52] and vascular compliance changes in the carotid sinus [53] are outcomes of exercise-induced shear stress and contribute to the normalization of baroreflex following AT. The increase in HRV indices can be attributed to the direct involvement of the sinus node as a result of the bradycardia seen during rest [54]. Afferent signals from the chemoreceptors in the muscles have been shown to control both sympathetic and vagal outflow to the heart via metaboreflex; thus autonomic activity is indirectly controlled by the working muscles [55]. The changes in the level of catecholamine from exercise might be responsible for autonomic modulation [56].

There are certain factors like the duration of diabetes [57], presence of hypertension [58], and obesity [59] that affect the exercise training outcomes in T2DM patients. Kanaley & colleagues reported that no gender differences were found in outcome measures of HRV & hemodynamic parameters after receiving 16 weeks of AT [32]. According to one study, greater effects of AT on HRV was reported in T2DM with hypertension group than in T2DM alone [42]. Two trials reported a positive change in sympathetic modulation & vagal modulation after receiving 16 weeks of AT in obese individuals independent of diabetes status [31, 33]. One study demonstrated that the change in outcome measures of cardiac autonomic function after 16 weeks of exercise in obese T2DM women did not differ from non-diabetic obese women [43].

It is important to note that, in all the included studies, majority of the items were compromised because none of the studies reported concealed allocation. Results of the study were also affected by absence of randomization as reported in six studies [31–33, 43–45]. A total of three trials failed to report intention to treat analysis [39, 40, 42]. The calculation of power and sample size was not mentioned in any of the trials. As a result, it is unclear if the studies had adequate sample power to detect variations in the outcomes. None of the studies reported blinding of participants and instructor blinding. In addition, only 2 [41, 44] studies had incorporated assessor blinding. The absence of participant and instructor blinding can be characterized as a bias of conduction & execution. However, this can be reduced by adding the blinding of the assessor.

Strengths and limitations

This is the first systematic review to look specifically at the effect of AT on CAC in people with T2DM. AT enhanced CAC in people with T2DM which was assessed using the HRV, BRS, and HRR which may reflect an improvement in the ANS function. Therefore, the findings of this review might help clinicians to incorporate AT programs for T2DM patients. The present review has some limitations. The RCTs, and nRCTs were also considered in this review in order to improve the representation of the existing literature, because randomization is a key part of studies, including the nRCTs in the review may have confounded the results. Due to insufficient data reporting meta-analysis was not done and the inability to calculate the CI for ES led to non-generalizability of the study findings. Using the PEDro scale, we discovered that the overall quality of the trials included in this review was low. Higher-quality trials with a randomized design and measures to reduce the effect of confounding variables are needed to corroborate these findings.

Recommendations for future research

A priori power analysis was not reported in any of the trials included in the review. Proper power and sample size calculation should be conducted and reported in future studies. According to this review, there is evidence pointing to the efficacy of AT in improving cardiac autonomic regulation. The strength of this evidence, however, is low due to the

scarcity of RCTs and high-quality studies. Furthermore, more studies with proper control group and randomization procedures are required to give conclusive evidence in support of the claim.

Conclusion

More large-scale, robust studies using randomization procedures that are appropriate enough to identify clinically relevant differences should be developed. Hence, high-quality studies are necessary to corroborate the review's findings. According to this review, the findings of the majority of included articles support the recommendation to incorporate aerobic exercise as a treatment strategy for managing CAC in T2DM people. The strength of this evidence is limited due to the scarcity of RCTs. Incorporating CAC measures like BRS, HRV, and HRR into exercise training protocols in diabetic people give information about the efficacy of AT. In addition, a higher number of clinical trials at various stages of CAN with various modes of aerobic exercise training are needed to offer clear proof for the same.

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Data Availability Data will be available on a reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Assessment of equations estimating average glucose among patients with diabetic kidney disease before dialysis

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Abstract

Objective Estimation of average glucose (AG) from hemoglobin A1c (HbA1c) helps guide diabetes management, and thus several AG-HbA1c equations have been constructed. However, it is not clear whether estimated AG calculated from existing AG-HbA1c equations could evaluate glycemic control in patients diabetic kidney disease (DKD) before dialysis. This study is aimed at evaluating the accuracy of estimated AG which is calculated from existing equations to assess glycemic control in DKD before dialysis. Additionally, we examined the relationship between AG and HbA1c in DKD before dialysis.

Methods In this retrospective study, we collected data of 71 Chinese patients with DKD before dialysis who had a complete flash glucose monitoring (FGM) data during hospitalization in a single center between August 2018 and August 2021 by casually sampling. Measured AG was derived from the FGM system and compared to estimated AG derived from a frequently used AG-HbA1c equation (that developed in ADAG study), in addition to a formula established in CKD (that of ADAG-CKD equation). Performance of AG-HbA1c equations was evaluated by mean absolute difference (MAD)/mean absolute relative difference (MARD) and Bland–Altman test. Linear regression analysis was used to investigate the relationship of AG and HbA1c in DKD before dialysis.

Results Among the 71 DKD before dialysis, 80% were type 2 diabetes. The mean age was 57 ± 13.8 years, and mean eGFR was 66.3 ± 32.3 mL.min/(1.73 m^2). Mean HbA1c was 8.4 ± 2.2 (%), and measured AG was 150.2 ± 40.3 (mg/dL). Measured AG was significantly overestimated by equations ADAG and ADAG-CKD. Both ADAG and ADAG-CKD equations did not reflect the measured AG accurately (MAD 2.42 vs. 3.42 mmol/L; MARD 33.3% vs. 46.7%, respectively; $p < 0.01$). We examined the relationship between AG and HbA1c in DKD before dialysis as follows: AG (mmol/L) $= 0.48 \times$ HbA1c (%) $+ 4.36$. In addition, using multiple regression analysis, HbA1c, diabetes type, body mass index (BMI), and CKD stage explained 42% of the variability in measured AG ($r = 0.68$, $R^2 = 0.42$, $p < 0.01$).

Conclusions HbA1c-derived estimated AG from existing equations may not accurately reflect measured AG in patients with DKD before dialysis. Diabetes type, BMI, and CKD stage should be considered when translating HbA1c into AG value in DKD before dialysis. It is advisable to adjust the AG-HbA1c equations for target population.

Keywords Average glucose · Flash glucose monitoring · Diabetic kidney disease · HbA1c

Introduction

Hemoglobin A1c (HbA1c) reflects glycemic condition over the preceding 8–12 weeks [1, 2], which is widely considered as a reliable indicator of chronic glycemia [3] since the Diabetes Control and Complications Trial (DCCT) [4] and U.K. Prospective Diabetes Study (UKPDS). HbA1c is viewed as gold standard for glycemic control, often expressed as a percentage of glycated hemoglobin [5, 6]. However, daily glucose measurements and insulin dosage adjustment are based on direct glucose concentration from flash glucose monitoring (FGM) or self-monitoring blood glucose (SMBG), usually referred as average glucose (AG) [7]. The relationship of HbA1c and AG helps understand how daily blood glucose level is associated with HbA1c, which is beneficial for achieving the target HbA1c value in diabetic management.

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In fact, the relationship of HbA1c and AG may be different in patients with DKD from those with normal renal function, since numerous DKD-related factors affect HbA1c. DKD is often accompanied with anemia [6], and both false high or low HbA1c value could be observed in DKD due to iron and erythropoietin supplement [8, 9]. Additionally, uremic toxin accumulation and dialysis affect HbA1c by shortening the lifespan of red blood cells (RBC), leading to less chance for RBC to chemically react with blood glucose [10]. Moreover, an article has reported that urinary albumin was an independent determinant of bias of HbA1c [11]. Thus, not only estimated glomerular filtration rate (eGFR) but also urinary albumin should be considered when interpreting the relationship between AG and HbA1c.

The relationship of AG and HbA1c has been explored by ADAG [7] (A1c-Derived Average Glucose) study. ADAG study [7] consisted of a total of 507 participants, including both diabetes and non-diabetes, exploring the relationship between HbA1c and AG by 7-point blood glucose and continuous glucose monitoring (CGM). The eAG-HbA1c formula established from ADAG was the most frequently used currently. However, the accuracy of estimated AG from ADAG equation to reflect glycemia status needs to be examined in population with DKD before dialysis. Also, there are several studies enrolled type 1 or 2 diabetes mellitus (DM) to establish AG-HbA1c relationship in patients with diabetes [12, 13]. Previous studies have also translated the HbA1c into estimated AG for patients with chronic kidney disease (eGFR $\leq$ 60 mL/min/1.73 m^2) or those on dialysis [7, 14]. However, little research studies the relationship of HbA1c and AG in diabetic kidney disease (include those with urinary albumin but normal eGFR) before dialysis.

Therefore, our study has two purpose. First, we aimed to evaluate the accuracy of eAG calculated from two commonly used AG-HbA1c equations to reflect glycemic condition in DKD before dialysis. Second, we examined the relationship between AG and HbA1c in DKD before dialysis.

Materials and methods

Study design

In this retrospective study, 71 patients with DKD before dialysis who performed FGM during hospitalization in Nanfang Hospital of Southern Medical University in Guangzhou, China, from August 2018 to August 2021 were enrolled by random sampling. Inclusion criteria included (1) subjects with T1D or T2D aged 18–80, (2) had complete FGM data, and (3) had a stable glycemic control over the preceding 3 months before wearing FGM, as evidenced by a stable glucose-lowering regimen or two HbA1c values within 1% of each other in the previous 3 months. Excluded criteria included (1) any factors affect glycemia pregnant status, steroid or chemotherapy therapy; (2) conditions which would make hemoglobin unstable were excluded such as erythropoietin stimulating agent (ESA) usage, iron deficiency or supplement, hemoglobinopathy, hypersplenism, and blood loss or transfusions; (3) patients on dialysis or undergone kidney transplantation. Diagnosis of diabetes was based on the criteria recommended by WHO in 1999. DKD was defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 or a urine albumin/creatinine ratio (UACR) of $\geq$ 3 (mg/mmol). eGFR was calculated based on the CKD-EPI equation (Chronic Kidney Disease Epidemiology Collaboration equation) [15].

Sample size calculation

This study is aimed at comparing the difference between estimated AG from existing equation and measured AG. The sample size was determined using MedCalc 20.0.22 using data from previous study which reported the discordance of eAG from equation ADAG and measured blood glucose level in patients with type 2 diabetes mellitus [16] (mean of difference = 0.2 mmol/L, standard deviation of difference = 0.04 mmol/L, maximum difference = 0.3 mmol/L, setting beta = 0.2, and Alpha = 0.1), which suggested that approximately 73 subjects would be required.

Measured AG assessment

The FGM system (Abbott FreeStyle Libre, Abbott Diabetes Care, USA) was used to detect the interstitial glucose concentration every 5 min for at least 3 days. Measured AG was derived from a mean duration of 8.9 days CGM data. This factory-calibrated system does not require fingertip glucose calibration [17].

AG-HbA1c equations

The estimated AG was calculated from two published AG-HbA1c equations. Estimated AG is calculated from equation of ADAG and ADAG-CKD named AG_{ADAG} and $AG_{ADAG\text{-}CKD}$, respectively [7, 14]. ADAG equation was chosen since it is commonly used. The ADAG-CKD formula was selected because it was established from participants with chronic kidney disease. We compared estimated AG calculated from these two equations with

measured AG in patients with DKD before dialysis. Details of the two original studies were summarized in Table 1.

Anthropometric and clinical measurement

Demographics and clinical parameters were obtained from electronic health record. Fasting blood samples were tested for HbA1c, hemoglobin (Hb), serum albumin (Alb), and serum creatinine (Scr) on the same day CGM were performed. HbA1c was measured using high-performance liquid chromatography with a reference range of 4–6%. eGFR was calculated using the creatinine-only CKD-EPI equation. Urine albumin and urine creatinine were collected from spot urine immediately after blood sample was drawn.

Table 1 Baseline demographic and clinical characteristics of enrolled patients

Characteristics	Overall ($n=71$)
Age (years)	57.0 ± 13.8
Male, *n* (%)	44 (62%)
Current smoke, *n* (%)	21 (30%)
BMI, kg/m^2	23.7 ± 3.4
Diabetes type, type 2, *n* (%)	57 (80%)
Diabetes duration, *n* (%)	
> 10 years	38 (53%)
≤ 10 years	33 (47%)
Diabetes treatments, *n* (%)	
Insulin injection	24 (34%)
Oral agent (s)	24 (34%)
Insulin + oral agent (s)	23 (32%)
CGM duration (days)	8.0 (6.0–12.0)
SCR (μmol/L), median (IQR)	98.0 (73.0–146.0)
eGFR stage, *n* (%)	
≥ 30 mL/min/1.73 m^2	61 (86%)
< 30 mL/min/1.73 m^2	10 (14%)
eGFR (mL/min/1.73 m^2)	66.3 ± 32.3
UACR stage, *n* (%)	
Normal albuminuria	4 (6%)
Microalbuminuria	33 (46%)
Macroalbuminuria	34 (48%)
UACR (mg/mmol)	21.1 (5.8–77.7)
AG (mmol/L)	8.4 ± 2.2
AG (mg/dL)	150.2 ± 40.3
HbA1c (%)	8.4 ± 2.2
HbA1c (mmol/mol)	66.1 (48.6–82.5)
FBG (mmol/L)	7.3 (5.5–11.8)
Hemoglobin (g/L)	121.5 ± 26.5
Anemia, (n,%)	37 (52%)
Albumin (g/L)	37.9 (34.6–42.2)

Numerical variable was presented as mean ± SD or as median (quartile range). Categorical variable was presented as number of subjects (percentage). *BMI*, body mass index; *AG*, average glucose derived from CGM; *CGM*, continuous glucose monitoring; *eGFR*, estimated glomerular filtration rate; *UACR*; urinary albuminuria creatine ratio; *SCR*, serum creatine; *HbA1c*, hemoglobin A1c; *FBG*, fast blood glucose

Statistical analysis

The Statistical Package for Social Sciences 27.0 (SPSS, https://www.spss.com) program and MedCalc 20.0.22 was employed. Demographic and laboratory data were summarized using proportions, mean (standard deviation, SD), or median (interquartile range, IQR) as appropriate.

We assessed the accuracy of two published equations for average glucose estimation from three aspects as follows. First, for the difference between measured AG and estimated AG from published AG-HbA1c equations, paired *t*-test was used to detect this difference. Second, mean absolute difference (MAD) and mean absolute relative difference (MARD) were calculated. MAD was calculated as the absolute value of | measured AG- estimated AG| and MARD was calculated as follows: MARD (%) = (|mAG-eAG|)/mAG × 100%. Then, paired *t*-test was used to compare MAD and MARD of estimated AG from two formulas. Third, Bland–Altman type of analysis was used to evaluate the agreement between the eAG and the difference between mAG and eAG from two equations as previous study reported [7]. Additionally, linear regression analysis was used to establish the AG-HbA1c equation for DKD before dialysis.

Results

Baseline characteristics

Study flow chart was shown in Supplementary Fig. 1. This study screened a total of 202 non-pregnant adult patients who performed CGM in hospitalization, among whom 71 subjects met the inclusion and exclusion criteria. Among the included patients with DKD before dialysis, 62% were men, and 80% were type 2 diabetes. The mean age was (57 ± 13.8) years, and mean BMI was 23.7 ± 3.4 kg/m^2. Mean eGFR was 66.3 ± 32.3 mL.min/(1.73 m^2), and 14% subjects had eGFR < 30 mL.min/(1.73 m^2). Median UACR was 21.1 mg/mmol, and 48% of participants were macroalbuminuria. Mean HbA1c was 8.4 ± 2.2%, and median FBG was 7.3 (5.5–11.8) mmol/L. Approximately 52% of patients were anemic. Demographic and clinical data were listed in Table 1.

Table 2 Comparison between measured AG and estimated AG calculated from ADAG and ADAG-CKD equations

Variables	Mean ± SD	*p* value
Measured AG	8.4 ± 2.2	-
AG_{ADAG}	11.8 ± 3.2	< 0.01**
AG_{CKD}	12.3 ± 4.4	< 0.01**

AG_{ADAG}, estimated average glucose from equation of ADAG; AG_{CKD}, estimated average glucose from equation of ADAG-CKD; *mAG*, measured average glucose derived from CGM. **Refers to $p < 0.01$ for the comparison between measured AG and estimated AG

Table 3 Mean MAD and MARD of AG_{ADAG} compared with AG_{CKD}

	MAD (mmol/L)		MARD (%)	
Variables	Mean	95% CI	Mean	95% CI
AG_{ADAG}	2.42**	1.65, 3.18	33.3**	22.8, 43.7
AG_{CKD}	3.42**	2.72, 4.11	46.7**	36.6, 56.8

AG_{ADAG}, estimated average glucose from equation of ADAG; AG_{CKD}, estimated average glucose from equation of ADAG-CKD; *mAG*, measured average glucose; *MARD*, mean absolute relative difference; *MAD*, mean absolute difference. **Refers to $p < 0.01$ compared with AG_{ADAG} or AG_{CKD}

Comparison of measured AG and estimated AG

An average of 8.9 days of FGM data was collected. Mean measured AG was 8.4 ± 2.2 mmol/L. Estimated AG_{ADAG} was 11.8 ± 3.2, and AG_{CKD} was 12.3 ± 4.4 mmol/L. Overestimated measured AG differed significantly from AG_{ADAG} and AG_{CKD} (Table 2).

The MAD and MARD are presented in Table 3. The equation of ADAG performed better, with MAD of 2.42 mmol/L and 33.3% MARD, lower than that of ADAG-CKD, with MAD of 3.42 mmol/L and 46.7% MARD ($p < 0.01$).

Figure 1 shows the Bland–Altman type of analysis, displaying the agreement between measured AG and estimated AG from the two existing formulas. For estimated AG of ADAG equation, the acceptable range was − 8.8 to 3.9 mmol/L, with 4.2% value fallen out of this range, similar to that of ADAG-CKD equation, with an acceptable range of − 9.2 to 2.4 mmol/L and 5.6% out of this range. This result indicated the inconsistency between measured AG and estimated AG from the equation of ADAG and ADAG-CKD.

Taking the MAD as objective variable, regression analysis found that BMI, eGFR < 30 mL/min/1.73 m^2, HbA1c, and diabetes type might explain the disparity between estimated AG and measured AG (Supplementary Table 1).

Variables predicting the AG level in patients with DKD before dialysis

Using linear regression analysis, we constructed AG-HbA1c formula for DKD before dialysis (Fig. 2). Univariate regression equation between AG and HbA1c was AG (mmol/L) = 0.48 × HbA1c (%) + 4.36 ($F = 18.001$, $p < 0.001$). Every 1% increase in HbA1c leads to a corresponding 0.48 mmol/L increase of AG (β, 0.48; 95%CI, 0.25–0.71).

After HbA1c, BMI, diabetes type (T1D or T2D), and eGFR stage (eGFR < 30 or eGFR ≥ 30 mL/min/1.73 m^2) were introduced by stepwise linear regression, the model's ability to predict AG level was improved by 24% ($F = 9.469$, $p < 0.01$). The improved equation could explain 42% of average glucose variability (Table 4).

Discussion

Translating HbA1c into corresponding AG level is important because daily glucose monitoring and insulin dose adjustment are based on glucose concentration (mmol/L) instead of percentage unit (%). However, DKD-related factors such as anemia, iron deficiency, or supplement would affect the relationship of HbA1c and AG. Thus, it is necessary to know whether commonly used AG-HbA1c equation could estimate AG accurately in DKD before dialysis. In the present study, the average glucose of patients with DKD before dialysis measured by FGM was compared to the average glucose estimated by two equations. We found that the two examined equations overestimated average glucose level in DKD before dialysis. In addition, we established AG-HbA1c formula in our population and found that diabetes type, CKD stage, and BMI contribute to AG variability. Therefore, diabetes type, renal function, and BMI should be taken into consideration when converting HbA1c to AG value in diabetes care.

Our result revealed that estimated average glucose overestimated measured AG in DKD before dialysis. Neither equation ADAG nor ADAG-CKD could estimate the AG level accurately in DKD before dialysis as CGM guideline have recommended that MARD should be lower than 15% [18]. Similar finding has been reported in diabetes with age > 70 years old. Munshi et al. conducted a retrospective study which found that the estimated AG from equation ADAG was higher than measured AG in older patients with T1D (193 ± 23 vs. 182 ± 23 mg/dL; $p = 0.008$) [19]. However, a research that enrolled a total of 80 patients with T2DM reported that the estimated HbA1c calculated as HbA1c (%) = [average glucose from CGM (mmol/L) + 2.59)/1.59] underestimated measured laboratory

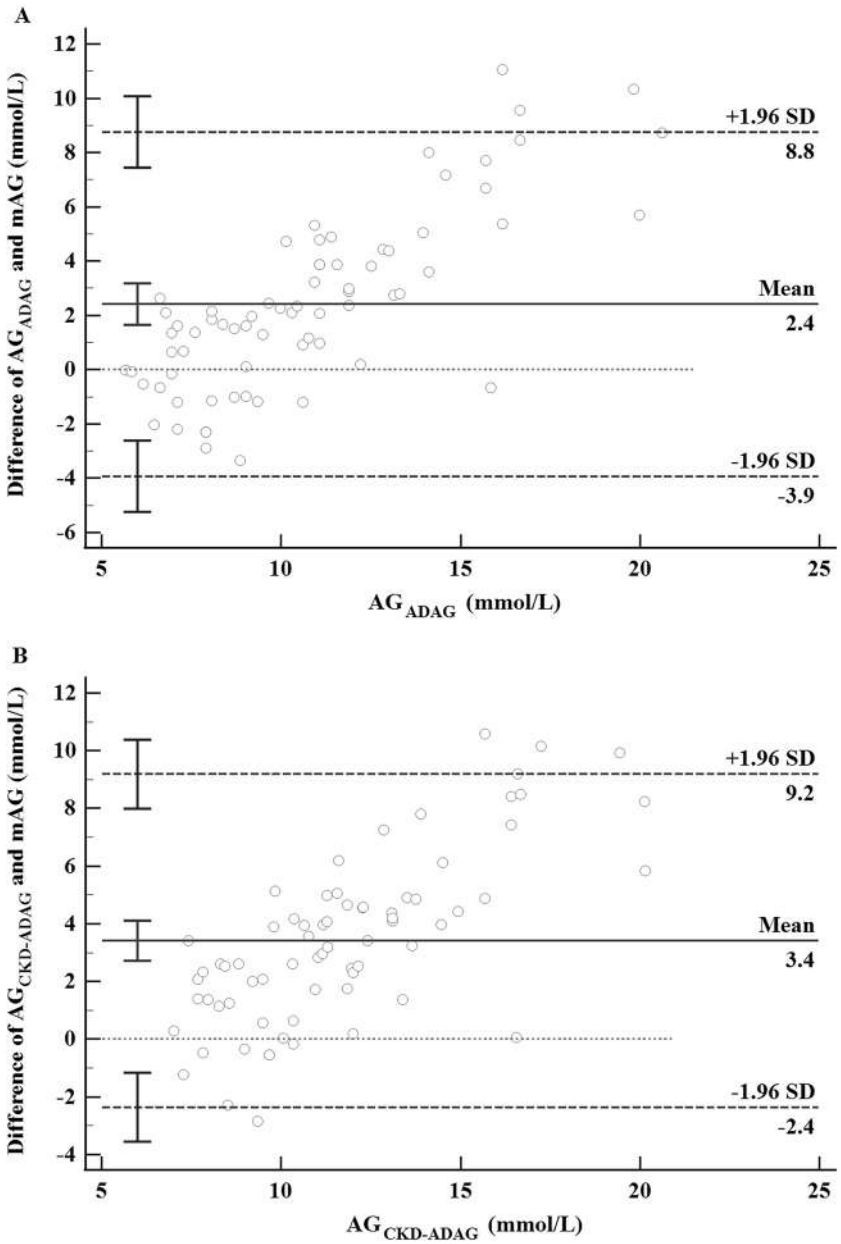

Fig. 1 Bland–Altman type of analysis for the difference of measured average glucose (mAG) and estimated average glucose (eAG) (*y*-axis) plotted against the eAG from equation ADAG and equation ADAG-CKD (*x*-axis). **A** Bland–Altman type of analysis between estimated AG_{ADAG} and mAG. **B** Bland–Altman type of analysis between estimated $AG_{CKD-ADAG}$ and mAG. Red dashed lines represent 95% limits of agreement, and blue solid line represents the mean discordance between eAG and mAG level

HbA1c (7.1 ± 1.3 vs. $7.7 \pm 1.3\%$; $p < 0.001$) [20]. Moreover, James et al. observed estimated AG underestimate measured AG with low hemoglobin glycation index (HGI) but overestimated AG in high HGI patients (low-HGI, 186 ± 31 vs. 163 ± 20 mg/dL; high-HGI, 199 ± 42 vs. 230 ± 31 mg/dL) [21]. We speculated that the discordance between estimated and measured glycemic indicators might be due to different characteristics across studies like anemic status, iron deficiency or treatment, and large glycemic excursion, which need large-scale research to verify.

In this study, there was significant discordance between measured AG and estimated AG from equation ADAG, and we found BMI, CKD stage, HbA1c, and diabetes type would explain the difference. Additionally, different characteristics between this study and ADAG research would also be the reason. First, the population in ADAG [7] study included subjects with both normal renal function or impaired renal function, while this study included only DKD participants. Second, patients in study ADAG came from multiple races (majority of white), while the eligible subjects in this trial were only Chinese people. It is acknowledged that the racial discordance affects the relationship between average glucose and HbA1c [22, 23].

Surprisingly, although ADAG-CKD focused on population with CKD, unacceptable difference between measured AG in our trial and estimated AG existed as well. We speculated that the following points maybe the causes. First, in ADAG-CKD study, a significant proportion of patients with

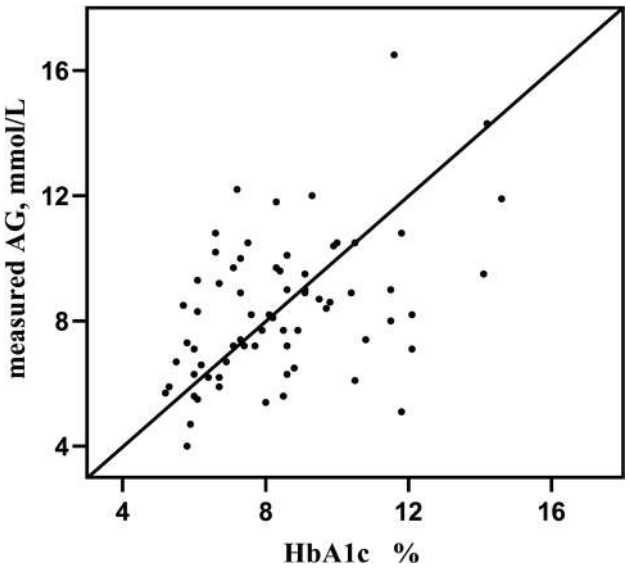

Fig. 2 Correlation of average glucose and hemoglobin A1c in DKD before dialysis. The formula was expressed as AG (mmol/L) = 0.48 × HbA1c (%) + 4.36

advanced CKD were enrolled, including dialysis patients. A previous study has demonstrated numerous dialysis-related factors would affect the HbA1c measurement [24, 25]. However, our study excluded those undergone dialysis. Second, the proportion of anemia in ADAG-CKD and our study differs (70% and 52%, respectively), indicating possible disparity in the erythrocyte turnover. Moreover, there are 44% of patients who use erythropoietin stimulating agent (ESA) and 44% have undergone iron infusions in ADAG-CKD study, which can bias HbA1c value greatly [26]. However, this study excluded the patients who use iron supplement or ESA to avoid factors influencing HbA1c.

Supported by linear regression test, this study established AG-HbA1c formula as AG (mmol/L) = 0.48 × HbA1c (%) + 4.36 ($r = 0.46$, $R^2 = 0.2$). After adjusting potential confounders like BMI, diabetes type, and CKD stage, the model's efficiency of predicting AG variability improved from about 20 to 42%. In fact, there have been several studies exploring the relationship between HbA1c and AG both in population without CKD or in those on dialysis. Rohlfing et al. conducted a trial in T1D ($n = 1439$) to explore the relationship of laboratory HbA1c and average glucose derived from 7-point blood glucose [AG (mmol/L) = 1.98 × HbA1c (%)-4.29; $r = 0.82$] [27]. An analysis from DaVita investigated the relationship of fast blood glucose and monthly HbA1c in 11,986 patients who had undergone dialysis and established the equation as follows: [AG (mg/dL) = 29.4 × HbA1c (%)-18.6; $R^2 = 0.468$] [28]. Zhao et al. examined HbA1c-AG relationship in a total of 305 patients on peritoneal dialysis and developed the formula: [AG (mmol/L) = 0.107 × HbA1c (mmol/mol) + 1.764; $R^2 = 0.494$] [29]. After adjusting potential confounders like serum albumin and serum creatinine, the correlation was stronger ($R^2 = 0.526$), which is similar to our study. Therefore, it is recommended to adjust potential factors when translating HbA1c into AG value for specific population.

There are some strengths of this study. First, to our knowledge, this is the first study to examine the accuracy of estimated AG calculated from published equations to assess glycemia status in patients with DKD before dialysis. Little study established formula for translation of HbA1c into AG in DKD before dialysis especially. The fact that estimated AG overestimates glycemia in DKD before dialysis helps understand potential hypoglycemia risk. Second, our result indicated that diabetes type, BMI, and renal function should be considered when estimating AG from HbA1c in DKD before dialysis, which helps glycemia assessment and diabetic treatment adjustment. Third, we recruited only those with stable HbA1c measurement over the past 3 months to make sure the HbA1c's stability.

The study also has limitations. First, the sample size in this study is relatively small and the recruited participants cannot represent all DKD before dialysis and external validity would be influenced by selection bias. Thus, the result needs to be further verified by large-scale research. Second, the FGM duration of 8.9 days in our study was expected to be close to 14 days as proposed by the recommendations from the International Consensus on Time in Range in 2019. However, a previous report demonstrated that 7 days could be enough to estimate HbA1c

Table 4 The relationship between the average glucose level and HbA1c in patients with DKD before dialysis

Model	AG-HbA1c equations	r	R^2	RMSE	F	ΔR^2
1	AG (mmol/L) = 0.48 × HbA1c (%) + 4.36	0.46	0.20	2.03	18.001	0.21
2	AG (mmol/L) = 0.41 × HbA1c (%) + 2.02 × type + 1.45 × eGFR stage-0.14 × BMI + 10.12 (× 1 if type = T1D; × 0 if type = T2D; × 1 if eGFR ≥ 30, × 0 if eGFR < 30 mL/min/1.73 m^2)	0.68	0.42	1.73	13.334	0.24

AG, average glucose; *HbA1c*, hemoglobin A1c; *type*, diabetes type (T1D or T2D); *BMI*, body mass index; eGFR stage (eGFR ≥ 30 or eGFR < 30 mL/min/1.73 m^2); *RMSE*, root mean square error

[30], which supports our method to some extent. Third, the accuracy of estimated AG in different subgroups was not further explored due to small sample size. Further study could help understand the usefulness of eAG in different subgroups such as diabetes type, CKD stages, and overweight by stratifications.

Conclusion

In conclusion, we found that estimated AG calculated from existing equations significantly overestimated measured AG derived from FGM in DKD before dialysis. The AG-HbA1c equation established in this study needs to be further verified. Potential confounding factors such as BMI, diabetes type, and renal function should be considered when interpreting the relationship between average glucose and HbA1c in DKD before dialysis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01305-1.

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Data availability The datasets analyzed during the current study are not publicly available because they are sampled from medical data of Nanfang Hospital of Southern Medical University but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The study protocol was approved by the Medical Ethics Committee of Nanfang Hospital of Southern Medical University. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki 1964 and its later amendments, Good Pharmacoepidemiology Practices, and applicable local laws and regulations.

Consent of patient The protocol for this retrospective study was approved by the Medical Ethics committee of Nanfang Hospital of Southern Medical University (approval number: NFEC-202204-K1-01). The study was conducted according to the Declaration of Helsinki.

Competing interests The authors declare no competing interests.

Authorship All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, have given their approval for this version to be published, and have agreed to be accountable for all aspects of the work.

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ORIGINAL ARTICLE

Differences in nutrition, handgrip strength, and quality of life in patients with and without diabetes on maintenance hemodialysis in Xi'an of China

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Abstract

Objective Malnutrition, reduced muscle function, and reduced quality of life are common problems among patients undergoing maintenance hemodialysis (MHD). This study aimed to evaluate nutritional risk, handgrip strength, and quality of life between patients with and without diabetes undergoing MHD to improve the nutrition, handgrip strength, and quality of life of patients with diabetic.

Methods This study was a descriptive cross-sectional design. Relevant survey scales were used to evaluate the nutritional status, handgrip strength, quality of life, and anthropometric measurements of patients receiving MHD. Logistic regression analysis was used to identify factors affecting MHD in patients with and without diabetes.

Results Compared to patients without diabetes undergoing MHD, patients with diabetes had a significantly higher malnutrition-inflammation score (MIS) ($p=0.003$), a higher proportion of malnourished patients ($p=0.011$), and weaker handgrip strength ($p<0.001$). Regarding quality of life, compared with patients without diabetes undergoing MHD, patients with diabetes undergoing MHD had a lower score of physical component summary (PCS) ($p=0.001$), mental component summary (MCS) ($p=0.005$), and 36-item short-form health survey (SF-36)($p=0.001$). In multivariate logistic analyses, MHD duration ($p=0.008$), handgrip strength ($p=0.001$), score of PCS ($p=0.013$), and employment status ($p<0.001$) were the major factors.

Conclusion Nutrition and quality of life of patients with diabetes undergoing MHD were worse than those of patients without diabetes. Additionally, patients with diabetes undergoing MHD had weaker handgrip strength and exercised less. Therefore, it is necessary to take effective measures to prevent further declines in nutritional status, handgrip strength, and quality of life.

Keywords Renal Dialysis · Complications of Diabetes Mellitus · Nutritional Status · Hand Strength · Quality of Life

Introduction

Diabetes-related renal complications are the main causes of end-stage renal disease (ESRD) [1]. Owing to the increase in life expectancy, lifestyle changes, and the importance of nutrition, the incidence of diabetes is gradually increasing worldwide [2, 3], leading to a significant increase in the incidence of diabetes-related complications [4].

Malnutrition is a common problem in patients undergoing maintenance hemodialysis (MHD) [5]. Measurement scores for patients undergoing MHD, such as the subjective global assessment (SGA) and malnutrition inflammation score (MIS), are recommended as tools for nutritional status assessment and management. These scores are effective and beneficial for predicting the prognosis of these patients. Malnutrition has a negative impact on the prognosis of patients, resulting in increased morbidity and mortality, as well as a decrease in quality of life (QOL) [6].

In terms of handgrip strength and prognosis, one study showed a negative relationship between handgrip strength and all-cause mortality in patients undergoing dialysis [7]. In some studies, handgrip strength measurement was selected as a screening tool for malnutrition in patients with chronic kidney disease (CKD). Moreover, studies that use handgrip

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strength measurement as an indicator of the outcomes of disease are significantly increasing [8, 9]. In patients undergoing MHD with other comorbidities, handgrip strength was negatively correlated with MIS [8]. In addition, there are significant correlations have been reported between handgrip strength and muscle function as well as the prediction of CKD complications [9].

Although the effects of malnutrition, handgrip strength, and QOL in patients undergoing MHD are conceivable, the relationship among different types of nutritional assessment scores, handgrip strength, and QOL in patients with and without diabetes undergoing MHD has not been sufficiently investigated.

Therefore, this study aimed to evaluate nutritional risk, handgrip strength, and QOL in patients with and those without diabetes who received MHD to improve the nutrition, handgrip strength, and QOL of patients with diabetes undergoing MHD. This study can help inform healthcare providers about intervention programs to enhance the nutritional status, handgrip strength, and QOL of patients undergoing MHD, thus helping these patients improve their psychological and physical problems.

Materials and methods

Study design

This study used a descriptive, cross-sectional design to investigate the QOL, handgrip strength, and nutritional status of patients with and without diabetes who received MHD between May and June 2021.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) undergoing MHD for at least 3 months, (2) aged ≥ 18 years, and (3) participation in the study on the basis of informed consent.

The exclusion criteria were: (1) other conditions, such as skeletal muscle problems, neurological problems, chronic liver disease, etc., that can lead to sarcopenia or affect handgrip strength; (2) patient refusal to participate in this study for various reasons; (3) age < 18 years old; and (4) inability to answer the questions.

Data collection

The data collection tools used in our study were comprehensive questionnaires that collected demographic, clinical, and laboratory data.

Sociodemographic information, including age, sex, marital status, primary renal diagnosis, smoking history, and drinking history, was collected through face-to-face interviews and searching electronic medical record systems. Blood pressure (BP) was measured before dialysis, and morning BP in patients undergoing MHD was recorded. Hypertension was defined by systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or regular use of antihypertensive drugs. Laboratory data for serum levels of hemoglobin (Hb), calcium (Ca), and phosphate (P) were collected upon patient enrollment.

Anthropometric measurements

Body mass index (BMI) was calculated according to the formula: BMI = body weight (kg)/height^2 (m^2).

Mid upper arm circumference (MAC) was used to select the upper arm without an arteriovenous fistula. The MAC was calculated by measuring the length of a circle of the part between the acromion of the scapula and the olecranon of the ulna with a non-elastic soft tape, and the measurements were taken twice in centimeters (cm) twice. The average value was then calculated [10].

Triceps skinfold thickness (TSF) was measured while the patient was standing, and the arm was allowed to relax and hang naturally. A skinfold caliper was used to the measure the thickness on the back of the upper arm at the midpoint between the acromion and olecranon, twice, and the average value was calculated. The measurements are in millimeters (mm).

Mid-armmuscle circumference (MAMC) was calculated in cm according tothe formula: MAMC = MAC − ([TSF/10] × л) [10].

Evaluation of handgrip strength

The handgrip strength of all patients was measured by the same researcher five to ten minutes before dialysis. The surveyed patient held a handgrip dynamometer with the non-fistulated hand before dialysis. The handgrip distance was adjusted to fit the patient's hand shape. The palm faced inward, the dial was oriented outward, the patient stood up right, and the arms naturally hung by the sides to avoid contact between the grip dynamometer, body, and clothing. Two measurements were performed. The time interval between each measurement was ten seconds or more, and the average value was recorded.

Evaluation of QOL

We used the Chinese version of the KDQOL-SF, Version 1.3 [11] to assess patients' QOL in two parts. One part of this scale evaluates QOL in 12 kidney disease-targeted areas (KDTA). The other part used a 36-item short-form health survey (SF-36) to evaluate overall QOL in eight fields. The KDTA contains 12 areas: symptoms/problems, effects of kidney disease on daily life, burden of kidney disease, work status, cognitive

function, quality of social interaction, sleep, social support, sexual function, dialysis staff encouragement, patient satisfaction, and overall health rating. The SF-36 assesses physical functioning, role limitations, physical health, bodily pain, general health perception, social functioning, emotional role, mental health, and vitality. The physical component summary (PCS) includes physical functioning, role limitations, physical health, bodily pain, and general health perception; the mental component summary (MCS) includes social functioning, role limitations-emotional, mental health, and vitality. The scoring standard and method of the KDQOL-SF, Version 1.3 were based on the scoring criteria reported by Hays et al. [12]. The scores on each scale ranged from 0 to 100. The higher the score, the better the QOL.

Evaluation of nutrition

Patient nutrition was assessed using the MIS. The MIS consists of four main parts, with 10 questions in total.The four parts include medical history, physical examination, BMI, and laboratory parameters. Medical history included weight changes, dietary intake, gastrointestinal symptoms, functional capacity, and presence of comorbidities. The number of years of hemodialysis treatment was also defined as a type of morbidity. Physical examination revealed loss of subcutaneous fat and muscle. Laboratory parameters included serum albumin level and total iron-binding capacity. The ten questions were scored on a scale of 0–3, with 0 points indicating normal and 3 points indicating very serious. The higher the total score, the more serious was the malnutrition [13].

Statistical analyses

Data are presented as the mean ± standard deviation, or percentages as appropriate. Continuous variables were compared in terms of nutrition, handgrip strength, and QOL in patients with diabetes undergoing MHD versus patients without diabetes undergoing MHD using t-tests or Mann–Whitney tests. Categorical variables were compared using the x^2 test. A binary logistic regression was performed to estimate the impact of multiple factors. The results are presented as odds ratios (OR) and 95% confidence intervals (CI). The level of significance was set at 0.05, and analyses were performed using SPSS software (version 16) (Chicago, IL, USA).

Results

Demographic and clinical characteristics of the participants

This cross-sectional study included 286 patients who underwent MHD. The mean age of the patients was 57.51 ± 16.05 years. More than half of the patients were male (66.8%) and had lower educational levels (65.0%). Most participants (81.5%) were unemployed. The most common ESRD etiology was diabetic nephropathy ($n = 113$, 39.5%). Other conditions included hypertensive nephropathy ($n = 67$, 23.4%), glomerulonephritis ($n = 58$, 20.3%), andother kidney diseases ($n = 48$, 16.8%).

Comparison of general characteristics between patients with and without diabetes undergoing MHD

There were no significant differences in sex, education, smoking history, drinking history, Ca, P, or Hb levels between the two groups. Compared with patients without diabetes, those with diabetes undergoing MHD were older ($p < 0.001$), had significantly higher mean SBP and DBP ($p = 0.004$, $p < 0.001$), had a shorter dialysis duration ($p = 0.002$), had a lower proportion who exercised ($p = 0.010$), had a higher proportion who were married ($p = 0.033$), and had a higher proportion who were retired ($p < 0.001$) (Table 1).

Comparison of nutrition differences between patients with and without diabetes undergoing MHD

There were no significant differences in BMI, MAC, MAMC, or TSF between the two groups. Compared with patients without diabetes undergoing MHD, those with diabetes had higher MIS scores ($p = 0.003$), had a higher proportion who were malnourished ($p = 0.011$), and had a weaker handgrip strength ($p < 0.001$) (Table 2).

Comparison of QOL differences between patients with and without diabetes undergoing MHD

As most patients did not respond to the KDQOL-SF questions on sexual function, we did not compare sexual function between patients with and without diabetes undergoing MHD. Regarding QOL, there were no significant differences in role-emotional status, work status, quality of social interaction, social support, dialysis staff encouragement, or patient satisfaction. Compared with patients without diabetes undergoing MHD, those with diabetes had higher scores for role-physical ($p = 0.034$), effects of kidney disease ($p = 0.013$), and burden of kidney disease ($p = 0.028$). Additionally, compared with patients without diabetes undergoing MHD, patients with diabetes scored lower for physical functioning ($p < 0.001$), bodily pain ($p < 0.001$), general health ($p = 0.034$), social functioning ($p = 0.012$), mental health ($p = 0.022$), vitality ($p = 0.041$), symptom/problem ($p = 0.001$), cognitive function ($p = 0.034$), sleep

Table 1 Comparison of general characteristics between patients with and without diabetes

	Patients with diabetes (n = 113)	Patients without diabetes (n = 173)	$t/Z/x^2$	p
Age, years	62.86 ± 12.78	53.95 ± 17.02	-5.018	< 0.001
Sex, n (%)			0.014	0.905
Male	75 (66.4)	116 (67.1)		
Female	38 (33.6)	57 (32.9)		
Education, n (%)			0.405	0.524
High school and below	76 (67.3)	110 (63.6)		
College and above	37 (32.7)	63 (36.4)		
Marital status, n (%)			4.524	0.033
Married	83 (73.5)	106 (61.3)		
Other(single, divorce, widowed)	30 (26.5)	67 (38.7)		
Employment, n (%)			22.329	< 0.001
Working	15 (13.3)	38 (22.0)		
Retired	59 (52.2)	43 (24.9)		
Other(unemployed、students)	39 (34.5)	92 (53.2)		
Exercise status, n (%)			6.561	0.010
Exercise	58 (51.3)	115 (66.5)		
No exercise	55 (48.7)	58 (33.5)		
Smoking history, n (%)			1.022	0.312
Yes	29 (25.7)	54 (31.2)		
No	84 (74.3)	119 (68.8)		
Drinking history, n (%)			0.042	0.837
Yes	20 (17.7)	29 (16.8)		
No	93 (82.3)	114 (83.2)		
MHD duration, months	29.03 ± 22.38	39.64 ± 33.50	3.205	0.002
SBP(mmHg)	154.99 ± 17.58	148.38 ± 19.07	-2.908	0.004
DBP(mmHg)	91.67 ± 81.20	86.37 ± 13.50	-4.162	< 0.001
Ca (mmol/L)	2.03 ± 0.36	1.96 ± 0.50	-0.902	0.367
P (mmol/L)	1.76 ± 0.58	1.86 ± 0.58	-1.044	0.296
Hb (g/L)	99.29 ± 23.63	100.91 ± 22.37	0.514	0.608

(SBP, systolic blood pressure; DBP, diastolic blood pressure; Ca: Serum Calcium; P, serum phosphorus; Hb: Hemoglobin)

Table 2 Comparison of nutrition and handgrip strength differences between patients with and without diabetes

Characteristics	Patients with diabetes (n = 113)	Patients without diabetes (n = 173)	$t/Z/x^2$	p
MIS	9.05 ± 2.90	8.06 ± 2.59	-2.943	0.003
Malnutrition	30 (26.5)	25 (14.5)	6.441	0.011
Normal nutrition	83 (73.5)	148 (85.5)		
BMI	21.99 ± 3.27	21.72 ± 3.46	-0.667	0.505
Handgrip strength	19.80 ± 6.94	24.25 ± 8.65	-4.422	< 0.001
MAC	26.99 ± 8.72	27.17 ± 6.94	-1.107	0.268
MAMC	22.97 ± 8.93	23.16 ± 5.21	-1.527	0.127
TSF	12.81 ± 5.63	12.78 ± 8.83	-0.371	0.711

(BMI, Body mass index; MIS,Malnutrition-Inflammation Score; MAC, Mid upper arm circumference; MAMC, mid-arm muscle circumference; TSF: Triceps skinfold thickness)

quality ($p = 0.001$), overall health rating ($p = 0.029$), PCS ($p = 0.001$), MCS ($p = 0.005$), and SF-36 ($p = 0.001$) (Table 3).

Binary multiple factors logistic regression

Using Wald logistic regression, the group of patients with or without diabetes was considered as the dependent variable y, and the meaningful index of the univariate analysis was considered as the independent variable x. In the multivariate logistic analyses, MHD duration ($p = 0.008$), handgrip strength ($p = 0.001$), PCS ($p = 0.013$), and employment status ($p < 0.001$) were the major factors. (Table 4).

Table 4 Multiple factors logistic regression on patients with and without diabetes

	B	*Wald* x^2	*p*	*OR*值	95% CI
MHD duration	-0.014	6.962	0.008	0.986	0.976 ~ 0.996
Handgrip strength	-0.060	10.263	0.001	0.942	0.908 ~ 0.977
PCS	-0.005	6.223	0.013	0.995	0.991 ~ 0.999
Employment (retired)	1.085	12.248	< 0.001	2.960	1.612 ~ 5.435
Constant	1.928	11.218	0.001	6.876	

(MHD, maintenance hemodialysis; PCS, physical component summary; OR, odds ratio; CI, confidence interval)

Discussion

In this study, 39.51% of the patients had diabetes. This rate was similar to that reported by Soleymanian et al. (41%) [6], indicating that diabetic nephropathy is a common cause of ESRD. According to the latest data (2021), the prevalence of diabetic nephropathy in patients undergoing MHD is 29% in the UK, 41% in Japan, 51% in Canada, and 64% in USA [14]. These data indicate that diabetes can aggravate renal function decline associated with CKD [15].Therefore, it is particularly important to strengthen the health management of patients with diabetes undergoing dialysis.

This study found that, when compared with patients without diabetes undergoing MHD, patients with diabetes were older and a higher proportion of them were retired (Table 1). This may be because most patients with diabetes are elderly. Aging is also associated with increased comorbidities. Compared with younger patients, the elderly usually suffer from more complications, such as diabetes, due to the decline in physical and cognitive power.

Table 3 Comparison of quality of life differences between patients with and without diabetes

Characteristics	Patients with diabetes (n = 113)	Patients without diabetes (n = 173)	*t/Z/x*^{2}	*p*
Physical functioning	62.50 ± 28.24	78.61 ± 21.68	-4.565	< 0.001
Role-physical	50.00 ± 47.43	34.72 ± 40.35	-2.125	0.034
Bodily pain	55.42 ± 29.56	77.64 ± 20.39	3.953	< 0.001
General health	50.00 ± 28.64	54.44 ± 23.69	2.129	0.034
Social functioning	58.33 ± 33.23	64.58 ± 30.69	-2.516	0.012
Mental health	63.33 ± 9.93	75.56 ± 17.29	-2.289	0.022
Role-emotional	55.56 ± 45.54	70.37 ± 45.57	-1.473	0.141
Vitality	63.33 ± 22.29	69.81 ± 23.79	-2.048	0.041
PCS	44.14 ± 19.30	51.46 ± 16.78	-3.439	0.001
MCS	58.40 ± 18.18	65.24 ± 16.35	-2.792	0.005
SF-36	52.55 ± 15.78	58.78 ± 13.83	-3.369	0.001
Symptom/problem	57.99 ± 28.85	80.79 ± 15.00	-3.231	0.001
Effects of kidney disease	53.65 ± 38.09	48.96 ± 17.25	2.508	0.013
Burden of kidney disease	41.67 ± 34.16	36.81 ± 23.86	2.206	0.028
Work status	51.67 ± 30.11	46.39 ± 31.33	-1.364	0.173
Cognitive function	71.11 ± 11.67	84.81 ± 19.51	-2.125	0.034
Quality of social interaction	64.44 ± 19.17	79.63 ± 20.26	-1.116	0.264
Sleep quality	56.25 ± 25.78	75.14 ± 16.68	3.369	0.001
Social support	83.33 ± 18.26	79.63 ± 25.28	-0.468	0.640
Dialysis staff encouragement	91.67 ± 20.41	97.92 ± 6.43	-0.924	0.356
Patient satisfaction	66.67 ± 14.89	83.32 ± 16.17	-0.087	0.931
Overall health rating	45.91 ± 19.74	51.29 ± 17.73	-2.185	0.029

(PCS, physical component summary; MCS, mental component summary; SF-36,36-item short-form health survey)

This study found that the nutritional status of patients with diabetes who undergoing MHD was worse than that of patients without diabetes. As shown in Table 2, compared with patients without diabetes undergoing MHD, patients with diabetes had a higher MIS score and a higher proportion of them were malnourished. This is because patients with diabetes are confronted with malnutrition due to low protein intake and a greater catabolic state. Therefore, attention should be paid to the nutritional status of patients undergoing MHD. According to the Kidney Disease: Improving Global Outcomes(KDIGO) Guideline for Diabetes Management in Chronic Kidney Disease, patients should have access to things and food resources, personalized diet plans for dialysis, and specific dietary restrictions tailored to special conditions, such as hyperkalemia, according to the patient's preferences and needs [16]. The diet plan for patients with diabetes undergoing MHD should take into account the opinions of nutrition providers, nutritionists, patients' attending doctors, nurses in charge, community health workers, and other health providers. Additionally, it should consider cultural differences, food intolerance, differences in food availability, limitations of complications on certain nutrients, and the economic costs of food production [16]. In contrast to other studies, this study used a variety of methods to assess the nutritional status of patients, including subjective assessment scale, such as MIS, and objective measurement indicators such as BMI, handgrip strength, MAC, MAMC, and TSF, and comprehensively assessed the nutritional differences between patients with and without diabetes undergoing MHD. The comparison in this study was more comprehensive.

This study found that diabetes could affect physical function. In our study, patients with diabetes undergoing MHD had weaker handgrip strength and a lower proportion of them exercised. Logistic regression analysis revealed a close relationship between handgrip strength and diabetic mellitus. This may be because diabetes affects muscle function through several mechanisms, such as reduction in glucose uptake by the muscles and anabolic rate in the muscle tissue due to peripheral insulin resistance [17]. The change in microvessels caused by diabetes leads to a decrease in the blood flow to muscles, thus reducing muscle function. In addition, other complications, such as visual acuity decline, cardiac function decline, neuropathy, and peripheral vascular disease caused by diabetes, can lead to reduced physical activity, leading to decreased activity in patients undergoing dialysis [18, 19]. Similar to this study, Isoyama et al. showed that diabetes is an influencing factor of handgrip and muscle strength [20]. Lee et al. showed that the prevalence of diabetes was higher in patients with weaker handgrip strength [21].Isoyama et al. [20] found that by comparing low-intensity muscle activity with moderate-intensity muscle activity, people with low-intensity muscle activity have more complications, such as cardiovascular disease, diabetes, and higher levels of inflammatory markers. In addition, handgrip strength is an independent predictor of all-cause mortality in patients undergoing MHD [20, 22]. According to this study, we should improve the evaluation of muscle function, such as handgrip strength, in patients with diabetes undergoing MHD to improve their prognosis. Moreover, simplicity, rapidness, and cost-effectiveness of this method make it more conducive to its promotion and application.

As shown in Table 3, this study found that patients with diabetes undergoing MHD had a significantly worse QOL. Except for the emotional role area; the other areas of the SF-36 subscale were significantly lower in patients with diabetes. In addition, both the PCS and MCS were significantly lower in patients with diabetes. In this study, owing to the influence of diabetes, kidney diseases, and the effects of various conditions such as hypertension, anemia, and diabetes complications, the disease burden of hemodialysis on patients is increased, and anxiety and depression are prone to occur, which greatly affects the physiological and psychological functions of patients. In addition, undergoing dialysis twice or thricea week requires more time. Further more, arteriovenous fistulas, jugular vein catheterization, diabetic retinopathy, and diabetic footcan greatly affect work, entertainment, social, and exercise activities of patients. As a result, patients struggle to find ways to relax and relieve their worries, which can aggravate their anxiety, depression, and other psychological problems. Consequently, these problems can reduce the QOL of patients with diabetes undergoing dialysis. Our results were similar to those reported by Soleymanian et al. [6]. Studies have confirmed that a decline in QOL is common in patients undergoing MHD. Lower QOL indicates more complications, which can independently and significantly affect the prognosis of patients with diabetes undergoing MHD [6]. Other studies have shown that patients with diabetes undergoing MHD have decreased physical and psychological health perception [23, 24]. Similarly, most studies have found that PCS is a predictor of mortality in patients with diabetes undergoing MHD. In addition, a few studies found that MCS has an impact on the prognosis of patients [23, 25].

Other studies mostly directly analyzed the status of nutrition, handgrip strength, QOL, and influencing-related factors of patients with diabetes undergoing MHD, but rarely compared the differences between patients with and without diabetes undergoing MHD. Compared to other studies, the strength of this study was that it evaluated handgrip strength, nutritional status, and various dimensions of QOL using different survey methods between patients with and without diabetes. This approach helps develop better interventions to improve the nutrition, grip strength, and QOL of patients undergoing MHD, especially those with diabetes, to help them improve their psychological and physiological

problems. However, our study has some limitations. First, it was cross-sectional, which excludes any discussion of causality. Therefore, more studies should further assess this correlation to establish a cause-effect relationship between the two aspects and investigate possible solutions to both problems. Second, our nutritional data relied solely on patient self-reports, which might have resulted in a recall bias.

Conclusions

In conclusion, we found that nutritional status, handgrip strength, and QOL of patients with diabetes undergoing MHD were worse than those of patients without diabetes.We used different methods to assess the nutritional status of the patients undergoing MHD. Additionally, we found that patients with diabetes undergoing MHD had a weaker handgrip and a lower proportion of them exercised. We recommend that healthcare officials, such as clinicians and nurses, consider our findings and pay more attention to patients with diabetes undergoing MHD to improve malnutrition, handgrip strength, and QOL. In addition, it is necessary to screen patients with diabetes undergoing MHD for malnutrition, handgrip strength, and QOL to take effective measures to prevent further declines in nutritional status, handgrip strength, and QOL.

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Declarations

Informed Consent Before the formal investigation, we introduced the purpose of this study and its possible benefits to the patients and promised to maintain patient privacy. Patients who agreed to participate signed an informed consent form.

Conflict of Interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Poor accuracy of HbA1c for the diagnosis of prediabetes in overweight and obese Bangladeshi adults

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Abstract

Background/Purpose The aim of the study was to assess the effect of obesity on the diagnostic accuracy of HbA1c.

Methods This retrospective cross sectional study was conducted in 108 overweight/obese and 40 normal weight Bangladeshi adults. Those satisfying the exclusion and inclusion criteria were included. Diabetes and pre-diabetes were diagnosed by oral glucose tolerance test (OGTT) and HbA1c using the 2006 World Health Organization (WHO) diagnostic criteria. HbA1c was estimated by capillary electrophoresis method.

Results 108 overweight and obese (mean body mass index (BMI) 36.33 ± 8.86 kg/m^2, mean age 29.12 ± 9.28 years) and 40 normal weight (mean BMI 20.35 ± 1.68 kg/m^2, mean age 28.13 ± 6.22 years) adults were included in the study. Significantly greater number of patients were diagnosed with prediabetes using HbA1c criteria than OGTT criteria (39.68% vs 19.05%, $p = 0.005$) in overweight and obese group. The concordance between OGTT and HbA1c for the diagnosis of prediabetes was low in overweight and obese adults [Ƙ with 95%CI = 0.031(-0.194 to 0.256), n = 52]. The specificity and discrimination of HbA1c for the diagnosis of prediabetes were low in overweight and obese compared to normal weight group (52.3% vs 93.9%; 0.64 vs 0.89, $p = 0.056$, 95% CI = -0.01 to 0.51, respectively). The specificity of HbA1c for the diagnosis of prediabetes in adults with BMI ≥ 23 kg/m^2 increased to 90% at a cut-off of 6.15%.

Conclusion HbA1c was not accurate in the diagnosis of prediabetes in adults with BMI ≥ 23 kg/m^2. A higher cut-off value for HbA1c should be used for the diagnosis of prediabetes, but not diabetes.

Keywords HbA1c · Overweight · Obese · Accuracy · Prediabetes

Introduction

HbA1c results from glycationof haemoglobin, and reflects average glyacemia over the last 3 months. However, unlike plasma glucose estimation, it is not a direct measure of glycaemia. Instead, it depends not only on the concentration of blood glucose, but also on the rate of glycation, haemoglobin quality and quantity [1]. Therefore HbA1c levels may not be representative of glycaemic status in conditions with enhanced glycation and altered red cell turn over. Oxidative

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stress and lipid peroxidation increase the rate of glycation [2]. Since there is increased lipid peroxidation in obesity, HbA1c has been found to be elevated in non-diabetic obese individuals [3].

Obesity is a state of chronic inflammation and oxidative stress. There are several mechanisms of increased oxidative stress and generation of free radicals in obesity. Adipose tissue generates proinflammatory cytokines such as tumour necrosis factor alpha, interleukin 1 and interleukin 6 [4]. There is enhanced mitochondrial and peroxisomal oxidation of fatty acids which in turn generate free radicals [5]. Excess fat deposition, increased oxygen consumption and mitochondrial dysfunction cause cellular damage, all leading to increased reactive oxygen species [6, 7].

HbA1c level is affected by lipid peroxidation, which is increased in obesity. Hence, HbA1c level may be inherently elevated in obese individuals, thus misdiagnosing them with prediabetes and diabetes. Therefore, this study aimed to investigate the diagnostic accuracy of HbA1c in diagnosing prediabetes and diabetes in overweight and obese adults.

Materials and methods

This retrospective cross-sectional study was conducted in the Obesity Clinic, Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka from May 2019 to August 2022 in 148 Bangladeshi adults (≥ 18 years).

Sample size for estimation of specificity was calculated based on the formula by Buderer et al., using 20% error, 95% confidence interval (CI), and 0% dropout [8]. Expected sensitivity and specificity of HbA1c for diagnosis of prediabetes in the general population was taken as 45% and 76% respectively [9]. The prevalence of prediabetes in Bangladesh is 13.3% [10]. Calculated sample size for estimation of specificity was 21, and sensitivity was 159.

Patients with anaemia, haemoglobinopathies, recent history of blood loss, chronic kidney disease, chronic liver disease, pregnancy and acute illness were excluded from the study. None of the participants were on any medications influencing glucose homeostasis. All participants meeting the inclusion and exclusion criteria were included consecutively.

The accuracy of Hba1c was determined using oral glucose tolerance test (OGTT) as reference standard, as it is the gold standard test for diagnosing glucose intolerance and is a direct measure of glycaemia. Plasma OGTT and HbA1c tests were done in normal weight, overweight and obese participants. Data on blood pressure, height, weight, BMI, waist circumference, neck circumference, low density lipoprotein cholesterol (LDL-c), serum triglyceride, fasting plasma glucose, OGTT and HbA1c of normal weight, overweight and obese participants were obtained from hospital records. All the biochemical tests including OGTT and HbA1c were done in a single centre. Plasma glucose was assayed by glucose oxidase (GluO) method with automated analyzer (Atellica CH Glucose Oxidase (GluO), Germany). HbA1c was done by capillary electrophoresis in free solution in an NGSP certified laboratory with analyzer (Sebia Capillary HbA1c, France). The coefficient of variation of reproducibility was 1.1 to 1.4%.Overweight and obese were defined by a body mass index (BMI) of ≥ 23 and 25 kg/m^2, respectively [11]. Abnormal glucose tolerance was used to denote prediabetes and diabetes. Diabetes mellitus and prediabetes were defined using the 2006 WHO diagnostic criteria. Prediabetes was defined by fasting plasma glucose 6.1–6.9 mmol/L, or 2 h plasma glucose 7.8–11 mmol/L or HbA1c 5.7–6.4%. Diabetes mellitus was defined by fasting plasma glucose ≥ 7 mmol/L, or 2 h plasma glucose ≥ 11.1 mmol/L or HbA1c ≥ 6.5% [12].

All values were expressed as means (SD) or frequencies. Cohen's kappa (Ƙ) was used to determine the agreement between OGTT and HbA1c. Diagnostic accuracy of Hba1c, determined with OGTT as gold standard, included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and discrimination. Discrimination was assessed by the area under the curve (AUC). The receiver-operating characteristic curveanalysis was performed to identify the optimal threshold of HbA1c for the diagnosis of prediabetes in overweight and obese adults. MedCalc, (DeLong method) was used to compare 2 AUCs. Pearson's correlation was done to see the relation between BMI, waist circumference and HbA1c. Missing data were entered as 999. All data were analyzed with SPSS version 23.

Results

Of the 148 cases enrolled, 108 were overweight or obese and 40 were normal weight. Table 1 shows the baseline clinical and biochemical characteristics of the participants. Age of the participants in normal weight, overweight and obese group was similar. Out of 108 overweight or obese participants, 99% had central obesity. Mean fasting plasma glucose (FPG) and the 2 h plasma glucose were normal in both groups. Mean HbA1c was in the prediabetic range in the overweight and obese group, but in the normal range in the normal weight group.

In adults without prediabetes and diabetes, HbA1c was significantly higher in overweight and obese compared to normal weight individuals [5.63 (0.35) vs 5.42 (0.22), $p = 0.004$, 95%CI -0.35 to -0.07), n = 78]. There was a significant positive correlation between HbA1c and BMI (r = 0.45, R2 = 20.16%, $p = 0.001$, $n = 43$), as well as waist circumference (r = 0.422, R2 = 17.81%, $p = 0.009$, $n = 37$) in overweight and obese adults without prediabetes or diabetes.

Table 1 Clinical and biochemical characteristics of the study population

	Overweight and obese (BMI ≥ 23 kg/m^2)	Normal weight (BMI 18.5–22.9 kg/m^2)	p
N	108	40	-
Age (years)	29.12 (9.28)	28.13 (6.22)	0.690
BMI (kg/m^2)	36.33 (8.86)	20.35 (1.68)	< 0.001
Waist circumference (cm)	108.51 (14.90)	75.95 (7.21)	< 0.001
Neck circumference (cm)	40. 92 (4.71)	-	-
Systolic blood pressure (mmHg)	124.62 (16.72)	103.25 (12.07)	0.069
Diastolic blood pressure (mmHg)	82.30 (10.84)	67.50 (8.47)	0.270
Low density lipoprotein (mg/dl)	111.11 (33.40)	-	-
Triglyceride (mg/dl)	168.25 (89.68)	-	-
Fasting plasma glucose (mmol/L)	5.54 (1.15)	5.06 (0.42)	0.016
Plasma glucose 2 h after 75 g glucose (mmol/L)	6.77 (2.29)	6.58 (0.73)	0.003
HbA1c (%)	6.15 (1.47)	5.44 (0.22)	0.002

All values expressed as mean (SD). *BMI* body mass index

Table 2 Glycaemic classification by OGTT and HbA1c criteria in adults

	OGTT			
HbA1c (ADA)	NGT	Prediabetes	Diabetes	Total
Normal weight (BMI 18.5–22.9 kg/m^2)				
NGT	31 (88.57%)	1 (2.86%)	0	32 (91.43%)
Prediabetes	2 (5.71%)	1 (2.86%)	0	3 (8.57%)
Diabetes	0	0	0	0
Total	33 (94.29%)	2 (5.71%)	0	35
Cohen's kappa (Ƙ) with (95%CI) = 0.36 (-0.21 to 0.92)				
Overweight and obese (BMI ≥ 23 kg/m^2)				
NGT	23 (36.51%)	5 (7.94%)	0	28 (44.44%)
Prediabetes	19 (30.16%)	5 (7.94%)	1 (1.59%)	25 (39.68%)
Diabetes	2 (3.17%)	2 (3.17%)	6 (9.52%)	10 (15.8%)
Total	44 (69.84%)	12 (19.05%)	7 (11.11&)	63
Cohen's kappa (Ƙ) with (95%CI) = 0.23 (0.09 to 0.42)				

Within parenthesis is percentage over total. *OGTT* oral glucose tolerance test, *NGT* normal glucose tolerance, *BMI* body mass index

Agreement between HbA1c and OGTT

Table 2 shows the glycaemic classification by OGTT and HbA1c criteria in normal weight South Asian adults. 8.57% and 5.71% adults were diagnosed as prediabetes by HbA1c and OGTT criteria, respectively ($p = 0.311$). No one was diagnosed with diabetes mellitus. 6.06% of adults with normal glucose tolerance (NGT) by OGTT criteria were diagnosed as prebiabetesby HbA1c criteria.

Table 2 also shows the glycaemic classification by OGTT and HbA1c criteria in overweight and obese (BMI ≥ 23 kg/m^2) South Asian adults. More patients had NGT with OGTT than HbA1c (69.84% vs 44.44%). Significantly greater number of patients were diagnosed with prediabetes using HbA1c criteria than OGTT criteria (39.68% vs 19.05%, $p = 0.005$). There was no difference in the rate of diabetes mellitus between the two criteria (HbA1c vs OGTT = 15.8% vs 111.11%, $p = 0.209$)0.36.51%, 7.94% and 9.52%of adults were diagnosed as NGT, prediabetes and diabetes mellitus by both tests, respectively. Of the adults diagnosed as NGT by OGTT criteria, 47.72% were misdiagnosed as glucose intolerant (43.18% asprediabetes, 4.54% as diabetes mellitus) by HbA1c criteria. Of the adults diagnosed as prediabetes by OGTT criteria, 7.94% were misdiagnosed as NGT and 3.17% as diabetes mellitus by HbA1c criteria. Of the adults diagnosed as diabetes mellitus by OGTT criteria, 1.59% was misdiagnosed as prediabetes by HbA1c criteria.

The agreement between OGTT and HbA1c for the diagnosis of abnormal glucose tolerance in normal weight, overweight and obese adults is shown in Table 2. Agreement was also seen across increasing quartiles of BMI in overweight and obese group. The concordance between OGTT and HbA1c in normal weight adults (Ƙ = 0.36)was higher than in overweight and obese adults (Ƙ = 0.23). In overweight and obese adults, it was very low for the diagnosis of prediabetes[Ƙ with 95%CI = 0.031(-0.19 to 0.26), n = 52], but high for diagnosis of diabetes [Ƙ with 95%CI = 0.82 (0.60 to 1.03)].The agreement did not decrease with BMI gain (0.13 (-0.37 to 0.62 in the 1st quartile (BMI 0–30.24 kg/m^2), 0.09 (-0.15 to 0.33) in the 2nd quartile (BMI 30.25–34.10 kg/m^2), 0.40 (0.08 to 0.72) in the 3rd quartile (BMI 34.11–40.05 kg/m^2) and 0.31 (-0.03 to 0.65) in the 4th quartile (BMI > 40.05 kg/m^2). Post hoc power analysis of the study gave an observed power of 0.803.

Diagnostic accuracy of HbA1c

Table 3 shows the diagnostic accuracy for HbA1c for the diagnosis of glucose intolerance with OGTT as standard in normal weight, overweight and obese adults, as well as in quartiles of BMI. HbA1c had a good specificity for the diagnosis of prediabetes (cut-off 5.7%) in normal weight (93.9%), but not in overweight and obese group (52.3%). The specificity of HbA1c for the diagnosis of prediabetes-decreased with increasing BMI (67% in the 1st quartile to 30.8% in the last quartile). When the cutoff of HbA1c for diagnosing prediabetes was increased to 6.15%, the specificity increased to 90% in the overweight and obese group. The agreement between OGTT and HbA1c at the cut-off of 6.15% was higher [κ with 95%CI = 0.19 (-0.11 – 0.48)] in the overweight and obese group. The sensitivity, PPV and NPVwere 25%, 20.8% and 82.1% in overweight and obese adults at an HbA1c cut-off of 6.15%.

The AUC of HbA1c for the diagnosis of prediabetes was higher in the normal weight than the overweight and obese group (0.89 vs 0.64, $p = 0.056$, 95% CI = -0.01 to 0.51) (Fig. 1). The AUC also decreased with increasing obesity (0.49 in 1st quartile to 0.29 in the last quartile). The specificity and AUC were both lowest when BMI was > 40.05 kg/m^2.

HbA1c, at a cut-off of 6.5% had a high sensitivity (85.7%), specificity (95.5%) and discrimination (AUC 0.97) for the diagnosis of diabetes mellitus in overweight and obese group. The diagnostic accuracy of HbA1c for the diagnosis of diabetes mellitus in normal weight group could not be done as none of the participants in that group had diabetes.

Females had a higher mean BMI than males (32.74 (9.86) vs 30.29 (11.57), $p = 0.158$). The sensitivity (54.3% vs 83%), specificity (64% vs 68%) and diagnostic accuracy [(0.61 (0.42–0.79) vs 0.89 (0.75–1)] of HbA1c for the diagnosis of prediabetes was lower in females than males. However, they were similar for the diagnosis of diabetes.

Discussion

HbA1c was higher in those with a BMI ≥ 23 kg/m^2, and overestimated the rate of prediabetes in this group. The current cut-off of 5.7% was not accurate for the diagnosis of prediabetes in overweight and obese adults, and instead a higher cut-off of 6.15% is suggested.

In the study, HbA1c was higher in overweight and obese adults without prediabetes and diabetes. Furthermore, HbA1c increased with increasing BMI and waist circumference in overweight and obese adults. In accordance with this study, HbA1c concentrations were disproportionately elevated in non-diabetic obese subjects, independent of glucose levels [3]. The level of HbA1c depends on the rate of glycation [1]. Oxidative stress and lipid peroxidation increase the rate of glycation [2]. Obesity is a state of chronic inflammation where there is increased lipid peroxidation [3]. In support of this, researchers found a positive correlation between MDA, the main product of lipid peroxidation and glycated

Table 3 Diagnostic accuracy of HbA1c for abnormal glucose tolerance with OGTT as standard

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95%CI)	n
Prediabetes (HbA1c cut-off 5.7%)						
Normal weight (BMI 18.5–22.9 kg/m^2)	50	93.9	33.33	96.88	0.89 (0.74 to 1)	35
Overweight and obese (BMI ≥ 23 kg/m^2)	58.3	52.3	20.8	82.14	0.64 (0.44 to 0.84)	62
1st quartile (BMI 0–30.24 kg/m^2)	20	67	25	66.67	0.49 (0.20 to 0.78)	17
2nd quartile (BMI 30.25–34.10 kg/m^2)	100	50	16.67	0	1 (1 to 1)	15
3rd quartile (BMI 34.11–40.05 kg/m^2)	100	53.8	28.57	0	0.67 (0.40 to 0.95)	15
4th quartile (BMI > 40.05 kg/m^2)	50	30.8	20	20	0.29 (0 to 0.68)	15
Diabetes (HbA1c cut-off 6.5%)						
Overweight and obese (BMI ≥ 23 kg/m^2)	85.7	95.5	75	100	0.97 (0.92 to 1)	51

PPV positive predictive value, *NPV* negative predictive value, *AUC* area under the curve, *OGTT* oral glucose tolerance test, *BMI* body mass index. There were no cases with diabetes mellitus in the normal weight group and few cases in different categories of BMI in overweight and obese group

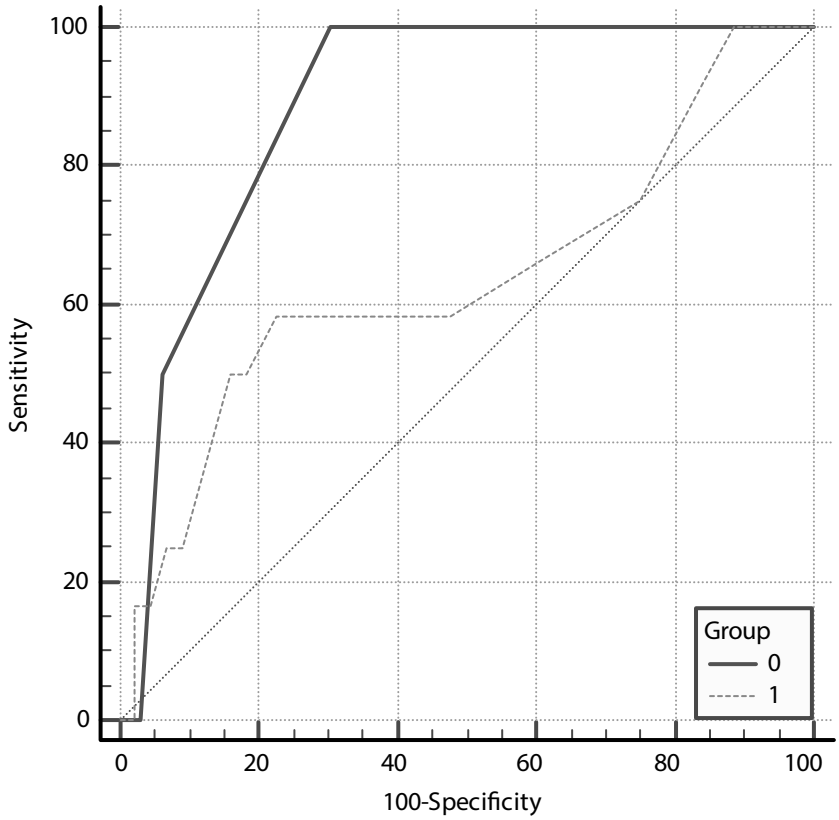

Fig. 1 Comparison of AUCs between normal weight (0) and overweight and obese adults (1) for the diagnosis of prediabetes. ($p = 0.056$, 95% CI = -0.01 to 0.5)

hemoglobin [3, 8]. Furthermore, they found that the effect of BMI on glycated hemoglobin was mediated through MDA [13]. This may explain the higher HbA1c levels in obese individuals.

Significantly greater number of overweight and obese adults was diagnosed with prediabetes, but not diabetes using HbA1c criteria. Almost 50% participants with BMI $\geq$ 23 kg/m^2 were misdiagnosed as prediabetes by HbA1c criteria. Agreement between OGTT and HbA1c for the diagnosis of abnormal glucose tolerance was low in overweight and obese adults. Moreover, the concordance for prediabetes was very low in overweight and obese adults. The specificity and discrimination of HbA1c for the diagnosis of prediabetes was lower in overweight and obese compared to normal weight adults, and they decreased with BMI gain. An HbA1c cutoff of 6.15% had 90% specificity for diagnosing prediabetes in overweight and obese Bangladeshi adults. Similar findings were seen in a study from Italy, where more adults with obesity (mean BMI 33.5 kg/m^2) were diagnosed with prediabetes by HbA1c (41%) than OGTT (19%) criteria. Insulin resistance was higher in those patients identified with OGTT than in those who were diagnosed with HbA1c [14]. Another study from China found that the agreement between HbA1c and OGTT decreased with increasing BMI. The same study also showed that the specificity of HbA1c for the diagnosis of prediabetes, but not diabetes was low in obese adults. For a specificity of 80%, the optimal cut-off for prediabetes (6%), but not diabetes was higher in obese individuals [13].This finding was similar to our study.

In further support of our findings, the diagnostic accuracy of HbA1c for prediabetes was lower in females. This may be explained by the fact that females have a higher percentage of body fat and thus higher lipid peroxidation [15].

An explanation of raised HbA1c and its low accuracy in diagnosing prediabetes may be the enhanced glycation of hemoglobin due to increased oxidative stress in obesity. Obesity induces increased systemic oxidative stress[16]. Biomarkers of oxidative stress such as C reactive protein are raised in obesity [17], whereas antioxidants such as superoxide dismutase, catalase and glutathione peroxidase are diminished [18]. Markers of oxidative stress such as malondialdehyde (MDA) and F-2 isoprostanes (F2-IsoPs) correlate with the body mass index, percent body fat and cardiovascular factors [3, 17]. Furthermore, a high fat diet has also shown to generate reactive oxygen species in humans [19]. The level of glycated hemoglobin depends on the rate of glycation, concentration of glucose and half life of the protein [20]. The rate of glycation is in turn affected by lipid peroxides and free radicals [2]. Therefore, it can be assumed that there is enhanced glycation of

hemoglobin in obesity due to enhanced lipid peroxidation in this disorder.

Impaired glucose tolerance (IGT) appears early in the natural history of diabetes. The concordance and diagnostic accuracy of HbA1c is lower for prediabetes than diabetes (K = 0.154, AUC = 0.54; K = 0.306, AUC = 0.74, respectively) [21].Concordance between OGTT and HbA1c is low with IGT (30%), intermediate with impaired fasting glucose (IFG) (44.9%) and higher with IFG + IGT (51.4%) [22]. The contribution of postprandial plasma glucose is more at a lower HbA1c [23]. Hence HbA1c reflects more post-glucose load values in the prediabetic range. However, both fasting and post-glucose values are raised in diabetes. This may explain why there is poor concordance between OGTT and HbA1c in the diagnosis of prediabetes, but not diabetes. In addition, hyperglycaemia may mask the contribution of BMI on HbA1c in diabetes [13].

The strength of this study was that it was done in a single race and ethnicity. OGTT and HbA1c were done in a single centre which was NGSP certified. HbA1c was measured by the capillary electrophoresis method. Although it was not possible to calibrate this method with the gold standard high performance liquid chromatography (HPLC) method, other studies have shown a good concordance between the two methods ($R^2 = 0.99$, $p < 0.0001$, good agreement with Bland–Altman plot) [24]. This method is accurate (between run coefficient of variation < 2%), reproducible and is not affected by labile HbA1c, carbamylatedHb, HbF, and hemoglobinopathies [25]. Still, other factors which may influence HbA1c were excluded from the study. However, it was not possible to do Hb electrophoresis in all the participants. A limitation of the study is the small sample size and retrospective nature of the study.

Conclusion

HbA1c was not accurate in the diagnosis of prediabetes in adults with BMI ≥ 23 kg/m^2. A higher cut-off should be used for the diagnosis of prediabetes, but not diabetes in this group. In summary, we should not rely solely on HbA1c for the diagnosis of prediabetes in overweight and obese adults.

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Data Availability Data is available on request from the corresponding author.

Declarations

Conflict of interest The authors do not have any conflict of interest.

Ethical clearance Consent for participation was obtained and the study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU) [registration number 651]. Data were obtained from hospital records.

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ORIGINAL ARTICLE

Parental monitoring status of the children with type 1 diabetes mellitus (DM) and their quality of life

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Abstract

Objective This study was carried out to determine the relationship between parental monitoring status of the children with type 1 DM and their quality of life.

Methods This descriptive-correlational type study was conducted in the pediatric endocrine outpatient clinic of a university hospital located in the northern region of Turkey and included 126 children with type 1 diabetes. The data of the study were collected with the "Parental monitoring of diabetes care scale (PMDC) in adolescents with type 1 diabetes" and "Pediatric quality of life inventory (PedsQL 3.0) diabetes module for children."

Results It was determined that 20.6% of the children were hospitalized for a reason related to diabetes and 7.1% received psychological support due to their disease. The mean score of the parents on the parental monitoring in diabetes care scale in adolescents with type 1 diabetes was found to be 65.40 ± 15.38, and the mean score on the pediatric quality of life inventory for children with type 1 diabetes was found to be 109.11 ± 16.99. No statistically significant correlation was determined between the parents' scores of the parental monitoring in diabetes care scale in adolescents with type 1 diabetes and the scores of the pediatric quality of life inventory for children with type 1 diabetes ($p > 0.05$).

Conclusion Although it was observed in the study that the levels of parental monitoring in type 1 diabetes care and pediatric quality of life were above the moderate level, parental monitoring was not found to affect children's quality of life.

Keywords Type 1 diabetes · Psychopathology · Parental monitoring · Quality of life

Introduction

Although the adolescence period is a period in which an individual's knowledge and self-care regarding type 1 diabetes can be at the highest level, it is also a period when the management and metabolic control of the disease are most difficult, and children and parents experience more problems. Studies have also shown that parents of children with type 1 diabetes experience problems with the management of the disease and have concerns about poor metabolic control during adolescence. In this context, parental monitoring and follow-up are especially important in chronic diseases such as type 1 diabetes [1]. Parental monitoring includes parental attitudes covering the communication between the adolescent and the parent and the importance given to the child's whereabouts, what he does, and the management of the disease. Another phase of parenting attitudes, that is the monitoring attitude, is the parent's knowledge of the activities that the adolescents do outside home. In type 1 diabetes, parental monitoring is expressed as a set of parental behaviors that include paying attention to monitoring the child's whereabouts, activities and compliances [2]. Studies have also stated that disease management and metabolic control will be better as parental monitoring increases [3–5]. It has been reported in a study by Ellis et al. that parental monitoring plays a protective role in adolescents with type 1 diabetes as well as in healthy adolescents [2]. Moreover, previous studies have shown that managing

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children's diabetic conditions requires changes in routines, diet, and lifestyle, and this new reality has direct implications for the entire family [1, 3, 6]. It is stated that parents of T1DM patients experience deep emotional distress; the daily routine may become a source of family discussions and conflicts; and especially adolescents may not always understand and meet parents' expectations regarding compliance with testing and care protocols [1, 5]. In addition, these families experience the difficulty of living with the constant threat of deterioration, repeated hospitalizations, and gradual loss of functionality, which creates additional stress and burden beyond the daily care of their children [3, 7]. Again, many studies have shown that treatment compliance and follow-up of the families of children with type 1 DM are effective in maintaining children's metabolic control [8–10].

Quality of life has become an important concept in the evaluation of children with chronic diseases. Chronic disease directly affects the individual's quality of life because it causes changes in lifestyle during the follow-up and treatment process of the disease [11]. Many studies have shown that the quality of life in children with diabetes is lower than in healthy children [12]. Quality of life reflects people's capacity and ability to manage diabetes treatment and achieve treatment goals. They appear to be effective in managing the disease, ensuring good metabolic control and preventing the development of secondary complications in children with diabetes who have a good quality of lifes [13]. When the quality of life is negatively affected in children with diabetes, they are at risk of psychological problems, decreased compliance with treatment, and also poor metabolic controls [11–14].

Studies have shown that parental support in the management of the disease in type 1 DM positively affects child's glycemic control, quality of life and general diabetes outcomess [10, 13, 15]. However, it has been observed that the effects of some characteristics of the parents (sociodemographic characteristics, communication characteristics, anxiety and anxiety states of the parents, and conflict situations) on the child's disease management (metabolic control, Hba1c levels, etc.) and quality of life were mostly investigated in the literature. No study has been found regarding the impact of the family's follow-up and monitoring of the disease on the child's quality of life. In this context, the study was carried out to determine the relationship between the disease monitoring status of the families of children with type 1 DM and the children's quality of life.

Material and methods

Study design

This was a descriptive and correlational-type study.

The study was carried out with children and their parents who admitted to pediatric endocrine outpatient clinic of a university hospital located in a large city in the northern part of Turkey and who were diagnosed with type 1 DM between September 2022–June 2023. The universe of the study was composed of 152 children with type 1 DM between 7 and 12 years old who were monitored for at least six months in this hospital for check up purposes; and the sample of the study included 126 children and their parents who brought their children to regular check-ups during the indicated time period. Participation rate was 83%. The following are the inclusion criteria: being able to read and write Turkish, having a child over 7 years old who has been monitored at least for 6 months due to the diagnosis of type 1 diabetes, and having no physical or psychological disease.

Data collection instruments

"Child-parent information form," "Parental monitoring in diabetes care scale in adolescents with type 1 diabetes," and "Pediatric quality of life inventory (PedsQL 3.0) diabetes module for children" were used as data collection instruments.

Child-parent information form

This form was developed by the researcher in line with the literature. It consists of questions including the sociodemographic characteristics of the families (5 questions) and the disease process (5 questions) [4, 16].

Parental monitoring in diabetes care scale in adolescents with type 1 diabetes (type 1 PMDC)

The scale, which was developed (2008) and revised by Ellis et al. (2012), and whose Turkish validity and reliability studies were conducted by Türk et al., consists of 27 items [2, 4, 17]. The scale is a five-point Likert type scale. "At least once a week" is scored as "1," and "more than once a day" is scored as "5." The highest score from the scale is 135, and the lowest score is 27; parental monitoring increases as the score increases. In the study conducted by Türk et al., the Cronbach alpha value of the scale was calculated as 0.85 [4]. In this study, it was found to be 0.89.

Pediatric quality of life inventory (type 1 PedsQL 3.0) type 1 diabetes module for children

The Turkish validity study of the scale, which was developed by Varni et al. (2001, 2003), was conducted by Ayar and Ozturk (2016) [18–20]. PedsQL 3.0 Diabetes Scale (28 items), which evaluates the quality of life in children with

type 1 and type 2 DM consists of five subscales including diabetes symptoms (11 items), treatment barriers (4 items), treatment adherence (7 items), worry (3 items), and communication (3 items). In this five-point Likert scale, 0 = 100 points indicate that it never creates a problem, 1 = 75 points indicate that it rarely creates a problem, 2 = 50 points indicate that it sometimes creates a problem, 3 = 25 points indicate that it often creates a problem, and 4 = 0 points indicate that it always creates a problem. Each item in the scale receives a score between 0 and 100, and it is thought that the higher the total score that can be obtained from the scale, the better the health-related quality of life is perceived. In the original child form of the scale, internal consistency coefficient of the total scale was 0.71; and the reliability coefficients of the subscales were found to be 0.81 for the diabetes symptoms, 0.66 for the treatment barriers, 0.66 for the treatment adherence, 0.63 for the worry, and 0.77 for the communication [19]. In this study, the internal consistency coefficient of the child form of the pediatric quality of life inventory diabetes module was found to be 0.88. In the study, the internal consistency coefficients for the subscales were found to be 0.83 for the diabetes symptoms, 0.45 for the treatment barriers, 0.82 for the treatment adherence, 0.81 for the worry, and 0.76 for the communication.

Implementation of data collection instruments

After obtaining the necessary ethics committee and institutional permissions to conduct the study, the researchers informed the parents about the study, and the parents who volunteered to participate were asked to fill out the necessary forms. The cover page of the data collection instruments is a document containing brief information about the research and obtaining the participant's voluntary consent in writing.

Ethical aspect of the study

Approval was obtained from the ethics committee of the relevant university to conduct the study (Date: 03.13.2020/ Ref. No: 2020–209). The data collection process in the study was initiated after obtaining the necessary ethics committee and institutional approval. Permission was also obtained for the scales used in the study. Additionally, written and verbal consents were obtained from the child and his/her family before the data collection forms were distributed.

Statistical analysis

The data were analyzed with SPSS 26.00 package program. While investigating the conformity of the variables for normal distribution, the skewness and kurtosis values of the scale scores were checked whether they were between +1 and −1, and it was determined that the data were not normally distributed. Descriptive statistics were carried out for the sociodemographic data. Differences between scale scores based on sociodemographic characteristics were analyzed using non-parametric tests (Mann-Whitney *U*, Kruskal Wallis test), and Spearman's correlation analysis was used to examine the correlation between the scales. When interpreting the results, the significance level was accepted as $p < 0.05$.

Results

91.3% of the parents participating in the research were mothers, and their mean age is 38.80 ± 4.71 years. The parents of 54% of the children were high school graduates, and the income of the family was lower than expenses in 56.3%. The mean age of the children is 9.94 ± 1.56 years, and 53.2% are females (Table 1).

It was determined that 49.2% of the children diagnosed with type 1 diabetes came for check-ups every 2–3 months and 94.4% were able to measure their own blood glucose. According to the statements of the parents, the rate of admitting to emergency service due to imbalance in the blood glucose of children in the last year was 11.9%, the rate of hospitalization for a diabetes-related reason was 20.6%, and the rate of receiving psychological support due to the disease was 7.1% (Table 2).

The mean score of parents on the parental monitoring in diabetes care scale in adolescents with type 1 diabetes was found to be 65.40 ± 15.38; and the mean score on the pediatric quality of life inventory for children with type 1 diabetes was found to be 109.11 ± 16.99. The mean scores of the subscales were found as 66.66 ± 15.00 for diabetes symptoms, 49.80 ± 20.01 for treatment barriers, 73.72 ± 22.45

Table 1 Sociodemographic characteristics of the parents and children

Parent's age ($X \pm SD$) (min–max)		38.80 ± 4.71 (27–52)	
Child's age ($X \pm SD$) (min–max)		9.94 ± 1.56 (7–14)	
		n	%
Parent's	Mother	115	91.3
	Father	11	8.7
Education status of the parents	Elementary and secondary school	46	36.5
	High school	68	54.0
	University and higher	12	9.5
Income status	Income lower than expenses	71	56.3
	Income equal to expenses	31	24.6
	Income higher than expenses	24	19.0
Child gender	Female	67	53.2
	Male	59	46.8

Table 2 Disease process-specific data of the children diagnosed with type 1 diabetes

Disease-specific data		n	%
The frequency of coming for check up due to the diagnosis of type 1 diabetes	0–1 month	2	1.6
	2–3 months	62	49.2
	3–6 months	51	40.5
	7 months and more	2	1.6
	When the doctor calls	9	7.1
Status of child to measure his/her own blood glucose	Able to	119	94.4
	Not able to	7	5.6
Status of admitting to emergency service due to the imbalance in blood glucose in the last year	Yes	15	11.9
	No	111	79.4
Status of hospitalization due to a diabetes-related reason in the last year	Yes	26	20.6
	No	100	79.4
Status of receiving psychological support due to the diagnosis of type 1 diabetes	Yes	9	7.1
	No	117	92.9

Table 3 The scores of parental monitoring in diabetes care scale in adolescents with type 1 diabetes and the pediatric quality of life inventory diabetes module for children with type 1 diabetes

	$X \pm$ SD (min–max)
Parental monitoring in diabetes care scale in adolescents with type 1 diabetes (type 1 PMDC)	109.11 ± 16.99 (72–135)
Pediatric quality of life inventory diabetes module for children with type 1 diabetes (type 1 PedsQL 3.0)	65.40 ± 15.38 (12–90)
Diabetes symptoms	66.66 ± 15.00 (20–90)
Treatment barriers	49.80 ± 20.01 (0–93)
Treatment adherence	73.72 ± 22.45 (0–100)
Worry	62.16 ± 30.10 (0–100)
Communication	2.14 ± 1.19 (1–5)

X mean, *Sd* standard deviation, *Min* minimum, *Max* maximum

Table 4 The correlation between type 1 PMDC and type 1 PedsQL

		Type 1 PMDC
Type 1 PedsQL	*r*	−0.045
	p	0.621

for treatment adherence, 62.16 ± 30.10 for worry, and 2.14 ± 1.19 for communication (Table 3).

No statistically significant differences were found between type 1 PMDC and type 1 PedsQL scores based on the sociodemographic characteristics of the parent and the child and some disease-specific data. No statistically significant relationship was determined between the parents' scores on the parental monitoring in diabetes care scale in adolescents with type 1 diabetes and the scores on the pediatric quality of life scale diabetes module for children with type 1 diabetes ($p > 0.05$) (Table 4).

Discussion

Parental monitoring in diabetes management plays a vital role in preventing direct and indirect diabetes-related complications and improving long-term health outcomes; and it is considered a strong indicator of adaptation to the disease [17, 21]. In this current study, the mean score of the parental monitoring in diabetes care scale in adolescents with type 1 diabetes was found to be above the moderate level (109.11 ± 16.99). Similarly, in a study conducted with parents of the adolescents with type 1 diabetes, the mean score of the parent diabetes monitoring scale was found to be above the moderate level (97.2 ± 15.2). In a study conducted in Poland, it was observed that some socioeconomic factors such as the income level of the families and living in the city center affected the follow-up and monitoring of the children by the families [22]. Another study showed that families experienced more concerns; and therefore, complied with treatments more as the age of the child decreased [23].

Chronic diseases are conditions that negatively affect the quality of life, are physically exhausting, cause negative emotional and spiritual effects on the child and their families, and require serious psychosocial support. In the current study, the mean score of the pediatric quality of ife inventory diabetes module for children with type 1 diabetes was found to be above the moderate level (65.40 ± 15.38). In literature reviews, it has been reported that quality of life scores varies between 59.2 and 73.8, and the development levels of countries are also effectives [13, 24, 25]. Studies have determined that the life quality of children diagnosed with different chronic diseases is lower than healthy children [26–30]. In addition, studies have also shown that children with diabetes have a lower quality of life compared to healthy children s

[12, 13, 16, 31, 32]. Duras et al. (2018) stated that the life quality of children diagnosed with type 1 diabetes mellitus is significantly lower than that of healthy childrens [27]. In the study by Bozbulut et al. (2022), quality of life was found to be 69.6 ± 14.9 compared to an adolescent [16].

This study showed that child and parent characteristics did not affect the life quality of the children with diabetes. Studies have shown that the quality of life of children with type 1 DM is affected by sociodemographic characteristics (age, gender) and some clinical indicators (HBA1c, frequency of hypo- and hyperglycemia attacks, etc.) [11, 13, 24, 25, 31, 33–35]. In an international cohort study, it was determined that three diabetes self-management behaviors (increasing the frequency of daily blood glucose monitoring, using carbohydrate counting, and exercising for at least 30 min per week) were significantly associated with better quality of life [36]. It has been observed that the low education level of the parents of children with type 1 diabetes and the presence of diabetes-specific family conflict negatively affect their quality of life [36]. In a study conducted in Brazil with a group of adolescents with type 1 DM between the ages of 10 and 19 years, it was determined that their quality of life was high; but the time since diagnosis, female gender, low family income, and parental education level affected their quality of life [37]. In a study conducted in Spain, it was determined that the quality of life was also negative in older children (> 11 years old) and patients with poor metabolic control [11]. Sundberg et al. (2014) found in their study that the life quality of underage children with type 1 DM was significantly lower than that of healthy children [33]. Moreover, in the study by Anderson et al. (2017), it was observed that the lowest quality of life was in the 19–25-year-old age group and female gender among the 8–12-, 13–18-, and 19–25-year-old age groups diagnosed with type 1 DM [36]. Additionally, studies have shown that the quality of life levels reported by the parents of children with diabetes is lower than the quality of life reported by their children [24, 32–34].

The study showed that parental monitoring in diabetes care did not affect the quality of life in children with type 1 diabetes. In another study, it was determined that the quality of life increased as parental monitoring increased [16]. In the study by Gorzny including adolescent patients with type 1 DM, it was found that adolescents, whose mothers followed their disease process more, had a higher quality of life than those who reported that their mothers monitored less; and it was also detected that adolescents had less depressive symptoms when their mothers participated in the diabetes management process more [15]. In a study conducted with adolescents with type 1 DM between the ages of 12 and 18 years, it was determined that adolescents who perceived that their parents had authoritarian parenting styles adapted better to the prescribed treatment plans and had a better perceived quality of life [38]. Besides, another study conducted with children with diabetes and their families showed that increased family conflict and less parental supervision negatively affected the child's diabetes self-care and glycemic control [21].

Study limitations

The limitations of the study are that it was conducted in a single center, and thus, the results cannot be generalized for Turkey. Again, since the research data were collected during the COVID-19 period, this resulted in the participation of fewer children with diabetes and their families coming to the outpatient clinic for check up. Comprehensive multicentric studies are needed in this field.

Conclusions

Although it was observed in the study that the levels of parental monitoring and quality of life for the children with type 1 diabetes were above the moderate level, parental monitoring was not found to affect children's quality of life.

Due to the effects of parental attitudes of children with diabetes on adaptation to the disease and quality of life, psychopathology, parental attitudes, perceptions of quality of life, and their relationship with each other should be taken into consideration in the monitoring of the disease in children with chronic diseases as well as DM. In order for school-age children with diabetes not to feel excluded, the school health nurse should provide informative trainings including the family and teachers (Duras et al., 2018). In addition, it is important for diabetes nurses, who contribute to the disease process together with children, to plan and implement initiatives to encourage parental monitoring in diabetes management. It is thought that these initiatives will contribute to preventing or reducing health behaviors and family conflicts that will negatively affect the adolescent's quality of life in the future.

Author contribution ETB, HU, and MK: conceptualization, methodology, writing—original draft preparation, investigation, supervision, and writing—reviewing and editing. RD: data curation, writing—original draft preparation, and investigation.

Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval Approval was obtained from the ethics committee of the relevant university to conduct the study (date: 03.13.2020/Ref. No: 2020-209).

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Factors affecting the prolongation of glycemic time in range among children with type 1 diabetes using continuous glucose monitoring systems: A case control study

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Abstract

Background Time in range is a reliable measure of the risk of diabetes complications. High percentage of patients with diabetes fail to achieve the recommended time in range (TIR) target of 70–180 mg/dl (3.9–10 mmol/l) >70%.

Objective This study aimed to identify factors influencing TIR prolongation.

Methods Children aged 1–17 years with >1-year type 1 diabetes (T1D) duration, treated with continuous subcutaneous insulin infusion (CSII) ≥3 months, using continuous glucose monitoring (CGM) or intermittently scanned CGM (is-CGM) ≥1 month, and with a registration time >70% were included. Data were collected during routine diabetology visits at an outpatient clinic. Insulin pump and CGM or is-CGM reports in the most recent 14 days were recorded using a dedicated software. Legal caregivers were also asked to complete a questionnaire on how the patients use the insulin pump functions and eating habits.

Results A sample of 110 patients was categorized into two groups: those with TIR >70% and TIR ≤70%. TIR ≤70% group presented with repeated hyperglycemia and a high glycemic variability coefficient of variation. We noted an acceptable hypoglycemia rate (3%), regardless of the TIR value. Patients with TIR >70% predominantly used predictive low glucose suspend system, maintained adequate intervals between insulin delivery and meal consumption, used the "bolus calculator" function, and more frequently created electronic reports.

Conclusions Hyperglycemia and high glycemic variability prevent patients from achieving the target TIR. Advanced features in the CGM systems, premeal insulin bolus, and patients' involvement in diabetes treatment are the main factors contributing to TIR prolongation.

Keywords Blood glucose self-monitoring · Continuous glucose monitoring system · Type 1 diabetes · Disease management · Therapeutics

Introduction

According to the US Type 1 Diabetes (T1D) Exchange Registry, continuous glucose monitoring (CGM) systems are currently the fastest growing technology for diabetes treatment [1]. Numerous benefits of CGM have been demonstrated; it has led to a reduction in the incidence of hypoglycemia and a decrease in glycated hemoglobin (HbA1c) levels up to 2.05% and reduce glycemic variability [2–4]. The increasing accuracy and advancement of CGM systems and their widespread availability have resulted in new indicators of proper glycemic control [5, 6].

In 2019, Battelino et al. published an international consensus on glycemic targets for patients using the CGM systems (Table 1), which has been accepted by many

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international societies, including the Polish Diabetes Association [6, 7]. The concept of the time spent in the target range of 70–180 mg/dl (3.9–10.0 mmol/l) or the time in range (TIR) warrants special attention. TIR was validated as an outcome measure in clinical trials complementing other components of glycemic control [8]. TIR is strongly associated with the occurrence of vascular complications and peripheral neuropathy [8–11]. Each 10% increase in TIR is associated with a 64% reduction in the risk of retinopathy and a 40% reduction in the risk of microalbuminuria [8]. TIR and HbA1c levels are correlated; each 10% increase in TIR is associated with a 0.6–0.8% decrease in HbA1c levels [8, 12, 13]. However, HbA1c levels may be affected by many conditions that influence the survival of the red blood cells independent of glycemia, including the glycation rates, uremia, pregnancy, smoking, and ethnicity [8]. Therefore, TIR is a more reliable measure of the risk of diabetes complications [13]. High percentage of patients with diabetes fail to achieve the recommended TIR target of >70%. Diabetes Control and Complications Trial (DCCT) obtained data from 1440 participants and demonstrated that a TIR is relatively low among patients with diabetes (52% vs. 31% for intensive vs. conventional treatment, respectively) [9]. Data from the Swedish Childhood Diabetes Registry revealed a mean TIR of 60.8% (±13.1%) [14].

This study aimed to identify factors that influence TIR prolongation, using CGM data among the pediatric population with T1D, on continuous subcutaneous insulin infusion (CSII).

Materials and Methods

The patients were recruited from the Department of Pediatric Diabetology and the Diabetic Outpatient Clinic at the Clinical Hospital. The study group were children aged 1–17 years with a >1-year T1D duration, treated with CSII ≥3 months, used CGM or intermittently scanned CGM (is-CGM) ≥1 month, and with registration time >70% (the percentage of time CGM is active, from the last 14 days) [6]. No restrictions were imposed on participation with respect to the type of CGM and is-CGM. Participants used the following devices: Medtronic Minimed: Guardian™ Sensor 3 with Guardian™ Link 3 transmitter (GL3); Sensor Enlite™ with Guardian™ 2 Link transmitter (GL2) or Guardian™ Connect (GC); Dexcom Inc: Dexcom G6 and Dexcom G5; and Abbott Diabetes Care: Free Style Libre (FSL). The records from the CGM system were registered using the dedicated software.

Polish citizens have equal access to healthcare services provided by the National Health Insurance System and managed by the National Health Fund. Treatment with insulin pumps is available and unpaid for, for children up to 26 years of age with T1D. The patients had access to insulin pumps Medtronic Minimed: Paradigm VEO, G640 and Roche Diabetes Care Accu-Chek Combo, free of charge.

The patients were under constant care at the outpatient clinic and had permanent access to medical assistance. Data were collected during routine clinical visit from January to April 2021. The study flow diagram is presented in Fig. 1. Insulin pump and CGM or is-CGM data were recorded using a dedicated software. CGM metrics were analyzed in the most recent 14 days, as per the recommendations of International Consensus on Time in Range [6, 8]. Moreover, legal caregivers were asked to complete a questionnaire (Appendix 1) on how the patients use the insulin pump functions and eating habits. Severe hypoglycemia was defined as an event with severe cognitive impairment (including coma and convulsions) requiring assistance by another person.

Anthropometric measurements (weight and height) were taken to calculate the body mass index standard deviation score (BMI-SDS), which was calculated using the World Health Organization child growth standards. A blood sample was taken for determining HbA1c levels. The test was performed at the hospital laboratory using a high-performance liquid chromatography (D-10 Hemoglobin Testing System, Bio-Rad Laboratories, USA) at a nondiabetic range of 4.1–6.4% (21–46 mmol/mol).

Statistical analysis

The sample of 110 patients was grouped into two categories: those with TIR >70% (study group, $n = 50$) and those with TIR ≤70% (control group, $n = 60$). Nominal variables were

Table 1 Glycemic targets for patients using CGM systems

	Time in range (TIR)	Time below range (TBR)		Time above range (TAR)	
Target values	70–180 mg/dl (3.9–10 mmol/l)	<70 mg/dl (<3.9 mmol/l)	<54 mg/dl (<3.0 mmol/l)	>180 mg/dl (>10.0 mmol/l)	>250 mg/dl (>13.9 mmol/l)
Percent of readings [%]	>70%	<4%	<1%	<25%	<5%
Daily time	>16 h 48 min	<1 h	<15 min	<6 h	<1 h 12 min

CGM continuous glucose monitoring, *h* hour, *min* minutes

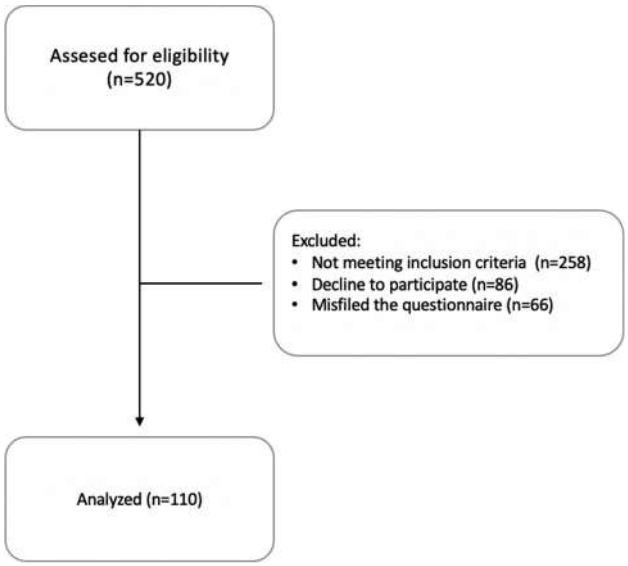

Fig. 1 The study flow diagram

presented as frequencies and percentages. Numeric variables were described using basic descriptive statistics, depending on the distribution (for those with normally distributed variables, mean ± standard deviation were reported; for other distributions, the median along with the first and third quartiles are reported). The normality of variable distribution as well as skewness and kurtosis were verified using Shapiro-Wilk's test. Homogeneity of variance was checked using Levene's test.

Groups were compared using Pearson's chi-square test, or Fisher's exact test in case of categorical variables. For assessing group similarity with numeric variables, the independent Student *t*-test, independent Welch's *t*-test, or Mann-Whitney *U* test were used, as appropriate. Assessment of group differences was additionally described using mean/median difference or risk ratio, with 95% confidence intervals (CIs).

Univariate logistic regression analyses were run for all variables to assess the impact of each factor on the risk of not exceeding 70% TIR. An additional multivariate logistic regression analysis was executed and presented to describe the simultaneous impact of selected variables. The variables were selected based on the significance level reached in the univariate model as well as the relationship between variables. Model quality assessment included chi-square test, Hosmer and Lemeshow goodness of fit (GOF) test, and R^2 Nagelkerke as well as variance inflation factor (VIF) measures. All calculations were conducted assuming 0.05 significance level and run using R software, version 4.1.2 [15].

Univariate logistic regression analyses were performed for all independent variables separately. Odds ratios (ORs) and their 95% CIs were presented to determine the odds of patients' TIR not exceeding the level of 70%. In the second step, a multivariate model including selected variables was built. Variables included in the second step were selected based on the significance level in the univariate models and on the relationships between the variables.

Results

Characteristic of the group

The sample of 110 patients was grouped into study and control groups with TIR >70% (study group, $n = 50$) and TIR ≤70% (control group, $n = 60$), respectively; both the groups had an equal sex distribution (50.0% each) and all participants were of Caucasian descent. Average age at diagnosis in the cohort was 7.06 ± 3.74 years, with no statistical difference between groups ($p = 0.057$). Significant difference occurred in the duration of the disease ($p = 0.002$), which was 3 years (median = 3.19) in the study group and 4 years (median = 4.34) in control group (median difference, MD = −1.15, 95% CIs [−2.58; −0.15], $p = 0.002$). Characteristic of the analyzed groups is presented in Table 2.

Patients had CSII implemented just after diabetes diagnosis without differences between groups ($p = 0.324$). Medtronic Paradigm VEO (49.1%) was the most common type of insulin pump used. There was no difference in insulin types ($p = 0.224$) but infusion set type differed significantly between groups ($p = 0.014$). Ninety-degree teflon cannulas were used by majority of the participants in the study group (72.0%). Majority of the patients (78.2%) reported exchanging the infusion set every 3 days. There was no statistical difference between the frequency of replacement of infusion sets between the two groups ($p = 0.559$). Median time of CGM system usage was 763.50 days and did not differ between the groups ($p = 0.318$). However, the groups used different types of CGM ($p = 0.022$). In the study group, most of the patients used GL3 (32.0%); in the control group, more than half (60.0%) the patients used FSL.

There was a significant difference ($p = 0.018$) between the study group (74.0%) and the control group (50%) in the number of patients who generated electronic reports based on CGM and insulin pump data in a domestic environment. Those who were unable to create electronic reports at home had 56% higher chance of being in TIR ≤70% group (RR = 1.56, 95% CIs = [1.12; 2.17]). The study group patients were more likely to use the "bolus calculator" function (56.0% vs. 33.3%, respectively). The lack of function usage tended to increase the chances of lower TIR by 55% (RR = 1.55, 95% CIs = [1.06; 2.27]). There was no difference in the proportion of patients counting their carbohydrate and fat-protein units (FPU) between the two groups ($p = 0.374$ and $p = 0.371$, respectively). Carbohydrate exchanges were

Table 2 Characteristics of the analyzed group

Variable	Total	TIR > 70%	TIR ≤ 70%	MD[5]/RR[6] (95% CI)	*p*
N	110	50	60		
Sex, *n* (%)					
Girl	55 (50.0%)	23 (46.0%)	32 (53.3%)	Reference	0.566[4]
Boy	55 (50.0%)	27 (54.0%)	28 (46.7%)	0.88 (0.62; 1.23)[7]	
Age, years, mean ± SD	11.96 ± 3.73	11.70 ± 3.56	12.18 ± 3.89	−0.48 (−1.9; 0.94)[6]	0.504[1]
BMI *Z*-score, mean ± SD	0.46 ± 1.03	0.63 ± 1.13	0.33 ± 0.93	0.30 (−0.09; 0.69)[6]	0.131[1]
Age (diagnosis), years, mean ± SD	7.06 ± 3.74	7.81 ± 3.80	6.44 ± 3.61	1.36 (−0.04; 2.77)[6]	0.057[1]
Disease duration, days, median (Q1; Q3)	1430 (827.75; 2382.75)	1164.50 (634.25; 1567.25)	1583.50 (1188.75; 2975.25)	−419.00 (−960.50; −57.50)[6]	0.002[3]
Disease duration, years, median (Q1; Q3)	3.92 (2.27; 6.53)	3.19 (1.74; 4.29)	4.34 (3.26; 8.15)	−1.15 (−2.58; −0.15)[6]	0.002[3]
Time from diagnosis to pump set up, days, median (Q1; Q3)	10.00 (7.00; 56.00)	12.00 (7.00; 56.00)	9.00 (7; 38.5.00)	3.00 (−2.00; 7.50)[6]	0.324[3]
Insulin pump type, *n* (%)					
Accu-Chek® Spirit Combo	21 (19.1%)	8 (16.0%)	13 (21.7%)	Reference	0.168[4]
MiniMed® PARADIGM VEO™	54 (49.1%)	21 (42.0%)	33 (55.0%)	0.99 (0.66; 1.47)[7]	
MiniMed® 640G	34 (30.9%)	20 (40.0%)	14 (23.3%)	0.67 (0.39; 1.12)[7]	
OmniPod®	1 (0.9%)	1 (2.0%)	0 (0.0%)	–	
Insulin type, *n* (%)					
NovoRapid, aspart. Novo Nordisk	35 (31.8%)	16 (32.0%)	19 (31.7%)	Reference	0.224[4]
Fiasp, faster insulin aspart, Novo Nordisk	26 (23.6%)	12 (24.0%)	14 (23.3%)	0.99 (0.62; 1.58)[7]	
Apidra, insulin glulisine, Sanofi-Aventis	13 (11.8%)	8 (16.0%)	5 (8.3%)	0.71 (0.33; 1.50)[7]	
Humalog, insulin lispro, Eli Lilly	21 (19.1%)	11 (22.0%)	10 (16.7%)	0.88 (0.51; 1.51)[7]	
Liprolog, insulin lispro, Eli Lilly	15 (13.6%)	3 (6.0%)	12 (20.0%)	1.47 (0.99; 2.19)[7]	
Infusion set type, *n* (%)					
Teflon cannulas 90°	65 (59.1%)	36 (72.0%)	29 (48.3%)	Reference	0.014[5]
Teflon angular cannulas 30–45°	7 (6.4%)	2 (4.0%)	5 (8.3%)	1.60 (0.93; 2.75)[7]	
Steel cannulas	36 (32.7%)	10 (20.0%)	26 (43.3%)	1.62 (1.15; 2.27)[7]	
OmniPod	1 (0.9%)	1 (2.0%)	0 (0.0%)	–	
Various	1 (0.9%)	1 (2.0%)	0 (0.0%)	–	
Infusion set change, *n* (%)					
Every 2 days	6 (5.5%)	4 (8.0%)	2 (3.3%)	Reference	0.559[5]
Every 3 days	86 (78.2%)	39 (78.0%)	47 (78.3%)	1.64 (0.52; 5.17)[7]	
Every 4 days	16 (14.5%)	7 (14.0%)	9 (15%)	1.69 (0.50; 5.67)[7]	
Every 5 or more days	2 (1.8%)	0 (0.0%)	2 (3.3%)	3.00 (0.97; 9.30)[7]	
Length of CGM usage, days, median (Q1; Q3)	763.50 (430.00; 1171.75)	665.00 (411.25; 1105.00)	784.50 (463.50; 1217.25)	−119.50 (−300.00; 153.77)[7]	0.318[3]
CGM type, *n* (%)					

Table 2 (continued)

Variable	Total	TIR > 70%	TIR ≤ 70%	MD[5]/RR[6] (95% CI)	*p*
Dexcom 6	9 (8.2%)	7 (14.0%)	2 (3.3%)	Reference	0.022[5]
Dexcom 5	4 (3.6%)	2 (4.0%)	2 (3.3%)	2.25 (0.47; 10.78)[7]	
Guardian™ Link 3 Transmitter and Guardian™	28 (25.5%)	16 (32.0%)	12 (20.0%)	1.93 (0.53; 7.04)[7]	
Free Style Libre	51 (46.4%)	15 (30.0%)	36 (60.0%)	3.18 (0.92; 10.92)[7]	
Mini Link 2 Transmitter and Enlite™ Sensor	14 (12.7%)	7 (14.0%)	7 (11.7%)	2.25 (0.60; 8.51)[7]	
Guardian Connect	4 (3.6%)	3 (6.0%)	1 (1.7%)	1.12 (0.14; 9.11)[7]	
Creating electronical reports in domestic environment, *n* (%)					
Yes	67 (60.9%)	37 (74.0%)	30 (50.0%)	Reference	0.018[4]
No	43 (39.1%)	13 (26.0%)	30 (50.0%)	1.56 (1.12; 2.17)[7]	
Bolus calculator function, *n* (%)					
Yes	48 (43.6%)	28 (56.0%)	20 (33.3%)	Reference	0.028[4]
No	62 (56.4%)	22 (44.0%)	40 (66.7%)	1.55 (1.06; 2.27)[7]	
Carbohydrate exchanges counting, *n* (%)					
Yes	105 (95.5%)	49 (98.0%)	56 (93.3%)	Reference	0.374[5]
No	5 (4.5%)	1 (2.0%)	4 (6.7%)	1.50 (0.93; 2.41)[7]	
Protein-fat units counting, *n* (%)					
Yes	62 (56.4%)	31 (62.0%)	31 (51.7%)	Reference	0.371[4]
No	48 (43.6%)	19 (38.0%)	29 (48.3%)	1.21 (0.86; 1.69)[7]	
Insulin/meal latency, *n* (%)					
Yes	63 (57.3%)	38 (76.0%)	25 (41.7%)	Reference	0.001[4]
No	47 (42.7%)	12 (24.0%)	35 (58.3%)	1.88 (1.33; 2.66)[7]	
Insulin/meal latency (time), *n* (%)					
0 min	47 (42.7%)	11 (22.0%)	36 (60.0%)	Reference	<0.001[4]
5 min	16 (14.5%)	9 (18.0%)	7 (11.7%)	0.57 (0.32; 1.02)[7]	
10 min	18 (16.4%)	14 (28.0%)	4 (6.7%)	0.29 (0.12; 0.70)[7]	
15 min or more	29 (26.4%)	16 (32.0%)	13 (21.7%)	0.59 (0.38; 0.90)[7]	
DKA, *n* (%)					
Yes	31 (28.2%)	15 (30.0%)	16 (26.7%)	Reference	0.862[4]
No	79 (71.8%)	35 (70.0%)	44 (73.3%)	1.08 (0.73; 1.60)[7]	
Severe hypoglycemia, *n* (%)					
Yes	16 (14.5%)	4 (8.0%)	12 (20.0%)	Reference	0.132[4]
No	94 (85.5%)	46 (92.0%)	48 (80.0%)	0.68 (0.48; 0.96)[7]	
Complications, *n* (%)					
Yes (retinopathy)	1 (0.9%)	1 (2.0%)	0 (0.0%)	–	0.455[5]
No	109 (99.1%)	49 (98.0%)	60 (100.0%)	–	
HbA1c, %, mean ± SD, mmol/mol	7.08 ± 0.96 54	6.36 ± 0.46 46	7.67 ± 0.86 60	−1.31 (−1.56; −1.05)[6]	<0.001[2]
AVG glycemia, mg/dl, median (Q1; Q3)	147.50 (131.5; 164.00)	133.50 (124.00; 139.00)	162.50 (152.75; 183.25)	−29.00 (−38.00; −21.50)[6]	<0.001[3]

Table 2 (continued)

Variable	Total	TIR > 70%	TIR ≤ 70%	MD[5]/RR[6] (95% CI)	*p*
CV, %, mean ± SD	36.06 ± 7.64	31.16 ± 4.67	40.13 ± 7.25	−8.97 (−11.25; −6.70)[6]	<0.001[2]
TIR, %, mean ± SD	67.76 ± 17.35	82.97 ± 7.38	55.08 ± 12.30	27.89 (24.12; 31.66)[6]	<0.001[2]
TAR180, %, mean ± SD	19.27 ± 9.66	11.75 ± 6.36	25.53 ± 7.15	−13.78 (−16.36; −11.20)[6]	<0.001[1]
TAR250, median (Q1; Q3)	5.00 (1.00; 11.00)	1.00 (0.00; 2.00)	11.00 (7.00; 19.00)	−10.00 (−15.00; −8.00)[6]	<0.001[3]
TBR70, %, median (Q1; Q3)	3.00 (2.00; 5.00)	3.00 (2.00; 5.00)	3.00 (1.00; 5.25)	0.00 (−2.00; 1.00)[6]	0.880[3]
TBR54, %, median (Q1; Q3)	1.00 (0.00; 1.00)	0.00 (0.00; 1.00)	1.00 (0.00; 2.25)	−1.00 (−1.00; −0.50)[6]	0.036[3]
TDD, IU/kg, mean ± SD	0.78 ± 0.23	0.71 ± 0.20	0.83 ± 0.24	−0.12 (−0.20; −0.03)[6]	0.007[1]
Base/kg, IU/kg, mean ± SD	0.29 ± 0.12	0.25 ± 0.12	0.32 ± 0.12	−0.07 (−0.11; −0.02)[6]	0.005[1]
Base/TDD, mean ± SD	0.37 ± 0.12	0.35 ± 0.12	0.38 ± 0.11	−0.03 (−0.08; 0.01)[6]	0.153[1]

AVG average, *DKA* diabetic ketoacidosis, *TIR* time in range, *TAR180* time above range >180 mg/dl ≦ 250 mg/dl, *TAR250* time over 250 mg/dl, *TBR70* time below 70 mg/dl ≧ 54 mg/dl, *TBR54* time below 54 mg/dl, *TDD* total daily dose of insulin, *CV* coefficient of variation, *IU* international unit, *SD* standard deviation, *Q1* first quartile, *Q3* third quartile. Group comparisons executed with independent *t*-Student test[1], independent *t*-Welch test[2], Mann-Whitney *U* test[3], chi-square Pearson's test[4] or exact Fisher test[5]. *MD*[6] mean or median difference (TIR ≤ 70% vs. TIR > 70%) with 95% confidence interval, *RR*[7] relative risk with 95% confidence interval

calculated by almost all patients (95.5%), while FPU were considered by more than half of them (56.4%).

The patients in the study group were more disciplined with regard to the latency period between insulin delivery and meal consumption (72.0%), when compared to the control group (41.7%, $p < 0.001$). Not maintaining a latency period between insulin delivery and meal consumption was correlated with an 88% higher chance of not reaching the 70% TIR level (RR = 1.88, 95% CIs = [1.33; 2.66], $p = 0.001$). Diabetic ketoacidosis (DKA) and severe hypoglycemic episodes were rare in both groups with no differences.

Data from CGM, except for time below the <70 mg/dl range, differed significantly between groups. Average glucose was higher in the control group and the difference ranged from MD = −1.00, 95% CIs [−1.00; −0.50], $p = 0.036$ for time spent below target glycemia (TBR, time below range) <54 mg/dl to MD = −13.78, 95% CIs [−16.36; −11.20], $p < 0.001$ for time spent above the target blood glucose level (TAR) >180 mg/dl. The total daily dose of insulin per kilogram (TDD) was significantly different between the two groups (MD = −0.12, 95% CIs [−0.20; −0.03], $p = 0.007$). Also, the level of basal insulin rate (BIR) per kilogram differed between the groups (MD = −0.07, 95% CIs [−0.11; −0.02], $p = 0.005$).

Logistic regression results

The longer disease duration is correlated with the worse TIR and one additional year increased the risk of not reaching 70% TIR by 20% (OR = 1.20, 95% CIs [1.06; 1.37], $p = 0.007$). Use of metal cannulas increased the risk of TIR ≤70% by three times, compared to usage of 90° teflon infusion sets (OR = 3.23, 95% CIs [1.37; 8.04], $p = 0.009$). The lack of both domestic report generation and usage of "bolus calculator" function almost tripled the risk of TIR ≤70% (OR = 2.85, 95% CIs [1.29; 6.55], $p = 0.011$ and OR = 2.55, 95% CIs [1.18; 5.60], $p = 0.018$, respectively). Lack of keeping a latency period between insulin delivery and meal consumption increased the risk by four times (OR = 4.43, 95% CIs [1.98; 10.45], $p < 0.001$). The HbA1c levels had a strong impact on the risk, with a 1% increase in HbA1c levels relating to a 161 times higher risk (OR = 161.93, 95% CIs [27.14; 1852.76], $p < 0.001$). Analyzing the CGM data, the strongest impact on the risk was noted for TAR >250 mg/dl; for every 1 pp rise, the risk increased four times (OR = 3.89, 95% CIs [2.31; 9.20], $p < 0.001$). Additionally, there was a significant risk of having a TIR ≤70% when both TDD and BIR increased with an impact of 11 and 101 times, respectively (TDD: OR = 11.75, 95% CIs [2.00 to 82.70], $p = 0.009$, BIR: OR = 101.08, 95% CIs [3.93; 3443.02], $p = 0.007$).

The multivariate model verified the simultaneous impact of HbA1c levels and different latency period categories on the risk of TIR ≤70%. The model identified high impact of additional 1 pp of HbA1c and the risk increased 278 times (OR = 278.81, 95% CIs [35.56 to 4915.73], $p < 0.001$). Further, maintaining a latency period of 5 or 10 min between insulin delivery and meal consumption reduced the risk by 95% (vs. not waiting). These showed OR = 0.05, 95% CIs [0.00 to 0.37], $p = 0.007$ for 5 min latency and OR =

0.05, 95% CIs [0.00 to 0.60], $p = 0.036$ for 10 min latency (Appendix 2).

Discussion

This study attempted to identify factors that contributed to prolonged TIR in children and adolescents using CSII. We found that the main problem of patients not achieving the >70% target range included repeated hyperglycemia and high glycemic variability defined by coefficient of variation (CV). Participants achieving TIR >70% predominantly used predictive low glucose suspend systems, maintained adequate interval between insulin delivery and meal consumption, used bolus calculator, and more frequently created electronic reports. Moreover, in this group, we observed lower daily and basal insulin requirements.

Furthermore, disease duration >3 years in our study participants lowered the probability of exceeding 70% TIR, and one additional year of diabetes duration increased the risk by 20%. The diabetes duration in the group was not long (median, 3 and 4 years for study and control groups, respectively) and the whole cohort had good metabolic control as assessed by HbA1c levels (median 7.08%; 54 mmol/mol). The possible reason for this trend is the effect of the patients' age (median age, 12 years); when they become adolescents, the glycemic control worsens compared to childhood. Adolescents have the highest glycemic variability and poorest metabolic control (especially those aged 13–18 years) [16, 17].

We found no differences between the insulin analogs used by the study participants. There is a huge interest now on faster acting insulin analogs. As demonstrated, faster acting insulin aspart (faster aspart) used in children prolonged TIR (38% vs. 50%) and is more effective than insulin aspart in reducing postprandial hyperglycemia during the first and second hour after consuming a meal [18, 19]. We did not note in our study group that more participants used faster aspart. We also found no influence of the type of insulin pump on the TIR.

Previous studies have not reported differences between steel and teflon infusion sets in their function over 7 days [20]. In this study, we found that teflon cannulas correlated with better TIR. The possible cause of the observed trend is the predominant use of teflon cannulas in our diabetology center, thus making them the first choice for most patients.

The general rule concerning length of use is 2 and 3 days for steel and teflon infusion sets, respectively [21, 22]. Our study participants declared changing their infusion sets regularly (78.2% declared every 3-day exchange), without differences between the two groups.

We recruited participants who used both types of CGM: real-time CGM (RT-CGM) and is-CGM. Some RT-CGMs work with insulin pump and have the following additional features: predictive low glucose suspend (PLGS) or low glucose suspend (LGS), that influence metabolic control. Among our study group, patients with TIR >70% predominantly used the PLGS system (GL3). We noted that not all participants who had the opportunity of using PLGS system took advantage of it. Thirty-four participants (in both groups) used Medtronic G640 insulin pump, but only 28 used compatible CGM system (GL3) with PLGS function. The possible cause of that is dissatisfaction with the CGM system due to inaccurate blood glucose measurements, need for calibration, and/or lack of mobile phone application. We observe that parents having younger children prefer the CGM system with a mobile phone application to manage diabetes remotely as it increases their sense of security. There is high quality evidence that PLGS leads to decreased time spent in hypoglycemia and nocturnal hypoglycemia, with no increase in the mean blood glucose concentration and hyperglycemia episodes [23–25].

Participants in the study group were more likely to calculate the insulin dose using "bolus calculator," an available feature of automated bolus calculation in most insulin pumps. Adult user data indicates that the use of a "bolus calculator" improved HbA1c levels, mean blood glucose levels, and glucose variability [26, 27]. On the other hand, a randomized controlled trial in a pediatric group did not reveal any additional effect of "bolus calculator" use with regard to HbA1c levels, postprandial blood glucose values, or other study outcomes [26].

The International Society for Pediatric and Adolescent Diabetes (ISPAD) recommends carbohydrate counting from the onset of diabetes, because it is correlated with improved glycemic control and quality of life among both adults and adolescents [28]. There are few methods of calculating carbohydrate, but research found no evidence to suggest that one particular method is superior to another [28]. In our diabetology center, during the first hospitalization, patients use 10 g carbohydrate portions and are introduced to carbohydrate counting and insulin dose calculations by using an individualized insulin-to-carbohydrate ratio. It is worth emphasizing that almost all study participants declared that they were counting carbohydrates (95.5%). Some patients in our center count also FPU because those macronutrients (fat and protein) lead to delayed hyperglycemia (up to 3–6 h after the meal) [29]. Usually, patients count that 1 FPU equals 100 kcal of fat or protein and requires the same amount of insulin (as an extended bolus) as 10 g of carbohydrates [29]. Over half (56.4%) of the participants declared that they counted FPU, without any differences between the two groups.

The timing of insulin bolus plays a crucial role in achieving stable glycemic values and long TIR. The recommended insulin timing is 15–20 min before meal consumption [28, 30]. Previous studies revealed that rapid-acting insulin

analogs before meals as opposed to after meals reduce postprandial glycemia by almost 30% [30]. We found also that participants in the study group were more disciplined with regard to maintaining a latency period between insulin delivery and meal consumption (72.0%) than participants in the control group.

We observed significantly higher insulin doses for both total daily dose and basal insulin dose among participants with poor metabolic control (TIR ≤70% group). Previous studies clearly indicate that uncontrolled glycemia (chronic hyperglycemia) is a risk factor of insulin resistance [29]. Interestingly, the study participants' insulin requirements are still being recommended by ISPAD at ranges of 0.7 to 1.0 IU/kg/day [29]. During puberty, the requirements may increase even up to 2 U/kg/day [29]. The study and control group patients required about 0.71 and 0.83 IU/kg/day, respectively.

Appropriate disease self-management is a crucial factor affecting good metabolic control in diabetes. Hence, it was not surprising that creating electronic reports for glycemic trends and insulin requirements using a dedicated platform in a domestic environment was related with longer TIR. Considering recent advancements in diabetes due to the use of technology such as smartphone applications and telemedicine, there is significant opportunity to achieve better patients' involvement in diabetes self-management and subsequently improve metabolic control and possibly ease the disease burden.

Conclusions

Maintaining an adequate interval between insulin delivery and meal consumption, usage of PLGS system with CSII, usage of "bolus calculator" function, and patients' involvement in the diabetes treatment (generating electronical reports in a domestic environment) may be the factors contributing to prolonged TIR.

Patients with shorter TIR have a higher insulin requirement.

Hyperglycemia and high glycemic variability are the main problems preventing patients from achieving the goal of treatment of TIR >70%.

Patients using CGM systems achieved an acceptable rate of hypoglycemia, regardless of the achieved TIR values.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-024-01310-y.

Author Contribution All authors contributed to the study conception and design. EK-K collected, analyzed and interpreted the data, and developed the first draft of the manuscript. AS conceptualized the study, coordinated the data collection process, and approved the final draft of the manuscript.

Data Availability Data available on request.

Declarations

Ethics approval The study was conducted in accordance with the ethical standards and principles of the Declaration of Helsinki, as revised in 2013. The Ethics Committee of the Medical University of Warsaw approved the study (KB/215/2020).

Consent to participate Written informed consent was obtained from the legal guardians and participants >16 years of age. Verbal informed consent was obtained from all the participants.

Competing interests The authors declare no competing interests.

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CORRECTION

Correction to: Factors affecting the prolongation of glycemic time in range among children with type 1 diabetes using continuous glucose monitoring systems: A case control study

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In the Results section of the Abstract, the statement "TIR >70%" should be "TIR ≤70%".

The Original article has been corrected.

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ORIGINAL ARTICLE

Iranian patients with diabetes and COVID-19-associated mucormycosis: Characteristics, manifestations, and mortality risk factors

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Abstract

Objective This study evaluated the mortality risk factors in Iranian patients with diabetes mellitus (DM) and COVID-19-associated mucormycosis (CAM).

Methods This retrospective study was conducted on confirmed CAM cases with DM. Only patients with a confirmed history of COVID-19 within the last 3 months were included. The patients were divided into the survived and deceased groups, and each group's characteristics were studied and compared. Patients were also studied according to their DM status (known or unknown case).

Results A total of 106 patients were included. The mortality rate was 25.5%. The most common underlying disease (hypertension, 41.5%) was significantly higher in the deceased group. Sixty-five patients (62.5%) were known cases of DM. The mean duration of DM was 12.46 years. There was a significant relationship between the DM history and mortality rate (84.6% vs. 15.4%, $p=0.007$). The history of ICU admission was 8 times higher in unknown DM patients ($p=0.011$, OR = 8.000, CI = 1.60–39.95). The mean HbA1C was significantly different in known DM cases (9.36 ± 2.03 vs. 8.02 ± 2.40, $p=0.004$). The mean first day FBS, mean first BS in emergency room, and mean FBS on the first hospitalization week were 171, 202, and 167.2 mg/dL, respectively. Although mortality was significantly related to hyperglycemic state of fasting and non-fasting BS levels ($p<0.05$), it was not related to HbA1C.

Conclusion Patients with diabetes and COVID-19 had uncontrolled fasting and non-fasting glucose levels during mucormycosis episode. Hypertension, history of DM, and the lack of glucose control during recent hospitalization can be associated with a poor outcome.

Keywords COVID-19 · Diabetes · Mucormycosis · Blood glucose levels

Introduction

COVID-19-associated mucormycosis (CAM) is a life-threatening complication observed in COVID-19 patients, with the rhino-orbital cerebral mucormycosis (ROCM) being the most common form of CAM [1, 2]. A mortality rate of 29.6% has been reported in CAM patients, and the majority of patients have been reported in middle-aged males with diabetes [1, 3]. The global prevalence of CAM has been estimated at 7 per 1000 patients, which is 50 times higher than the highest documented mucormycosis prevalence in the pre-COVID era in the Indian diabetic population (0.14 per 1000) [3]. This increase can be attributed to glucocorticoid overuse in COVID-19 patients [4]. Most CAM patients have been reported in India, where the high burden of diabetes mellitus (DM) is likely to be the primary reason [1].

Diabetes mellitus was clearly one of the most common predisposing factors in patients with mucormycosis before the COVID-19 area, associated with an increased mortality rate [5, 6]. Phagocytosis impairment and ketoacidosis are known to be the main mechanisms associated with the high prevalence of mucormycosis in patients with diabetes [7]. Diabetes mellitus remains the most common comorbidity in CAM patients [1, 8]. Other comorbidities in CAM patients

Extended author information available on the last page of the article

are hypertension, heart disease, chronic kidney disease, immunosuppressive states, and malignancies [2, 9].

While most studies show higher mortality in CAM patients with diabetes, a study reported no significant difference in the mortality rate between patients with and without diabetes [1, 2, 9, 10]. Several mechanisms, such as immunosuppression resulting from glucocorticoid administration or previous comorbidities, exacerbation of hyperglycemia, lymphopenia, iron disequilibrium, and endothelial injuries, have been proposed to link COVID-19 and subsequent or simultaneous mucormycosis infections [11, 12]. A recent study by Patel et al. (2022) suggested that new-onset diabetes was a more prominent risk factor for developing CAM than pre-existing diabetes [13]. Although the studies performed in Iran have identified DM as an important risk factor for CAM [9, 14, 15], the literature review shows that the association of DM-related factors with the mortality rate of CAM has not been fully studied. In the present study, we aimed to evaluate the effect of different characteristics, including DM-related factors, on the mortality rate of CAM patients with diabetes.

Materials and methods

Study design

This retrospective study was conducted on confirmed CAM cases with DM who were admitted to Imam Khomeini Hospital Complex, affiliated with Tehran University of Medical Sciences, as a referral center in Tehran, Iran, from April 2021 to January 2022. Only patients with diabetes and mucormycosis who had a confirmed history of the SARS-COV2 infection in the last 3 months were included in the study. SARS-COV 2 infections were confirmed through RT-PCR (Real Time Reverse Transcription Polymerase Chain Reaction) from nasopharyngeal swabs, and mucormycosis infections were confirmed based on the histopathological examination or isolation of zygomycetes on mycological cultures. Demographic, clinical, and laboratory results of survived and deceased patients and the related risk factors for death were studied. The patients included in the study were also divided into two groups: patients with known DM and patients without a known history of DM whose laboratory test results during hospitalization were compatible with the definition of diabetes according to the ADA (American Diabetes Association) criteria, which are a random blood glucose of ≥ 200 mg/dL, fasting glucose of ≥126 mg/dL, or hemoglobin A1c (HbA1c) of ≥ 6.5% [16]. The characteristics of both groups were also studied and compared. Patients with other risk factors, such as hematologic malignancy and organ transplantation, were excluded from the study. During the first week of admission, the levels of fasting blood sugar (FBS) and BS before dinner, before launch, and at bedtime for all recruited patients were measured using a glucometer and were recorded on a blood glucose chart. The patient's data regarding COVID-19, diabetes condition, and mucormycosis were obtained through phone interviews, in-person interviews, and patients' records.

Data analysis

In order to analyze the data, descriptive and inferential statistics were used according to the type of variables. First, the Shapiro-Wilk test was used to check the normality distribution of quantitative variables. To compare the mean variables between two groups, the unpaired independent *t*-test was used if the data distribution was normal, and the non-parametric Mann-Whitney test was used if the data distribution was not normal. Also, to compare the mean of the dependent variables between three or more groups, the one-way analysis of variance test was used if the data distribution was normal, and the Kruskal-Wallis test was used for the variables a non-normal distribution. The Chi-square and Fisher's exact tests were used to compare the qualitative variables. Bivariate-logistic regression was used to examine the association between the dependent and independent variables. Finally, multiple logistic regression was used to identify the significant predictors of the outcome variable.

Statistical indices were reported as mean ± standard deviation for normally distributed variables, median (Quartile1, Quartile3) for non-normally distributed variables, and frequency percentage for categorical variables. Data analysis was performed with SPSS software version 28, and a significant level of 5% was considered significant.

Results

Demographics and underlying diseases

Of 142 cases with CAM, 106 patients with DM (74.6%) were included in this study, of whom 27 patients died (mortality rate, 25.5%), and 79 patients survived. Fifty-four patients (50.9%) were male, and there was a significant relationship between sex and the rate of death, so that the rate of death among females was more than that among males ($p = 0.034$). The results showed that the chance of death in female patients was 2.64 times higher than that in male patients ($p = 0.037$, OR = 2.64, CI = 1.05–6.61).

The median age was 57.0 (48.0, 64.0) years, and there was a statistically significant relationship between age and the rate of death, so that the rate of death among patients > 50 years old was more than that among patients ≤ 50 years old ($p = 0.039$). The results showed that the mortality chance in patients > 50 years old was 2.99 times higher than that in patients ≤ 50 years old ($p = 0.044$, OR = 2.99, CI = 1.02–8.73).

The most common underlying disease was hypertension (41.5%), which was significantly higher in the deceased group (59.30% vs. 35.40%, $p=0.030$). The results showed that the mortality chance in patients with hypertension was 2.64 times higher than in patients without hypertension ($p=0.033$, OR= 2.64, CI = 1.08–6.48). Demographic characteristics of patients can be found and compared in Table 1.

COVID-19 features

The median duration of COVID-19 hospitalization was 8 days, and the median time interval between COVID-19 and mucormycosis was 20 days. Forty-nine patients (46.7%) needed oxygen therapy during COVID-19 treatment, while only 10 patients (9.5%) had a history of ICU admission. In terms of pulmonary

Table 1 Demographic and clinical characteristic of CAM patients with DM

Variables	Categories	Survived (*n*=79)	Deceased (*n*=27)	Total	*p*-value
Sex n (%)	Female	34 (43.0)	18 (66.7)	52 (49.1)	0.034*
	Male	45 (57.0)	9 (33.3)	54 (50.9)	
Age (year) n (%)	<=50	32 (40.5)	5 (18.5)	37 (34.9)	0.039*
	>50	47 (59.5)	22 (81.5)	69 (65.1)	
	Median (Q1, Q3)	60.0 (52.0, 65.0)	56.0 (47.0, 62.0)	57.0 (48.0, 64.0)	
Duration of COVID-19 hospitalization (days)	Median (Q1, Q3)	9.0 (5.0, 17)	5.0 (2.7, 10.0)	8 (5.0, 14.0)	0.177
Time interval between COVID-19 and mucormycosis (days)	Median (Q1, Q3)	20.0 (7.0, 30.0)	21.0 (7.0, 40.0)	20.0 (7.0, 30.0)	0.440
DM history n (%)	Yes	43 (55.1)	22 (84.6)	65 (62.5)	0.007*
	No	35 (44.9)	4 (15.4)	39 (37.5)	
Duration of DM (years) n (%)	Mean ± SD	12.65 ± 6.86	11.93 ± 9.91	12.46 ± 7.56	0.833
Family history of DM n (%)	Yes	26 (33.3)	12 (44.4)	38 (36.2)	0.300
	No	52 (66.7)	15 (55.6)	67 (63.8)	
COVID-19 ICU admission n (%)	Yes	8 (10.1)	2 (7.7)	10 (9.5)	1.000
	No	71 (89.9)	24 (92.3)	95 (90.5)	
Adherence to DM regimen in COVID-19 time n (%)	Yes	26 (35.1)	7 (28.0)	33 (33.3)	0.513
	No	48 (64.9)	18 (72.0)	66 (66.7)	
Oxygen support in COVID-19 time n (%)	Yes	36 (46.2)	13 (48.1)	49 (46.7)	0.858
	No	42 (53.8)	14 (51.9)	56 (53.3)	
COVID-19 Pulmonary involvement n (%)	No	7 (9.7)	5 (20.8)	12 (12.5)	0.468
	Mild	21 (29.2)	7 (29.2)	28 (29.2)	
	Moderate	33 (45.8)	8 (33.3)	41 (42.7)	
	Severe	11 (15.3)	4 (16.7)	15 (15.6)	
Comorbidities n (%)					
HTN	Yes	28 (35.4)	16 (59.3)	44 (41.5)	0.030*
	No	51 (64.6)	11 (40.7)	62 (58.5)	
IHD	Yes	16 (20.3)	10 (37.0)	26 (24.5)	0.080
	No	63 (79.7)	17 (63.0)	80 (75.5)	
RF	Yes	5 (6.3)	0 (0.0)	5 (4.7)	0.326
	No	74 (93.7)	27 (100.0)	101 (95.3)	
Mucormycosis symptoms n (%)					
Ptosis	Yes	24 (30.4)	8 (29.6)	32 (30.2)	0.942
	No	55 (69.6)	19 (70.4)	74 (69.8)	
Headache	Yes	45 (57.0)	11 (40.7)	56 (52.8)	0.145
	No	34 (43.0)	16 (59.3)	50 (47.2)	
Facial numbness	Yes	40 (50.6)	12 (44.4)	52 (49.1)	0.579
	No	39 (49.4)	15 (55.6)	54 (50.9)	
Orbital pain	Yes	43 (54.4)	8 (29.6)	51 (48.1)	0.026*
	No	36 (45.6)	19 (70.4)	55 (51.9)	

HTN, hypertension; *IHD*, ischemic heart disease; *RF*, renal failure

* Statistically significant

involvement in COVID-19, 42.7% of cases were moderate and only 15.6% patients were severe. Only 33 patients (50.7%) were able to adhere to their diabetic diet when they were involved with COVID-19. Out of 65 known cases of diabetes, 24 patients (36.9%) did not adhere to the medical treatment of their metabolic disease at the time of COVID-19. No significant difference was seen between the two groups (survived and deceased) in COVID-19 features.

Mucormycosis manifestations

The most common site (72.5%) of mucormycosis involvement was the paranasal sinuses, while the least common site (4.7%) was the cerebral involvement, and all patients with cerebral involvement died. The most frequent symptoms were ptosis, headache, facial numbness, and orbital pain, among which orbital pain was significantly higher in the survived group (54.40% vs. 29.60%, $p=0.026$). Ninety-six patients (90.6%) received Amphotericin B and 44 patients (41.5%) received Posaconazole. Nineteen patients (17.9%) were admitted to ICUs for mucormycosis management, with the mean length of stay of 11.4 (±11.8) days.

Diabetes characteristics

Sixty-five patients (62.5%) had a history of DM (known case of DM). The mean duration of DM for patients with a history of DM was 12.5 years. The results indicated a significant relationship between DM history and the rate of death. Among patients who died, most patients had a DM history (84.6% vs. 15.4%, $p=0.007$). The univariate-logistic regression analysis also showed that CAM patients with a DM history had more mortality rate than those with newly diagnosed DM ($p=0.011$, OR = 4.47, CI = 1.41–14.21). Thirty-eight patients (36.8%) had a family history of DM. Sixty-two patients (58.5%) had a history of taking diabetes treatment (43% on insulin therapy).

The mean FBS of the patients on the first day of admission was 171 ± 86.7 mg/dL, and the mean non-fasting blood glucose of the patients on the first day of admission in the emergency room was 202.2 ± 93.3 mg/dL. The results also showed that there was no significant difference between the survived and deceased patients in the HbA1C level and blood gas parameters. The mean levels of the first week's FBS, BS before dinner, and BS before sleeping were significantly different in the two groups ($p<0.05$), being significantly higher in the deceased group (Table 2).

Patients with and without the DM history were further studied, and the results showed that age, family history of DM, and pulmonary involvement in COVID-19 period were significantly higher in CAM patients with a DM history, while ICU admission was significantly higher in patients without the DM history ($p=0.011$, OR = 8.000, CI=1.60–39.95). The mean HbA1C level was also significantly higher in patients with the DM history than in the group without the history (9.36 ± 2.03 vs. 8.02 ± 2.40, $p=0.004$) (Table 3).

The results also showed that the chance of death in patients with a history of DM was 3.67 times more than that in patients without a history of DM when the effect of other variables was constant (Table 4).

Discussion

We studied different characteristics of CAM patients with DM in an Iranian referral hospital in Tehran, with a focus on mortality risk factors in these patients. Our findings indicated that DM is a serious risk factor in developing mucormycosis in COVID-19 patients, which is consistent with previous studies [17]. A significant difference is observed in the risk factors for mucormycosis between the western and the eastern world before the COVID-19 pandemic [8]. While DM was the most common risk factor for mucormycosis only in Asian countries before the COVID-19 pandemic [18, 19], it is now recognized as the most common risk factor for CAM worldwide [9, 20].

The mortality rate in CAM patients with DM in our study was 25.5%, unexpectedly lower than the mortality rate in CAM patients, which has been reported to be between 29 and 50% [1, 3, 21]. The difference can be related to different risk factors for death in different studies. In a report on 73 patients with CAM

Table 2 The blood gases and diabetes-related results of CAM patients with DM (Mean ± SD)

Variable	Survived	Deceased	Total	*p*-value
HbA1C	8.88 ± 2.35	8.97 ± 2.12	8.90 ± 2.28	0.867
pH	7.37 ± 0.06	7.39 ± 0.06	7.38 ± 0.07	0.072
HCO3	21.69 ± 4.20	21.15 ± 4.74	21.56 ± 4.32	0.593
PCO2	35.32 ± 8.36	36.03 ± 7.52	35.49 ± 8.13	0.715
FBS*	161.11 ± 42.52	198.15 ± 44.84	167.28 ± 44.89	0.004*
BS before lunch*	193.57 ± 59.33	211.23 ± 59.36	196.68 ± 59.37	0.299
BS before dinner*	194.16 ± 51.40	233.25 ± 66.08	200.60 ± 55.59	0.015*
BS before sleeping*	194.70 ± 46.87	248.04 ± 63.76	203.15 ± 53.20	0.001*

* On the first week of admission

Table 3 The comparison of characteristics between CAM patients with and without a DM history

Variables	Categories	DM history			
		NO (*n*=41)	Yes (*n*=65)	Total	*p*-value
Sex n (%)	Female	15 (38.5)	36 (55.4)	51 (49.0)	0.095
	Male	24 (61.5)	29 (44.6)	53 (51.0)	
Age (year) n (%)	<=50	25 (64.1)	11 (16.9)	36 (34.6)	<0.001*
	>50	14 (35.9)	54 (83.1)	68 (65.4)	
	Median (Q1, Q3)	49.0 (46.0, 59.0)	59.0 (52.0, 64.5)	57.0 (48.0, 64.0)	
Duration of COVID-19 hospitalization (days)	Median (Q1, Q3)	10.0 (5.5, 20.0)	6.0 (2.0, 12.0)	8.0 (5.0, 14.0)	0.055
Time interval between COVID-19 and Mucor mycosis (days)	Median (Q1, Q3)	20.0 (7.0, 30.0)	20.0 (7.0, 30.0)	20.0 (7.0, 30.0)	0.696
Family history of DM n (%)	Yes	5 (12.8)	32 (49.2)	37 (35.6)	<0.001*
	No	34 (87.2)	33 (50.8)	67 (64.4)	
COVID-19 ICU admission n (%)	Yes	8 (20.5)	2 (3.1)	10 (9.7)	0.006*
	No	31 (79.5)	62 (96.9)	93 (90.3)	
Oxygen support in COVID-19 time n (%)	Yes	16 (41.0)	32 (50.0)	48 (46.6)	0.376
	No	23 (59.0)	32 (50.0)	55 (53.4)	
COVID-19 pulmonary involvement n (%)	No	4 (11.8)	8 (13.3)	12 (12.8)	0.035*
	Mild	5 (14.7)	22 (36.7)	27 (28.7)	
	Moderate	16 (47.1)	25 (41.7)	41 (43.6)	
	Severe	9 (26.5)	5 (8.3)	14 (14.9)	
History of corticosteroid n (%)	Yes	30 (76.9)	43 (66.2)	73 (70.2)	0.245
	No	9 (23.1)	22 (33.8)	31 (29.8)	
HbA1c	Mean ± SD	8.02 ± 2.40	9.36 ± 2.03	8.89 ±2.28	0.004*

Table 4 The multiple logistic Regression analysis

	B	SE	*p*-value	OR	95% CI	
					Lower	Upper
Sex (female)	0.639	0.504	0.205	1.89	0.706	5.089
HTN (yes)	0.433	0.503	0.389	1.54	0.575	4.128
History of diabetes (yes)	1.302	.607	.032	3.68	1.118	12.092

from India, the most important risk factors for death were severe COVID-19, orbital involvement, and uncontrolled DM [22]. Our findings showed that aging, female sex, a longer duration of DM, uncontrolled diabetes, and the co-existence of hypertension in CAM patients with DM were associated with a higher mortality rate.

Diabetes is a serious underlying disease associated with severe illness, respiratory failure, and increased mortality in COVID-19 patients [23, 24]. More than 46% of patients in our study needed oxygen support when infected with COVID-19, while less than 10% required ICU admission. In other studies, a higher number of patients with severe COVID-19 and the need for O_2 support with mechanical ventilation have been reported [25, 26]. It seems that the risk factors of mucormycosis in COVID-19 patients can depend on the severity of COVID-19 and the type of underlying diseases.

According to the reports, the most common type of mucormycosis involvement is rhino-orbital [27], while rhinosinusitis was the most common type in our patients. Moreover, the pulmonary mucormycosis was reported in none of our patients, whereas a study conducted in Germany reported that 7 patients had pulmonary involvement. The difference in site involvement might be attributed to the presence of diabetes as a predisposing factor in our patients, whereas the majority of cases among German patients were associated with malignancies [25].

In our study, 37.5% of patients with no DM history developed diabetes after an average of 20 days following the COVID-19 infection and during the occurrence of mucormycosis. The finding that the rate of ICU admission (during the COVID-19 episode) was eight times higher in patients with no history of diabetes supports the hypothesis that COVID-19 can induce diabetes by provoking inflammatory processes and insulin resistance (stress hyperglycemia) [28].

COVID-19 has been reported to be associated with poor blood glucose control in people with diabetes and can be

manifested in both hyperglycemia and hypoglycemia [4, 29]. The dietary and medication non-adherence seen in a significant number of CAM patients with diabetes in our study can be among the reasons for poor control of diabetes in COVID-19 era. The recommended level of FBS in COVID-19 patients with DM is 110–140 mg/dL [30]. The CAM patients in our study did not have acceptable mean FBS levels on the first day of hospitalization or during the first week. It is now clear that hyperglycemia at the time of admission increases the risk of severe COVID-19, regardless of previous proper control of diabetes [31]. For DM patients with COVID-19, especially in severe cases, a non-fasting blood glucose level of 140–180 mg/dl is recommended, and insulin is the preferred treatment [30]. However, only 43% of CAM patients with DM in our study received insulin for hyperglycemia, and the mean level of non-fasting blood glucose was not adequately controlled.

Some experts have emphasized the importance of checking HbA1c level during hospitalization for COVID-19 patients [32]. Some studies have suggested a potential association between high HbA1c levels and severe COVID-19 and mortality. However, this association has not been confirmed yet [33, 34]. In our study, HbA1c was not found to be a risk factor for poor outcomes.

This study had some limitations. First, it was a retrospective study subject to the inherent limitations and shortcomings of this type of study. Second, we did not have a control group (without mucormycosis) with a history of known diabetes and COVID-19 infection to compare the glycemic control indices. Future studies with a prospective design and a defined control group are required to confirm our findings.

Conclusion

Aging, female sex, the coexistence of hypertension, and DM history were identified as risk factors for a higher mortality rate in CAM patients with diabetes. Most patients did not have adequate glucose control during the last few months before or at the time of hospitalization. Although the mortality rate was related to fasting and non-fasting hyperglycemia, it was not associated with HbA1C levels. The adequate control of blood glucose during hospitalization can reduce the mortality rate in CAM patients with DM.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval This study was approved by the Institutional Ethics Committee (ethics number: IR.TUMS.IKHC.REC.1400.355). Although this study was conducted retrospectively, patients were informed about the possible use of their demographic and clinical information for research purposes. Only data from patients who signed the consent form were included in this study.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Clinical risk factors analysis and prevention of osteoporosis as a complication of diabetes

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Abstract

Objective Osteoporosis is a common complication of type 2 diabetes. This study aimed to provide a basis for the clinical prevention and treatment of osteoporosis as a complication of type 2 diabetes.

Methods We retrospectively analyzed patients hospitalized with type 2 diabetes with or without osteoporosis. Binary logistic regression was performed to assess the relationship between the assessed indexes and the risk of type 2 diabetes with osteoporosis. Receiver operating characteristic curves were created to evaluate the efficacy of these indexes in predicting osteoporosis in patients with type 2 diabetes.

Results The cohort comprised 1,811 patients with type 2 diabetes and 1,758 with type 2 diabetes combined with osteoporosis. The basic indexes (height and weight) and glucose metabolism indexes (glycated hemoglobin A (HbA1c) and fasting plasma glucose/fasting blood glucose) were positively correlated with the bone metabolism indexes. Receiver operating characteristic curve analysis showed that HbA1c was an effective predictor of osteoporosis risk, with an area under the curve of 70.1%. When the HbA1c of patients with type 2 diabetes was between 6% and 6.45% or reached 6.45% at a long-term stable state, the risk of osteoporosis was increased. The risk of osteoporosis was also increased in patients with type 2 diabetes who were older than 59.5 years.

Conclusion HbA1c and fasting plasma glucose/fasting blood glucose were significantly correlated with bone metabolism in patients with type 2 diabetes with and without osteoporosis. Clinical monitoring of the HbA1c may be useful in preventing osteoporosis in patients with type 2 diabetes.

Keywords Type 2 diabetes · Osteoporosis · Glycated Hemoglobin A · Fasting plasma glucose · Fasting blood glucose

Background

Type 2 diabetes mellitus (T2DM), previously referred to as non-insulin-dependent diabetes, is a chronic inflammatory disease with the main characteristics of disorders of glucose metabolism and insulin resistance [1, 2]. T2DM is a global public health problem that brings a high socioeconomic burden [3]. It is estimated that 451 million adults worldwide had diabetes in 2017, and the prevalence is increasing annually [4, 5]. Although the bone mineral density (BMD) is increased in patients with T2DM with a high BMI, the microfractures caused by bone fragility and microvascular complications also increase the risk of fracture in most patients with T2DM compared with patients with type 1 diabetes mellitus and healthy individuals [6–8]; this leads to an increased prevalence of osteoporosis (OP) and related fractures in patients with T2DM [9]. T2DM has been proven

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to be one of the risk factors for OP-related fractures, which indicates a significant association between diabetes and OP [10–12]. Therefore, it is very important to determine the clinical risk factors for OP in patients with T2DM to prevent the complications of diabetes combined with OP.

OP is a common chronic bone disease that is mainly characterized by reduced bone density, increased porosity, and increased susceptibility to fracture [13, 14]. Clinicians need to monitor for early diagnostic indicators of OP in older adults who have additional comorbidities such as T2DM [15]. In general, because the disease process of OP has no obvious symptoms, it is only diagnosed after the first fracture [16]. Preventing fractures is the main purpose of treating OP and early assessment of OP is performed to reduce the risk of fractures. Studies have shown that the risk factors for fractures in patients with T2DM are different from those in individuals without diabetes. Compared with individuals without diabetes, patients with T2DM have poorer bone quality, which increases the risk of fracture [11, 17]. Therefore, there is an urgent need to evaluate the risk factors for OP in patients with T2DM.

T2DM and OP are becoming increasingly common concomitant diseases among the older adult population and have become a substantial socio-economic burden [12]. However, most previous studies evaluating the relationship between diabetes and fractures have compared the incidence of fractures in patients with diabetes versus people without diabetes [18–20]. To fill the knowledge gap, we aimed to identify the risk factors for T2DM combined with OP through Spearman correlation analysis and logistic regression analysis of the clinical indicators related to bone metabolism in patients with T2DM and patients with both T2DM and OP. We hope to provide evidence for the clinical treatment and prevention of OP as a complication of T2DM.

Materials and Methods

Study population

We conducted a retrospective study of patients with T2DM and patients with T2DM combined with OP who were treated in the Second Xiangya Hospital of Central South University from January 2016 to September 2019. We collected data on 3,176 patients with T2DM and 2,607 patients with T2DM combined with OP. After assessing patients in accordance with the inclusion and exclusion criteria, the final study cohort comprised 1,811 patients with T2DM and 1,758 patients with T2DM combined with OP.

Inclusion criteria: patients with T2DM and patients with OP admitted to the Department of Geriatrics, Department of Orthopedics and Department of Endocrinology, Second Xiangya Hospital of Central South University from January 2016 to September 2019. T2DM was diagnosed using the diagnostic criteria for T2DM published by the World Health Organization in 1999. Based on the diagnostic criteria for OP published by the World Health Organization in 1994, OP was diagnosed when the BMD was 2.5 standard deviations (SD) lower than the peak value of healthy adults of the same sex and race (T value < -2.5 SD); that is, a T value of -2.5 SD to -1.0 SD was taken to indicate bone loss, while a T value of -1.0 SD indicated normal bone mass. A Hologic dual-energy x-ray absorptiometry device was used to measure the BMD of the vertebrae and femur.

Exclusion criteria: data from multiple admissions of the same patient; severe data loss; type 1 diabetes mellitus, acute complications of diabetes, liver and kidney dysfunction, malignant tumor diseases, or diseases affecting calcium and phosphorus metabolism; use of drugs affecting bone metabolism (such as glucocorticoids); treatment with medications for OP.

Study design

A retrospective case analysis was performed to analyze the effects of basic demographic characteristics and indicators of bone metabolism, lipid metabolism, and glucose metabolism on the vertebral and femoral T values. Referring to previous studies, the following data were extracted from the hospital records: basic indexes (age, sex, height, weight, systolic pressure, diastolic pressure, smoking status, and drinking status), bone metabolism indexes (serum 25–hydroxyvitamin D, parathyroid hormone at 0 min, parathyroid hormone at 20 min, C-terminal cross-linking telopeptide of type I collagen, procollagen type 1 N-terminal propeptide, bone Gla protein, vertebral BMD, femoral BMD, vertebral T value, femoral T value, alkaline phosphatase (ALP), and albumin), lipid metabolism indicators (serum total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (HDL-C), and glucose metabolism indicators (glycated hemoglobin A (HbA1c), fasting plasma glucose/fasting blood glucose (FPG/FBG)), C-peptide release (CP) at 0 min, CP at 60 min, and CP at 120 min, urine protein, and estimated glomerular filtration rate (eGFR)).

Statistical analysis

The distribution of variables in the T2DM group and the T2DM combined with OP group were compared. The t-test and Mann–Whitney U test were used for comparisons between the two groups. Categorical variables were tested by the chi-squared test or Fisher's exact test. Pearson correlation analysis was used to analyze the correlations among continuous variables. Binary logistic regression was then used to evaluate the relationship between indicators and the risk of T2DM with OP. $p < 0.05$ was considered statistically

significant. The statistical analysis was performed with IBM SPSS Statistics 26 and Python 3.9.

Results

The total cohort comprised 3,569 patients with valid data, namely 1,811 patients with T2DM and 1,758 patients with T2DM combined with OP (Fig. 1). Table 1 summarizes the clinical characteristics of the patients, including baseline characteristics, bone metabolism indexes, and lipid and glucose metabolism indicators. There were significant differences in most variables between the T2DM group and the T2DM combined with OP group ($p < 0.05$). Compared with the T2DM combined with OP group, the T2DM group had a younger average age, greater average height and weight, and lower proportion of females (Table 1). The glucose metabolism indexes also significantly differed between the T2DM group and the T2DM combined with OP group ($p < 0.001$). The average HbA1c and FPG/FBG were higher in the T2DM group than the T2DM combined with OP group.

Table 2 shows the results of Spearman correlation analyses performed to further analyze the relationships between variables and bone-related indicators. The basic indexes (height and weight) and glucose metabolism indexes (HbA1c and FPG/FBG) were positively correlated with the bone metabolism indexes, namely the vertebral BMD, femoral BMD, vertebral T value, and femoral T value. The index C-terminal cross-linking telopeptide of type I collagen, procollagen type 1 N-terminal propeptide, and bone Gla protein that are used clinically to determine bone metabolism-related conditions were negatively correlated with the bone metabolism indexes (vertebral BMD, femoral BMD, vertebral T value, and femoral T value). Our results were consistent with the clinical results, so we did not analyze these indicators in depth during follow-up. The patient age, liver function index ALP, and lipid metabolism indicator HDL-C were also negatively correlated with the bone metabolism indexes. Other basic characteristics such as systolic blood pressure, diastolic blood pressure, and metabolic indicators (serum 25-hydroxyvitamin D, serum total cholesterol, and CP) were not correlated with the bone metabolism indicators (Fig. 2A). These results were similar in both the T2DM group and the T2DM combined with OP group (Fig. 2B-C). To further verify the correlations between variables, we removed indicators with several missing data and conducted a network correlation analysis. The result was consistent with that of the heat map (Supplemental Fig. 1).

Combining the results of statistical analysis, correlation analysis, and clinical significance, we used binary logistic regression to analyze the risk factors for T2DM combined with OP. The Box-Tidwell method was used to test whether the continuous independent variable and the logit (P) conversion value of the dependent variable were linear. The linearity test results showed that there was a linear relationship between all continuous independent variables and the logit conversion value of the dependent variable. The preliminary analysis showed that sex, weight, and HbA1c may be risk factors for T2DM combined with OP ($p < 0.05$) (Fig. 3). Binary logistic regression analysis using the stepwise

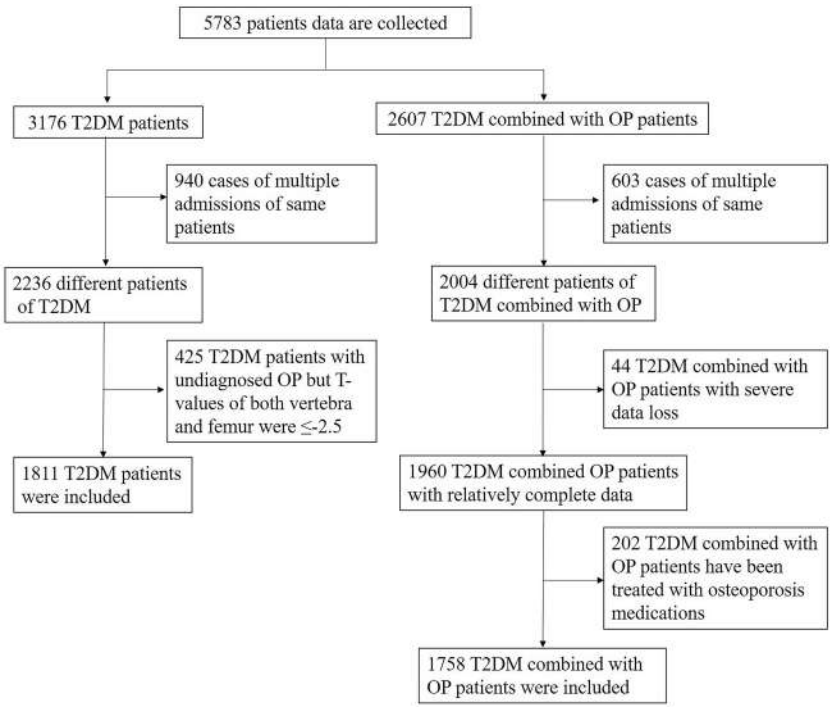

Fig. 1 Flowchart of patient inclusion and exclusion

Table 1 Clinical characteristics of patients with type 2 diabetes (T2DM) and those with T2DM combined with osteoporosis (OP)

Disease	T2DM	T2DM + OP	*p*
N	1811	1758	
Age (years)	(1811) 57.0 (50.0–65.0)	(1758) 65.0 (56.0–71.0)	< 0.001
Gender			< 0.001
Male	1161 (64.1%)	539 (30.7%)	
Female	650 (35.9%)	1219 (69.3%)	
Height (cm)	(1456) 162.0 (156.0–168.0)	(1518) 155.0 (150.0–161.5)	< 0.001
Weight (kg)	(1470) 60.0 (53.0–66.7)	(1647) 55.0 (48.6–62.0)	< 0.001
Systolic pressure (mmHg)	(1801) 133.0 (120.0–150.0)	(1652) 135.0 (121.0–150.0)	0.209
Diastolic pressure (mmHg)	(1796) 80.0 (72.0–87.0)	(1648) 78.0 (70.0–86.0)	< 0.001
Smoker			< 0.001
No	1047 (64%)	1259 (81.4%)	
Yes	590 (36%)	288 (18.6%)	
Drinker			< 0.001
No	1198 (74.5%)	1345 (87.2%)	
Yes	410 (25.5%)	197 (12.8%)	
Course of diabetes (years)	(1792) 9.0 (3.0–9.0)	(1245) 10.0 (5.0–15.0)	< 0.001
Serum 25 hydroxyvitamin D (ng/mL)	(1335) 39.0 (27.0–53.0)	(1549) 40.0 (28.0–54.0)	0.078
PTH0 (ng/L)	(358) 4.5 (3.0–7.7)	(1068) 3.9 (2.8–5.7)	< 0.001
PTH20 (ng/L)	(129) 4.2 (2.8–7)	(598) 4.0 (2.8–5.8)	0.208
β-CTX (ng/L)	(125) 465.0 (314.5–821.5)	(1101) 557.0 (347.0–839.0)	0.130
P1NP (μg/L)	(118) 43.4 (30.1–69.1)	(1028) 49.5 (35.5–75.5)	0.027
BGP (ng/L)	(101) 14.0 (11.0–21.0)	(1009) 17.0 (12.0–24.5)	0.005
Vertebra BMD (g/cm2)	(512) 1.0 (0.9–1.1)	(1740) 0.7 (0.7–0.8)	< 0.001
Femur BMD (g/cm2)	(512) 0.8 (0.6–0.9)	(1741) 0.7 (0.6–0.8)	< 0.001
Vertebra T value	(512) -0.3 (-1.0- -0.6)	(1756) -2.2 (-2.9- -1.5)	< 0.001
Femur T value	(512) -0.8 (-1.8–0.1)	(1752) -1.8 (-2.6- -1.3)	< 0.001
ALP (U/L)	(180) 66.4 (53.3–81.1)	(437) 73.5 (58.0–99.0)	< 0.001
ALB (g/L)	(1622) 36.1 (32–39)	(1645) 36.0 (33.0–39.6)	0.754
TC (mmol/L)	(1559) 4.2 (3.5–5.0)	(1583) 4.3 (3.6–5.1)	0.003
LDLC (mmol/L)	(1529) 2.6 (2.0–3.3)	(1564) 2.7 (2.1–3.3)	0.257
HDLC (mmol/L)	(1545) 1.0 (0.9–1.3)	(1562) 1.1 (0.9–1.3)	< 0.001
HbA1c (mmol/L)	(1539) 8.4 (6.9–10.5)	(1758) 7.3 (6.0–9.1)	< 0.001
FPG/FBG (mmol/L)	(810) 6.8 (5.3–9.0)	(1500) 6.3 (5–7.9)	< 0.001
CP 0 min (ng/L)	(1419) 309.2 (191.5–495.4)	(1311) 364.9 (223.2–576.3)	< 0.001
CP 60 min (ng/L)	(512) 552.5 (291.9–849.5)	(404) 604.5 (278.5–1078.3)	0.132
CP 120 min (ng/L)	(1387) 683.9 (351.8–1124.2)	(1274) 859.1 (408.2–1477.2)	< 0.001
Urine protein (mg/24 h)	(1078) 12.0 (2.0–139.3)	(516) 7.1 (1.7–29.7)	< 0.001
eGFR (mL/min)	(457) 97.1 (62.4–117.0)	(221) 90.5 (65.3–111.8)	0.024

Continuous variables are expressed as mean ± standard deviation (normal distribution) or median and quartile (non-normal distribution), while categorical variables are expressed as frequency and percentage

Abbreviations: *PTH* parathyroid hormone, *β-CTX* C-terminal cross-linking telopeptide of type I collagen, *P1NP* procollagen type 1 N-terminal propeptide, *BGP* bone gla protein, *BMD* bone mineral density, *ALP* alkaline phosphatase, *ALB* albumin, *TC* serum total cholesterol, *LDLC* low-density lipoprotein cholesterol, *HDLC* high-density lipoprotein cholesterol, *HbA1c* glycated hemoglobin, *FPG/FBG* fasting plasma glucose/fasting blood glucose, *CP* C-peptide release, *eGFR* estimated glomerular filtration rate

$p < 0.05$ is considered statistically significant

regression method (forward method) further showed that sex, weight, and HbA1c were independent risk factors for T2DM combined with OP (Table 3). Compared with that of T2DM, the risk of T2DM with OP was 82.8% lower in men than in women. With increases in the weight and HbA1c, the risk of T2DM combined with OP showed a downward trend.

We then divided the patients into three age groups (younger than 40 years, 40–65 years, and older than

Table 2 Spearman correlation analysis between variables and T values

	BMD		T value	
	vertebra	Femur	Vertebra	Femur
Age (years)	-0.135**	-0.234**	-0.127**	-0.226**
Height (cm)	0.460**	0.405**	0.413**	0.274**
Weight (kg)	0.404**	0.420**	0.381**	0.368**
Systolic pressure (mmHg)	0.010	-0.039	0.016	-0.023
Diastolic pressure (mmHg)	0.063**	0.101**	0.056**	0.100**
Course of diabetes (years)	0.02	-0.069**	0.037	-0.063**
	0	0	0	0
	0	0	0	0
Serum 25 hydroxyvitamin D (ng/mL)	0.017	0.088**	0.011	0.082**
PTH0 (ng/L)	0.047	-0.047	0.042	-0.043
PTH20 (ng/L)	0.047	-0.018	0.033	-0.023
β-CTX (ng/L)	-0.184**	-0.114**	-0.178**	-0.116**
P1NP (μg/L)	-0.171**	-0.143**	-0.162**	-0.146**
BGP (ng/L)	-0.172**	-0.150**	-0.169**	-0.135**
ALP (U/L)	-0.195**	-0.194**	-0.204**	-0.213**
ALB (g/L)	0.019	0.077**	0.041	0.113**
TC (mmol/L)	-0.034	-0.019	-0.028	0.008
LDLC (mmol/L)	0.006	0.010	0.010	0.031
HDLC (mmol/L)	-0.109**	-0.111**	-0.094**	-0.084**
HbA1c (mmol/L)	0.215**	0.125**	0.233**	0.163**
FPG/FBG (mmol/L)	0.201**	0.164**	0.204**	0.162**
CP 0 min (ng/L)	-0.053*	-0.048*	-0.056*	-0.044
CP 60 min (ng/L)	-0.057	-0.073	-0.057	-0.047
CP 120 min (ng/L)	-0.065**	-0.021	-0.068**	-0.014
Urine protein (mg/24 h)	0.094**	0.263**	0.074*	0.134**
eGFR (mL/min)	0.039	0.113*	0.059	0.145**

Abbreviations: *PTH*parathyroid hormone, *β-CTX* C-terminal cross-linking telopeptide of type I collagen, *P1NP* procollagen type 1 N-terminal propeptide, *BGP* bone gla protein, *BMD* bone mineral density, *ALP* alkaline phosphatase, *ALB* albumin, *TC* serum total cholesterol, *LDLC* low-density lipoprotein cholesterol, *HDLC* high-density lipoprotein cholesterol, *HbA1c* glycated hemoglobin, *FPG/FBG* fasting plasma glucose/fasting blood glucose, *CP* C-peptide release, *eGFR* estimated glomerular filtration rate

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

65 years), and revalidated our results at different age stages. We conducted correlation analyses on various indicators in the three age groups as shown in Supplemental Fig. 2. Compared with those in the general population, the factors closely related to bone metabolism were increased in patients younger than 40 years. The duration of diabetes and urine protein were negatively correlated with the bone metabolism indexes. The CP at 60 min and eGFR were positively correlated with the bone metabolism indexes. Compared with those in the total population, the urinary protein and eGFR were positively correlated with the bone metabolism indexes in patients aged 40 to 65 years. The factors related to bone metabolism in patients older than 65 years were basically the same as those in the total population. The stratified age group results verified the findings of our previous analysis. Additionally, patients younger than 40 years had more indicators related to bone metabolism.

We investigated whether glucose metabolism was able to assess the clinical transformation of patients with normal bone mass (both the vertebral T value and femoral T value were $\geq$ -1) into patients with OP (both the vertebral T value and femoral T value were $\leq$ -2.5) by performing receiver operating characteristic (ROC) curve analysis. The ROC curve analysis showed that compared with the patients with normal bone mass, HbA1c was an effective predictor of the risk of OP with an area under the curve of 70.1% (95% CI 65.8%–74.4%) (Fig. 4A). When the HbA1c value was between 6% and 6.45% or reached 6.45% at a long-term stable state, clinicians should start to monitor patients with T2DM for the clinical occurrence of OP. In

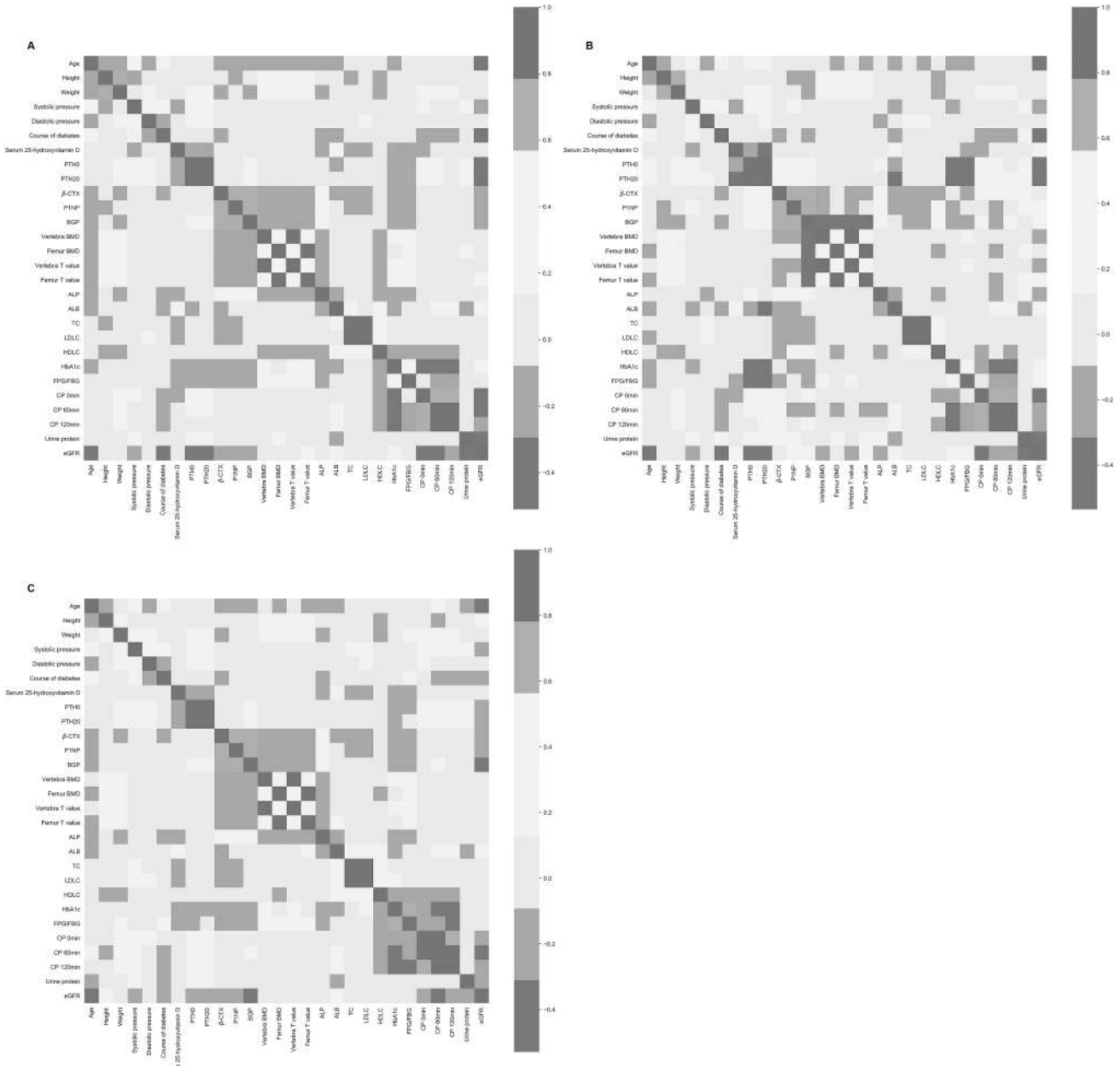

Fig. 2 Heatmap of index correlation analysis. Correlations between various indicators in all patients (**A**), patients with type 2 diabetes (**B**), and patients with type 2 diabetes combined with osteoporosis (OP). Deeper green indicates more positive correlations, while deeper red indicates more negative correlations. Abbreviations: PTH, parathyroid hormone; β-CTX, C-terminal cross-linking telopeptide of type I collagen; P1NP, procollagen type 1 N-terminal propeptide; BGP, bone gla protein; BMD, bone mineral density; ALP, alkaline phosphatase; ALB, albumin; TC, serum total cholesterol; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; FPG/FBG, fasting plasma glucose/fasting blood glucose; CP, C-peptide release; eGFR, estimated glomerular filtration rate

order to exclude the influence of gender on the prediction results, we analysed female patients in the OP group and the T2DM combined OP group separately, and the ROC curve analysis showed that HbA1c was still a valid predictor of the risk of OP in females with an area under the curve of 72.3% (95% CI 66.4%–78.2%) (Supplemental Fig. 3A), compared with female patients with normal bone mass. Thus, we suggest that in future clinical treatment, HbA1c may be used to predict the transformation from normal bone mass to OP. In addition, the risk of OP in patients with T2DM was increased when they were older than 59.5 years, with an area under the ROC curve of

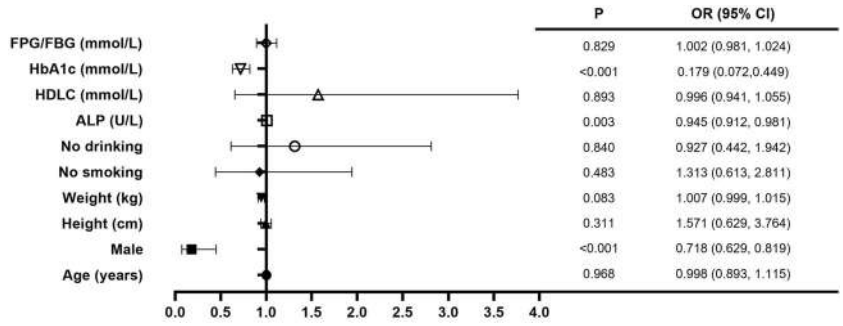

Fig. 3 Binary logistic regression analysis to evaluate the relationship between various indicators and the risk of type 2 diabetes combined with osteoporosis (OP). $p < 0.05$ is considered statistically significant. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; ALP, alkaline phosphatase; HDLC, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; FPG/FBG, fasting plasma glucose/fasting blood glucose

Table 3 Binary logistic regression analysis of the factors influencing type 2 diabetes combined with osteoporosis

		B	S.E	Wald	P value	OR	95% CI
Step 1a	Gender	-2.186	0.268	66.376	< 0.001	0.112	(0.066, 0.190)
	Constant	2.225	0.215	107.187	< 0.001	9.25	
Step 2b	Gender	-2.209	0.286	59.444	< 0.001	0.11	(0.063, 0.193)
	HbA1c	-0.309	0.055	31.172	< 0.001	0.734	(0.659, 0.818)
	Constant	4.727	0.535	77.94	< 0.001	112.948	
Step 3c	Gender	-1.759	0.305	33.335	< 0.001	0.172	(0.095, 0.313)
	Weight	-0.063	0.016	16.157	< 0.001	0.939	(0.911, 0.968)
	HbA1c	-0.343	0.058	34.379	< 0.001	0.71	(0.633, 0.796)
	Constant	8.383	1.126	55.382	< 0.001	4370.57	

The gender in this table represents females. B, regression coefficient value; S.E., standard error; Wald, Wald chi-squared value; OR, odds ratio; 95% CI, 95% confidence interval. $p < 0.05$ is considered statistically significant

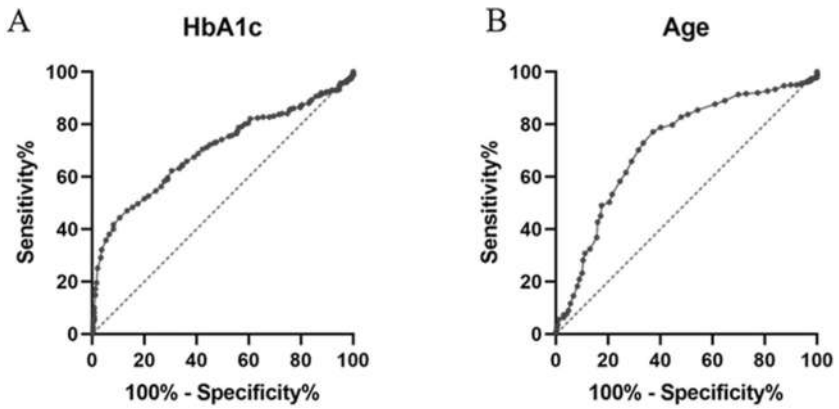

Fig. 4 Receiver operating characteristic (ROC) curves to analyze the indicators used for the clinical prediction of osteoporosis. **A** HbA1c was a predictor of osteoporosis with an area under the curve of 70.1% compared with patients with normal bone mass. **B** Age was a predictor of osteoporosis with an area under the curve of 72.1%

72.1% (95% CI 67.8%–76.3%). Therefore, patients with T2DM who are older than 59.5 years should be closely monitored in clinical practice (Fig. 4B). Similarly, when we excluded the effect of gender and analysed women separately, the results still showed age as a risk factor, when the area under the ROC curve for female patients was 83.1% (95CI 77.7%–88.5%) (Supplemental Fig. 3B).

Discussion

T2DM and OP are both harmful and potentially disabling diseases [21]. T2DM is an increasingly prevalent metabolic disease that is causing a major global economic burden. Diabetic osteopathy is characterized by the increased risk of OP and fragility fractures in diabetic patients,

which is a serious complication of diabetes [22]. It is essential to understand the interactions of T2DM and OP to provide optimal treatment for patients. Previous studies have not clarified the risk factors for T2DM combined with OP. Therefore, we investigated these risk factors in the present study. Our results showed that the major risk factors for T2DM combined with OP are increased height and weight, older age, and increased glucose metabolism indicators (HbA1c and FPG/FBG). Secondary risk factors were increased liver function indicators (ALP) and lipid metabolism indicators (HDL-C). On the basis of our data analysis results and clinical significance, we are likely to focus on age and glucose metabolism indicators in further analyses.

Previous studies have shown that female sex and an increased BMI are risk factors for OP in patients with and without diabetes [23, 24]. Our patients with T2DM had independent risk factors for T2DM combined with OP (such as female sex and increased weight). Poor glycemic control in patients with T2DM is associated with the risk of fracture [25]. Some studies have shown that the risk of fracture is increased in patients with diabetes-related complications (i.e., diabetic macrovascular complications, such as stroke and coronary heart disease, and microvascular complications, such as retinopathy and neuropathy), and that patients with these complications have decreased BMD [26]. Our study confirmed the correlation between T2DM and OP; namely, the glucose metabolism indicators were closely related to the bone metabolism indexes (vertebral T value, femoral T value, vertebral BMD, and femoral BMD), and may affect the occurrence of OP by affecting the changes in the bone metabolism indexes. However, in contrast to previous studies, we further analyzed the correlations to identify the clinical OP predictors and monitoring indicators with specific guiding significance.

HbA1c variability is related to increased risks of all-cause and cardiovascular mortality and complications of diabetes, such as cardiometabolic and microvascular complications [27–29]. HbA1c is also one of the important biomarkers of fracture [30]. Clinical trials (DCCT, UKPDS) have clearly showed the relationship between glycemic control, HbA1c, and diabetic complications [31]. This is consistent with our finding that HbA1c was a clinical evaluation index associated with T2DM combined with OP. Our study was the first to use ROC curve analyses to find that the HbA1c may be useful to clinically assess the risk of normal bone mass transitioning to OP. When the HbA1c value is between 6% and 6.45% or reaches 6.45% at a long-term stable state, clinicians should start to monitor for the occurrence of OP in patients with T2DM. Our results suggest that clinical monitoring of glucose metabolism indicators (HbA1c and FPG/FBG) might be able to predict the risk of OP in patients with T2DM and aid in the clinical prevention of OP as a complication of diabetes.

Prior studies have found that the prevalence of OP in T2DM is related to age [32] and increases with age [24]. Based on these previous study findings, our study further analyzed the risk factors affecting bone metabolism indicators in patients with T2DM at different ages. In addition to HbA1c and FPG/FBG, which affected bone metabolism at every age stage, we found that patients under 40 years old had more factors influencing bone metabolism-related indicators than other age groups. One study reported that bone mass increases fastest during puberty [33], while another study revealed that there is higher calcium absorption and lower calcium excretion during puberty, making puberty an important period for maximizing bone density [34]. Alghadir et al. showed that endocrine function, nutrition, body weight, daily solar radiation, physical activity, and genetic factors are all associated with increased bone mass during youth to prevent OP [35]. Our findings were in line with their findings that there were more factors affecting bone mass and the body can self-regulate in various ways to achieve balance in younger age groups; thus, there was not a high risk of developing OP in the younger age group. We further found that the risk of OP in patients with T2DM was increased when they were older than 59.5 years, with an area under the ROC curve of 72.1% (95% CI 67.8%–76.3%), suggesting that early management of diabetes might be vital to prevent complications such as OP.

An increasing number of researchers have investigated the effect of hypoglycemic drugs on bone metabolism. Mu et al. showed that metformin promotes bone formation/quality by stimulating the differentiation of osteoblasts and preventing them from being affected by diabetic conditions such as hyperglycemia [36, 37]. Combined with the results of our correlation and ROC curve analyses, we concluded that metformin treatment before the age of 59.5 years may be beneficial for the prevention of OP in patients with T2DM. Insulin has anabolic effects on bone and plays a vital role in regulating bone metabolism and turnover. Each class of new non-insulin agents added to initial therapy generally reduces the HbA1c by approximately 0.7% to 1.0% compared with metformin alone [38]. Our study found that patients with T2DM had an increased risk of OP when the HbA1c was between 6% and 6.45% or reached 6.45% at a long-term stable state. Therefore, the use of insulin may prevent OP in patients with T2DM by regulating HbA1c to normal levels. This result may provide a prediction for subsequent clinical treatment and provide support for future clinical studies and possible research directions.

Conclusions

In summary, our study suggested that clinical monitoring of glucose metabolism indexes (HbA1c and FPG/FBG) may predict the risk of OP in patients with T2DM and aid in the clinical prevention of OP. When the HbA1c is between 6% and 6.45% or reaches 6.45% at a long-term stable state in patients with T2DM who are older than 59.5 years, clinicians should implement strategies to prevent OP and start anti-OP treatment as soon as possible in accordance with the individual situation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01303-3.

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Author contributions Jiaojiao Wang: conceptualization, data curation, writing-original draft preparation. Hang Li: methodology, data curation. Haihong Zhu: investigation. Xinyan Xie: conceptualization. Qiyue Zheng: data curation. Jian Qu: methodology. Haiyan Yuan: conceptualization, data curation. Ting Liu: contributed to the discussion and editing. Qiong Lu: writing-review and editing. All authors accept responsibility for the entire content of this manuscript and have approved its submission.

Data availability Data are available upon request due to privacy and ethical restrictions.

Declarations

Competing interests Authors declare no competing interests.

Ethical clearance The Second Xiangya Hospital of Central South University of Changsha, China, approved the present study and waived the need for informed consent.

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ORIGINAL ARTICLE

Fasting plasma glucose and 2-h postprandial plasma glucose characteristics in a large multi-ethnic Chinese population

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Abstract

Objective During an oral glucose tolerance test (OGTT), typically fasting plasma glucose is lower than 2-h postprandial plasma glucose. However, postprandial plasma glucose (PPG) levels lower than fasting plasma glucose (FPG) levels may also occur. This study aims to describe the prevalence, clinical characteristics and contributing risk factors for PPG ≤ FPG in a large diverse Chinese population.

Methods We conducted a cross-sectional analysis of baseline data from a nationwide cohort study conducted in China. In addition to sociodemographic and anthropometric data collection, individuals had OGTT and blood chemistry tests. We determined the prevalence of PPG ≤ FPG ('Low Post Load' group) and PPG > FPG ('High Post Load' group) and used logistic regression to evaluate the association of risk factors with the occurrence of Low Post Load.

Results The prevalence of Low Post Load was 26.04% ($n = 3773$) and High Post Load was 73.96% ($n = 10{,}714$). Low Post Load was found to be related to younger age, male, lower BMI, lower blood pressure, higher HDL cholesterol levels and lower triglycerides levels. Compared with participants in the High Post Load group, participants in Low Post Load group had lower PPG (4.59 ± 0.83 mmol/L vs 7.15 ± 1.41 mmol/L) and HbA1c (5.30 ± 0.43% vs 5.39 ± 0.45%). People in Low Post Load group were more likely to have hypoglycaemic episodes (2.12% vs 0.01%) and impaired fasting glucose (12.30% vs 4.81%) compared with people with High Post Load, all $p < 0.001$.

Conclusions We found a high prevalence of people with Low Post Load glucose (26.04%) in a Chinese population cohort. The relationship between Low Post Load and the progression to or protection from diabetes and related complications and future incidence of cardiovascular disease needs further exploration in longitudinal analyses.

Keywords Fasting plasma glucose · Diabetes · OGTT · Glucose tolerance · Cross-sectional study

Highlights
- A quarter (26.04%) of participants in a Chinese population had plasma glucose levels following a 75 g glucose load that were equal to or less than their fasting plasma glucose value.
- Low Post Load was associated with a beneficial cardiometabolic profile with a lower BMI, lower blood pressure and favourable lipid profile.
- IFG occurred more frequently in participants without hypoglycaemia (12.54% vs 1.25%, $p = 0.004$).
- The relationship between this phenomenon and High Post Load glucose and the progression to prediabetes, diabetes and 'hard' cardiovascular outcomes in a Chinese population requires further longitudinal investigation.

Extended author information available on the last page of the article

Introduction

The oral glucose tolerance test (OGTT) is a test commonly used to diagnose diabetes or prediabetes, in which plasma glucose is tested after an overnight fast and again 2 h following ingestion of a 75 g glucose solution. The 2-h post glucose load plasma glucose measure (hereafter referred to as postprandial plasma glucose (PPG)) is usually found to be higher than the fasting plasma glucose levels (FPG). A PPG result higher than 7.8 mmol/L and less than 11.1 mmol/L

indicates impaired glucose tolerance (IGT), which is a well-established risk factor for type 2 diabetes [1]. The relationship between the PPG and FPG is thought to be related to the risk of developing type 2 diabetes as subjects whose PPG levels return to fasting levels more quickly have demonstrated a lower risk for the development of type 2 diabetes [2].

PPG levels that are lower than or equal to FPG levels 2 h after a glucose load have been described in people with and without diabetes [3]. Several factors have been associated with this phenomenon including abnormal liver function, pancreatic dysfunction, late dumping syndrome and patients who have had gastrectomy or bariatric surgery [4–10]. In some cases, PPG levels can fall low enough to cause hypoglycaemia, referred to as reactive hypoglycaemia. Plasma glucose levels are maintained within a narrow normal range by the coordinated physiological responses of multiple organs [11]. The liver maintains plasma glucose level in the fasted state via activation of metabolic pathways like gluconeogenesis and glycogenolysis, and the pancreas releases insulin to promote uptake of glucose into the tissues [12–16]. PPG lower than FPG has been observed in some patients with liver disease [17, 18] and pancreatic dysfunction such as high insulin sensitivity, over-reaction of glucagon-like peptide 1 and deficiencies of counter-regulatory hormones which can lead to PPG being lower than FPG [19–22]. In addition to antidiabetic medications, vigorous activity before sampling and insufficient food intake, late dumping syndrome, related to a rapid rate of gastric emptying, results in the exposure of the distal gut to more carbohydrates and leads to postprandial hyperglycaemia, which stimulates the pancreas resulting in hyperinsulinaemia, leading to late hypoglycaemia or reactive hypoglycaemia [23, 24]. Reactive hypoglycaemia has been associated with a number of linked insulin resistance-related conditions such type 2 diabetes, non-alcoholic fatty liver disease, polycystic ovarian syndrome and hypertriglyceridaemia [17, 25].

Although the relationship between PPG and FPG and the risks of developing diabetes have been described in research settings, no studies have reported the population prevalence of PPG ≤ FPG. In this study, we used an FPG value and a PPG value measured 2-h after a 75 g OGTT as glycaemic gap to define 'Low Post Load' (PPG ≤ FPG) and 'High Post Load' (PPG > FPG) groups. We aimed to evaluate the population prevalence of 'Low Post Load' and the clinical characteristics of people with 'Low Post Load'.

Materials and methods

Study design

All subjects were participants of the Study on Evaluation of Innovative Screening tools and determination of optimal diagnostic cut-off points for type 2 diabetes in Chinese multi-ethnic population (SENSIBLE) and SENSIBLE-Addition studies (the National Key R&D Program of China (2016YFC1305700)) [26, 27]. It was conducted in 8 provinces from different regions of China across several ethnic groups [26, 27]. A multi-stage cluster and simple randomisation method were used to invite subjects aged 20 to 70 years who had been living in their residence for 5 years to participate. Participants enrolled in the baseline study between November 2016 and June 2017. After providing written informed consent, participants completed a questionnaire, anthropometric examination and laboratory evaluation. Individuals who refused to sign the informed consent, were pregnant, had a significant psychiatric illness (mild depression was not an exclusion) or had any other diseases that could not complete the investigation procedures; they were excluded from the study. In this paper, we use the baseline data of the cohort to conduct a cross-sectional analysis.

A total of 17,629 participants were recruited into this study. We excluded participants with missing data on sociodemographic information (e.g. age, gender, ethnicities, family history of diabetes) and plasma glucose value (fasting plasma glucose and/or 2-h plasma glucose), the outliers (> 99.9 percentile or < 0.1 percentile), including anthropometric examination characteristics (BMI < 15.605 kg/m^2 or BMI > 48.423 kg/m^2, waist < 55 cm or waist > 126 cm) and participants with self-reported diabetes or who had been diagnosed with diabetes by the baseline OGTT.

Data collection

Eligible participants were invited to attend a study day and advised to maintain their usual lifestyle/physical activity for at least 3 days prior and maintain an overnight fast of at least 10 h. On the study day, the following assessments were conducted: (1) heart rate and blood pressure were measured using electronic sphygmomanometers (YE680E, Jiangsu Yuyue Medical Equipment Inc., Nanjing, Jiangsu, China); (2) fasting plasma glucose; (3) blood chemistry tests and biochemistry examination; (4) a standard 75 g glucose solution for an oral glucose tolerance test (OGTT) was given and plasma glucose measure taken 120 min later and (5) completion of a face-to-face structured

questionnaire and anthropometric examination using standardized procedures [28].

Blood sample collection and analysis

All blood samples were centrifuged on-site within 30 min after collection. For the serum and the whole blood samples, they were shipped at 4 °C by air to the central laboratory in Nanjing Adicon Clinical Laboratories. All the blood specimens were analysed immediately after arrival using an automatic chemistry analyser (Synchron LX-20, Beckman Coulter Inc., CA, USA). HbA1c was measured with high-performance liquid chromatography (HPLC; D-10™ Haemoglobin Analyser, Bio-Rad Inc., CA, USA).

Questionnaire

The questionnaire was designed to collect demographic characteristics (age, gender, educational level), lifestyle behaviour (smoking status, drinking status, regular physical activity) and health-related characteristics. This standardized questionnaire was administered by trained interviewers to all enrolled participants.

Occupations were categorized into three groups: professional occupations (including researcher, doctor, teacher, administrative leader and office staff), manual workers (including commerce or service man, farmer, fisherman, soldier and workman) and students. Lifestyle and behaviour questions, i.e. smoking, alcohol, exercise, and diet information, were categorical variables, scored on several points scales or classified as 'yes' or 'no'.

Health-related characteristics were self-reported by participants and included medical conditions (the start time, control time or end time and severity level) and medication use (the start time and end time).

Definitions of diabetes and type 2 diabetes

Diabetes mellitus, impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and normal glucose tolerance (NGT) were defined using the Chinese guideline 2020 edition (based on WHO 1999 diagnostic criteria) [29]. The fasting plasma glucose (FPG) of normoglycaemia people has been set at 3.9–6.1 mmol/L and the 2-h postprandial plasma glucose at 7.8 mmol/L or less; impaired fasting glucose (IFG) was defined as an FPG of greater than or equal to 6.1 mmol/L and less than 7.0 mmol/L, and PPG at 120 min after oral glucose load less than 7.8 mmol/L; impaired glucose tolerance (IGT) was defined as an FPG of less than 6.1 mmol/L, and PPG at 120 min after oral glucose loading of equal or greater than 7.8 mmol/L and less than 11.1 mmol/L. Diabetes was defined as FPG $\geq$ 7.0 mmol/L or 2-h postprandial blood $\geq$ 11.1 mmol/L; the diagnostic criteria for hypoglycaemia were plasma glucose < 2.8 mmol/L.

Statistical analyses

Continuous variables were described as means ($\pm$ SD) or median (interquartile range), and categorical data are presented as number and percentage. Statistical differences in continuous data were determined using Student's *t*-test and Welch's *t*-test. Categorical data were compared using the chi-squared test or Fisher's exact test.

A multivariate logistic regression was used to identify factors associated with Low Post Load. All variables with significant differences at univariate logistic regression analysis ($p < 0.05$) were included in multivariate logistic regression analysis, and the odds ratio (OR) and 95% CI were calculated for each factor. We further divided into those with and without hypoglycaemia and used logistic regression model to compare respondent characteristics.

All statistical analyses were performed with SAS version 9.4 (SAS Institute Inc.). Results were considered significant when the *p*-value was less than 0.05 at the two-sided test.

Results

In total, 17,629 people participated in the original study. A total of 14,487 participants (Fig. 1) were included in this analysis after exclusion of 93 subjects without FPG, 1281 subjects without PPG, 4 subjects without HbA1c, 49 subjects without sociodemographic information, 67 subjects without BMI information, 17 outliers in BMI, 2 subjects lacking diet information, 68 subjects who self-reported with diabetes and 1561 subjects who were found to have diabetes in the OGTT.

Table 1 shows data on clinical, socio-economic and behavioural characteristics of High Post Load and Low Post Load groups. The mean age of participants was 49.34 $\pm$ 11.92 years and 9702 (66.97%) were female. There were 3773 participants (26.04%) in the Low Post Load group and 10,714 participants (73.96%) in the High Post Load group. Participants in the Low Post Load group were significantly younger; were more likely to be male; non-Han ethnicity; had a lower BMI, a heart rate less than 100 beats/min, higher HDL and lower TG levels; and were more likely to drink alcohol and exercise regularly compared with participants in the High Post Load group.

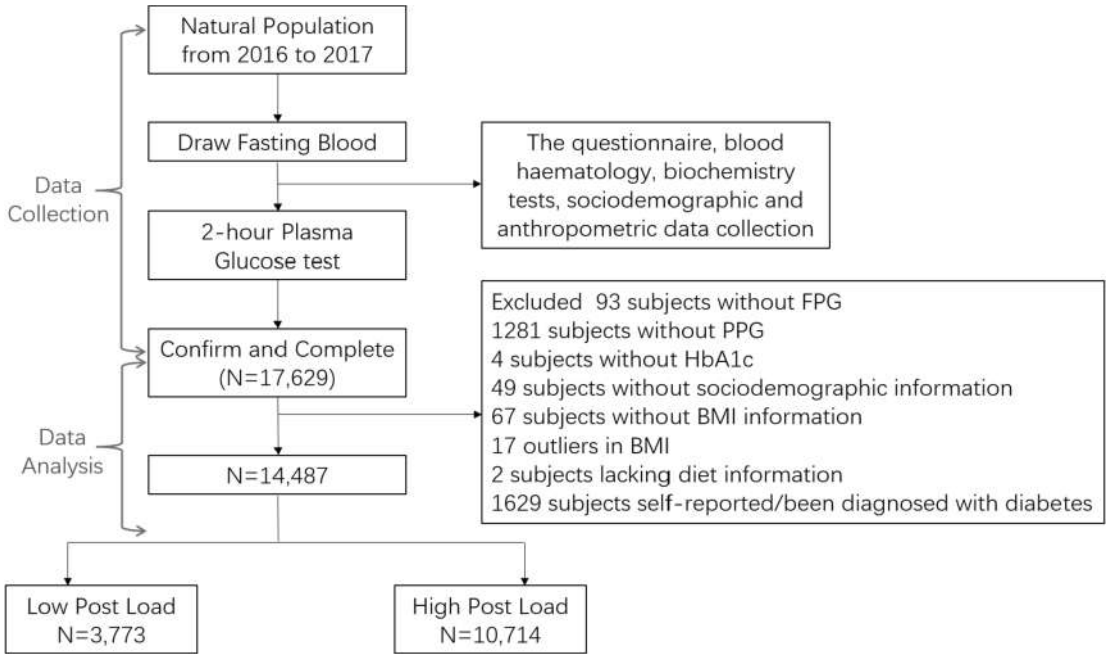

Fig. 1 Flow chart of this research

Figure 2 shows the number of plasma values in the Low Post Load group and High Post Load group. Compared with participants in the High Post Load group, participants in the Low Post Load group had lower PPG (4.59 ± 0.83 vs 7.15 ± 1.41) and HbA1c (5.30 ± 0.43 vs 5.39 ± 0.45), with higher hypoglycaemia incidence (all $p < 0.001$). In the Low Post Load group, 87.70% ($n = 3309$) had normal glucose tolerance compared with 66.18% ($n = 7090$) in the High Post Load group. There were no participants in the Low Post Load group with IGT and 464 (12.30%) participants had IFG. In the High Post Load group, 515 (4.81%) participants had IFG, 2313 (21.59%) participants had IGT and 796 (7.43%) participants had both IFG and IGT ($p < 0.001$).

In Table 2, an adjusted logistic regression model indicated that most of the evaluated factors had significant and independent positive or negative associations with Low Post Load. For the phenomenon of Low Post Load, the significant factors were age < 44 years; male; people of Uyghur or Zhuang ethnicity; BMI < 24 kg/m^2; normal blood pressure; heart rate < 100 beats/min; status as never or former drinker; HDL cholesterol ≥ 1.55 mmol/L and triglycerides < 1.70 mmol/L, all $p < 0.001$.

Within the Low Post Load group, a small number of participants ($n = 80/3773$; 2.12%) had hypoglycaemia, defined as a blood glucose of < 2.8 mmol/L in the OGTT.

As shown in Table 3, for participants in the Low Post Load group, the following variables had a significant association with hypoglycaemia: age < 44 years old, male, non-Han population, manual-worker, BMI < 24 kg/m^2 and cigarette smoking. Although participants with hypoglycaemia has lower FPG (5.13 ± 0.53 vs 5.44 ± 0.56, $p < 0.001$) and PPG (2.49 ± 0.30 vs 4.64 ± 0.78, $p < 0.001$), there was no difference in HbA1c compared with participants without hypoglycaemia.

Discussion

In this analysis, we found a quarter (26.04%) of participants in a Chinese population had plasma glucose levels following a 75-g glucose load that were equal to or less than their fasting plasma glucose values. Although Low Post Load has been described in individuals with specific clinical conditions, it can occur without any precipitating factors, and until now, there has been a lack of published data that provides estimates of the prevalence of this phenomenon in the general population. This large cohort study now establishes the prevalence in a multi-ethnic Chinese population.

In our study, people with Low Post Load were more likely to be younger, have lower BMI and have a number

Table 1 Baseline information of participants by Low Post Load and High Post Load

	All ($N=14,487$)	Low Post Load, $N=3773$ (26.04%)	High Post Load, $N=10,714$ (73.96%)	p-value
	Mean ± SD/N (%)	Mean ± SD/N (%)	Mean ± SD/N (%)	
Age (years)	49.34 (11.92)	46.60 (12.69)	50.31 (11.48)	< 0.001
Gender (female)	9702 (66.97%)	2136 (56.61%)	7566 (70.62%)	< 0.001
Ethnicity (Han)	7056 (48.71%)	1590 (42.14%)	5466 (51.02%)	< 0.001
Education levels (illiteracy)	1415 (9.77%)	274 (7.26%)	1141 (10.65%)	< 0.001
Occupation type				< 0.001
Professional	2364 (16.32%)	711 (18.84%)	1653 (15.43%)	
Manual-worker	12,011 (82.91%)	3004 (79.62%)	9007 (84.07%)	
Student	112 (0.77%)	58 (1.54%)	54 (0.50%)	
Body mass index (kg/m^2)	24.87 (3.79)	24.17 (3.65)	25.12 (3.80)	< 0.001
SBP (mmHg)	130.8 (20.45)	128.0 (19.87)	131.7 (20.57)	< 0.001
DBP (mmHg)	80.86 (12.14)	79.47 (12.05)	81.34 (12.14)	< 0.001
Heart rate (beats/min)	78.55 (11.38)	77.27 (11.18)	79.00 (11.42)	< 0.001
Total cholesterol (mmol/L)	5.03 (1.09)	4.92 (1.11)	5.06 (1.08)	< 0.001
HDL cholesterol (mmol/L)	1.57 (0.39)	1.61 (0.41)	1.55 (1.54)	< 0.001
LDL cholesterol (mmol/L)	2.84 (0.81)	2.77 (0.82)	2.87 (0.80)	< 0.001
Triglycerides (mmol/L)	1.55 (1.53)	1.35 (1.47)	1.62 (1.54)	< 0.001
Smoking				< 0.001
Never	11,764 (81.20%)	2873 (76.15%)	8891 (82.98%)	
Former	453 (3.13%)	135 (3.58%)	318 (2.97%)	
Current	2270 (15.67%)	765 (20.28%)	1505 (14.05%)	
Alcohol				< 0.001
Never	10,991 (75.87%)	2721 (72.12%)	8270 (77.19%)	
Former	534 (3.69%)	168 (4.45%)	366 (3.42%)	
Current	2962 (20.45%)	884 (23.43%)	2078 (19.40%)	
Vigorous exercise	5259 (36.30%)	1420 (37.64%)	3839 (35.83%)	0.047
Regular meals	10,992 (75.87%)	2784 (73.79%)	8208 (76.61%)	< 0.001
Family history of diabetes	2021 (13.95%)	496 (13.15%)	1525 (14.23%)	0.097
Hypoglycaemic drugs use	16 (0.11%)	4 (0.11%)	12 (0.11%)	1.000
Self-report liver disease	294 (2.03%)	65 (1.72%)	229 (2.14%)	0.120
Self-report pancreatic disease	3 (0.02%)	1 (0.03%)	2 (0.02%)	1.000
Self-report upper digestive track disease	114 (0.79%)	32 (0.85%)	82 (0.77%)	0.621
FPG (mmol/L)	5.43 (0.57)	5.44 (0.56)	5.43 (0.58)	0.317
PPG (mmol/L)	6.49 (1.71)	4.59 (0.83)	7.15 (1.41)	< 0.001
Hypoglycaemia	81 (0.56%)	80 (2.12%)	1 (0.01%)	< 0.001
Glycaemic Gap (2-h PPG-FPG)	1.06 (1.59)	− 0.85 (0.70)	1.73 (1.24)	< 0.001
HbA1c (%)	5.37 (0.45)	5.30 (0.43)	5.39 (0.45)	< 0.001
Status (WHO 1999)				< 0.001
NGT	10,399 (71.78%)	3309 (87.70%)	7090 (66.18%)	
IFG	979 (6.76%)	464 (12.30%)	515 (4.81%)	
IGT	2313 (15.97%)	0 (0.00%)	2313 (21.59%)	
IFG + IGT	796 (5.49%)	0 (0.00%)	796 (7.43%)	

Data are presented as n, n (%), mean ± SD or median (IQR)

Occupation type: professional occupation: researcher, doctor, teacher, administrative leader and office staff; manual worker: commerce or serviceman, farmer, fisherman, soldier and workman; student: current student

Significance of differences at p-value < 0.05

BMI body mass index; *SBP* systolic blood pressure; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein; *FPG* fasting plasma glucose; *PPG* postprandial plasma glucose; *HbA1c* haemoglobin A1C

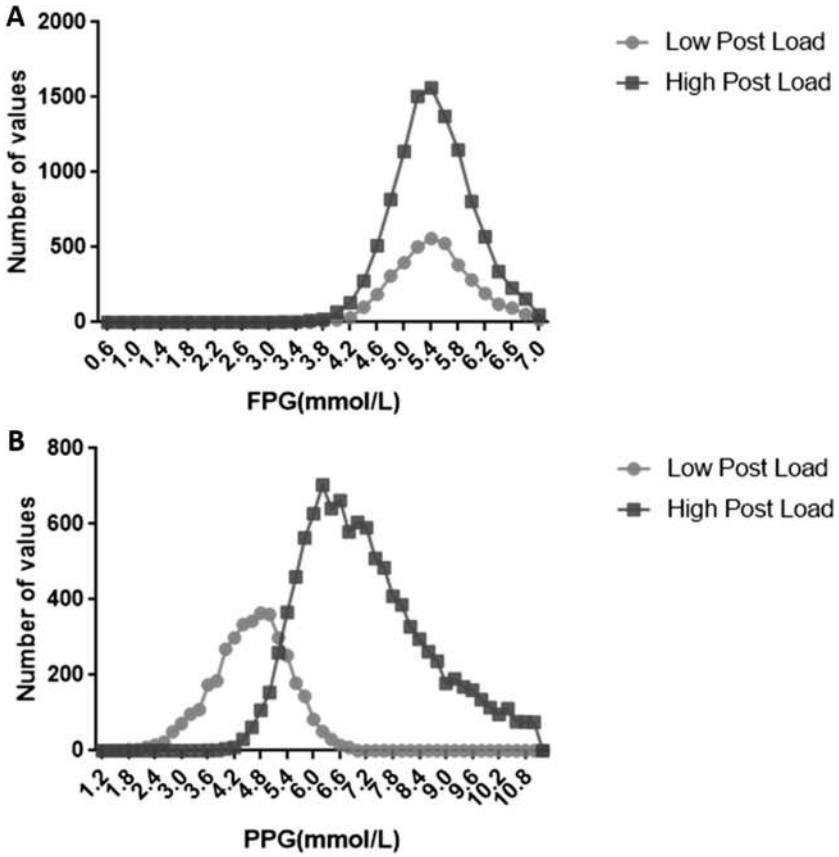

Fig. 2 Distribution of blood glucose values in the Low Post Load group and High Post Load group

of characteristics such as lower triglycerides, LDL, blood pressure and higher HDL suggesting that Low Post Load has a beneficial cardiometabolic profile. People with High Post Load were more likely to have prediabetes. The Low Post Load group had a higher proportion of people with normal glucose tolerance than the High Post Load group (87.7% vs 66.2%); 464 (11.93%) participants had IFG, and no participants had IGT; while in the High Post Load group, there was a lower proportion with IFG (4.21%), higher proportions with IGT (18.92%) and both IFG and IGT (6.51%). Prediabetes is an intermediate hyperglycaemic state between normal glucose tolerance and overt diabetes. Over a lifetime follow-up, about 70% of people with prediabetes develop type 2 diabetes, with the risk of developing diabetes twofold higher in those with IFG and IGT than in those with isolated IFG or isolated IGT [31–33]. IGT is associated with more severe insulin resistance and beta-cell dysfunction [34–36]. The San Antonio Heart Study demonstrated that participants with normal glucose tolerance and High Post Load had 2.33-fold odds of developing type 2 diabetes when compared with normal glucose tolerance participants with Low Post Load, during 7 to 8 years of follow-up [2]. In 20 years of follow-up in the CARDIA (Coronary Artery Risk Development in Young Adults) study, normal glucose tolerance participants with Low Post Load had a lower risk of developing type 2 diabetes [37].

We defined 'Low Post Load' as 2-h post load plasma glucose lower than fasting plasma glucose. Using this definition, there are two sub-categories of people: (1) those with adequate insulin reserve who are able to quickly normalise their plasma glucose after an oral load and (2) those with over-correction of their plasma glucose with post load plasma glucose in the hypoglycaemic range, i.e. reactive hypoglycaemia. We found that IFG occurred less frequently in participants with hypoglycaemia (1.25% vs 12.54%, $p = 0.004$). This might due to the higher insulin secretion or insulin response and a different metabolic trajectory in participants with hypoglycaemia. We will follow these participants in subsequent cohort studies to find if they are more prone to diabetes progression.

Smoking, caffeine intake, insufficient food intake, antidiabetic medications and heavy exercise have been demonstrated as pre-analytical factors that affect plasma glucose levels [38, 39]. We excluded participants with diabetes (self-reported or diagnosed by OGTT). Use of glucose lowering agents could affect glucose levels during OGTT. Also, people with diabetes have insulin resistance and relative insulin insufficiency resulting in raised fasting and post

Table 2 Effects of respondent characteristics on Low Post Load in a sample of 14,487 Chinese population

Characteristics	OR (95% CI)	*p*-value
Age		
Young age (< 44 years)	1.000	
Middle age (44–59 years)	0.629 (0.567, 0.697)	< 0.001
Older adults (60–74 years)	0.478 (0.420, 0.545)	< 0.001
Elderly (≥ 75 years)	0.225 (0.073, 0.697)	0.010
Gender		
Female	1.000	
Male	2.007 (1.819, 2.215)	< 0.001
Ethnicity		
Han	1.000	
Dai	0.842 (0.721, 0.982)	0.029
Kazakh	1.136 (0.921, 1.401)	0.234
Korean	1.057 (0.889, 1.257)	0.528
Uyghur	1.900 (1.639, 2.203)	< 0.001
Zhuang	1.655 (1.436, 1.906)	< 0.001
Other	1.822 (0.816, 4.070)	0.144
Education levels		
Illiteracy	1.000	
Primary school	1.000 (0.840, 1.189)	0.996
Middle school	1.025 (0.865, 1.215)	0.774
High school	1.088 (0.904, 1.311)	0.372
Junior college, undergraduate and above	1.228 (1.018, 1.482)	0.032
Occupation		
Professional occupation	1.000	
Manual-worker	0.825 (0.727, 0.935)	0.003
Student	1.494 (0.915, 2.442)	0.108
BMI		
Underweight (< 18.5kg/m^2)	1.000	
Normal range (18.5–23.9 kg/m^2)	0.826 (0.620, 1.102)	0.194
Overweight (24–27.9 kg/m^2)	0.537 (0.401, 0.718)	< 0.001
Obese(≥ 2 8kg/m^2)	0.425 (0.314, 0.576)	< 0.001
Blood pressure		
Normal	1.000	
High normal	0.865 (0.773, 0.967)	0.011
Grade 1 hypertension	0.898 (0.789, 1.022)	0.102
Grade 2 hypertension	0.738 (0.623, 0.872)	< 0.001
Grade 3 hypertension	0.781 (0.607, 1.005)	0.054
Isolated systolic hypertension	0.940 (0.825, 1.072)	0.356
No data	0.768 (0.272, 2.164)	0.617
Heart rate (beats/min)		
< 60	1.000	
60–100	0.811 (0.630, 1.043)	0.102
> 100	0.518 (0.363, 0.738)	< 0.001
No data	1.177 (0.558, 2.484)	0.668
Total cholesterol (mmol/L)		
< 5.17	1.000	
5.17–6.46	0.981 (0.892, 1.078)	0.689
≥ 6.47	0.982 (0.841, 1.146)	0.815
HDL cholesterol (mmol/L)		
< 0.91	1.000	
0.91–1.54	1.276 (0.945, 1.722)	0.112

Table 2 (continued)

Characteristics	OR (95% CI)	*p*-value
≥ 1.55	1.694 (1.251, 2.292)	< 0.001
LDL cholesterol (mmol/L)		
< 3.37	1.000	
3.37–4.13	0.930 (0.833, 1.038)	0.196
≥ 4.14	1.018 (0.863, 1.202)	0.828
Triglycerides (mmol/L)		
< 1.70	1.000	
1.70–2.25	0.667 (0.584, 0.762)	< 0.001
≥ 2.26	0.552 (0.483, 0.630)	< 0.001
Smoking		
Never	1.000	
Former	0.938 (0.737, 1.195)	0.605
Current	0.949 (0.830, 1.085)	0.444
Alcohol		
Never	1.000	
Former	1.031 (0.801, 1.327)	0.813
Current	0.839 (0.733, 0.959)	0.010
Vigorous exercise‡	0.894 (0.823, 0.972)	0.009
Regular meals ‡	1.070 (0.971, 1.179)	0.171
Family history of diabetes‡	1.117 (0.982, 1.271)	0.092

Occupation type: professional occupation: researcher, doctor, teacher, administrative leader and office staff; manual worker: commerce or serviceman, farmer, fisherman, soldier and workman; student: current student

Blood Pressure (mmHg): normal: SBP < 130, DBP < 85; high normal: 130 ≤ SBP < 140, 85 ≤ DBP < 90; grade 1 hypertension: 140 ≤ SBP < 160, 90 ≤ DBP < 100; grade 2 hypertension: 160 ≤ SBP < 180, 100 ≤ DBP < 110; grade 3 hypertension: SBP ≥ 180, DBP ≥ 110; isolated systolic hypertension: SBP ≥ 140, DBP < 90 [30]

Adjusted by continuous age variable, gender and BMI, the significance of differences at p-value < 0.05

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein

‡ p-value for comparison between no data or no vs yes

load glucose by definition. We found that current drinking and vigorous exercise were related to hypoglycaemia after adjustment for age, gender and BMI in our study.

The study has several limitations. About 70% of the study participants were women. This study was conducted in areas where there is a lot of mobility of young men who travel to urban areas for work, leaving predominantly women to be sampled in the study areas. In this study, there were 294 (2.03%) participants with liver disease, 3 (0.02%) participants with pancreatic disease and 114 (0.79%) participants with upper digestive tract disease. These conditions have been associated with the occurrence of Low Post Load; however, as the prevalence was limited and derived from self-reporting by subjects in questionnaires, they may be underestimated, and we were not able to fully examine the contribution of these conditions to the occurrence of Low Post Load. Fasting or postprandial insulin was not measured in this study, and we could not investigate the association between insulin secretion, insulin resistance and Low or High Post Load. Additionally, there were missing data on waist-hip ratio, and we were unable to examine the relationship between central obesity and this phenomenon.

Conclusion

In conclusion, we found a significant prevalence of people with Low Post Load glucose (26.04%) in population-based baseline data of a cohort in China. Low Post Load was associated with a beneficial cardiometabolic profile with a lower BMI, lower blood pressure and favourable lipid profile. The relationship between this phenomenon and High Post Load glucose and the progression to prediabetes, diabetes and 'hard' cardiovascular outcomes in a Chinese population requires further longitudinal investigation.

Table 3 Participants with/without hypoglycaemia in Low Post Load group

	With hypoglycaemia, $N=80$ (2.12%) Mean ± SD/N (%)	Without hypoglycaemia, $N=3693$ (97.88%) Mean ± SD/N (%)	p-value
Age (years)	42.68 (14.42)	46.68 (12.64)	0.016
Gender (female)	27 (33.75%)	2109 (57.11%)	< 0.001
Ethnicity (Han)	11 (13.75%)	1579 (42.76%)	< 0.001
Education levels (illiteracy)	3 (3.75%)	271 (7.34%)	0.315
Occupation type			< 0.001
Professional	3 (3.75%)	708 (19.17%)	
Manual-worker	73 (91.25%)	2931 (79.37%)	
Student	4 (5.00%)	54 (1.46%)	
Body mass index (kg/m^2)	23.47 (3.04)	24.18 (3.66)	0.041
SBP (mmHg)	125.8 (17.75)	128.1 (19.91)	0.304
DBP (mmHg)	76.49 (11.56)	79.54 (12.05)	0.025
Heart rate (beats/min)	76.43 (12.15)	77.29 (11.15)	0.492
Total cholesterol (mmol/L)	5.00 (1.17)	4.92 (1.11)	0.515
HDL cholesterol (mmol/L)	1.69 (0.48)	1.61 (0.40)	0.148
LDL cholesterol (mmol/L)	2.83 (0.85)	2.76 (0.82)	0.510
Triglycerides (mmol/L)	1.15 (0.90)	1.35 (1.48)	0.053
Smoking			0.009
Never	50 (62.50%)	2823 (76.44%)	
Former	3 (3.75%)	132 (3.57%)	
Current	27 (33.75%)	738 (19.98%)	
Alcohol			0.200
Never	53 (66.25%)	2668 (72.24%)	
Former	2 (2.50%)	166 (4.49%)	
Current	25 (31.25%)	859 (23.26%)	
Vigorous exercise	34 (42.50%)	1386 (37.53%)	0.364
Regular meals	60 (75.00%)	2724 (73.76%)	0.803
Family history of diabetes	10 (12.50%)	486 (13.16%)	0.863
Hypoglycaemic drugs use	0 (0.00%)	4 (0.11%)	1.000
Self-report liver disease	1 (1.25%)	64 (1.73%)	1.000
Self-report pancreatic disease	0 (0.00%)	1 (0.03%)	1.000
Self-report upper digestive track disease	0 (0.00%)	32 (0.87%)	1.000
FPG (mmol/L)	5.13 (0.53)	5.44 (0.56)	< 0.001
PPG (mmol/L)	2.49 (0.30)	4.64 (0.78)	< 0.001
Glycaemic Gap (2-h PPG-FPG)	− 2.64 (0.70)	− 0.81 (0.65)	< 0.001
HbA1c (%)	5.22 (0.54)	5.31 (0.42)	0.171
Status (WHO 1999)			0.004
NGT	79 (98.75%)	3230 (87.46%)	
IFG	1 (1.25%)	463 (12.54%)	
IGT	0 (0.00%)	0 (0.00%)	
IFG + IGT	0 (0.00%)	0 (0.00%)	

Data are presented as n, n (%), mean ± SD or median (IQR)

Occupation type: professional occupation: researcher, doctor, teacher, administrative leader and office staff; manual worker: commerce or serviceman, farmer, fisherman, soldier and workman; student: current student

Significance of differences at p-value < 0.05

BMI body mass index; *SBP* systolic blood pressure; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein; *FPG* fasting plasma glucose; *PPG* postprandial plasma glucose; *HbA1c* haemoglobin A1C

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Data availability The data that support the findings of this study are not openly available due to reasons of sensitivity, but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The study was conducted in accordance with the Declaration of Helsinki, and this study protocol was reviewed and approved by the Human Research Ethics Committee of Zhongda Hospital, Southeast University, approval number: 2016ZDSYLL092-P01. The names of the other institutions indicated in the Ethical approval were listed in the supplement.

Consent to participate Written informed consent has been obtained from the patients to publish this paper.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Influence of cerebral small vessel disease on functional outcome and recurrence of cerebral infarction in patients with type 2 diabetes

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Abstract

Objective To estimate the effect of the total cerebral small vessel disease score in the functional outcome and recurrence of cerebral infarction in patients with type 2 diabetes.

Methods A comparative study was used to review the initial cerebral infarction cases of patients with type 2 diabetes undergoing cranial MRI during 2016–2019, follow up their recovery for 3 months, and count the events of cerebral infarction recurrence within 24 months. MRI with lacunes, enlarged perivascular space (EPVS), cerebral microbleeds (CMBs), and white matter hyperintensities (WMHs) were defined as cerebral small vessel disease (CSVD). Chi-square tests, t-tests, rank-sum tests, and Logistic regression were used for the statistical analysis.

Results A total of 208 patients were included in the analyses. Mean age was 65.2 ± 11.8 years, and 62% were men. The distribution of the total SVD score from 0 to 4 was 26.9%, 23.6%, 26.4%, 16.3%, and 6.7%. Multivariate Logistic regression showed that the cumulative CSVD score was independently associated with poor outcome 3 months after cerebral infarction (OR:2.193, 95% CI:1.673–2.875) and recurrence within 2 years (OR:2.715, 95% CI: 1.363–2.979). Lacunes, CMBs, WMHs but not EPVS were associated with the modified Rankin Scale (mRS) scores at 3 months after cerebral infarction, Lacunes was associated with recurrence within 2 years. However, the impact of each CSVD marker on functional outcome and stroke recurrence was smaller than that of the total CSVD score.

Conclusion Cumulative CSVD burden exert important influences on the functional outcome and recurrence of cerebral infarction in patients with type 2 diabetes.

Keywords Cerebral microvascular disease · Type 2 diabetes · Stroke · Cerebral infarction

Introduction

With the rising number of adult patients with diabetes worldwide, type 2 diabetes has become a worldwide public health problem [1–3]. It is especially important to prevent the occurrence and recurrence of diabetic vascular complications, strokes, and ischemic heart disease. The risk of cerebral infarction in the diabetic population is more than twice as high as in the non-diabetic population, and long-term subclinical cerebrovascular injury exists prior to their cerebral infarction [4]. Numerous studies have shown that diabetes can lead to cerebral small vessel injury through various mechanisms such as abnormal polyol metabolism, hyper-glycolytic response, oxidative stress, abnormal transport of β-amyloid across the blood–brain barrier, and protein kinase C activation [5]. In clinical practice, experts currently define MRI manifesting lacunes of vascular origin, white matter hyperintensities (WMHs), enlarged perivascular

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space (EPVS), and cerebral microbleeds (CMBs) as CSVD [6]. Clinical studies have found that these image manifestations may be related to cerebrovascular events, but there is still a lack of systematic evidence [7]. In this study, various imaging manifestations of cerebrovascular disease in patients with type 2 diabetes were cumulatively scored, and their relationship with functional outcome and recurrence of ischemic cerebrovascular events was evaluated, so as to provide evidence for the prevention of diabetic cerebrovascular disease.

Materials and Methods

General data

The data of patients with primary cerebral infarction of type 2 diabetes who underwent head MRI examination in our hospital from January 2016 to December 2019 were reviewed. The modified Rankin Scale (mRS) score and ischemic stroke recurrence events within 2 years were recorded during follow-up. Clinical data such as gender, age, underlying diseases, cerebral infarction type and CSVD imaging markers were collected. This study was reviewed and approved by the hospital Ethics Committee.

Inclusion criteria and exclusion criteria

The 2 neurologists who were not involved in the case data collection read the MRI images respectively and then checked the unified opinion. The imaging markers of cerebrovascular disease were any one or more of lacunes of vascular origin, white matter hyperintensities, enlarged perivascular space, and cerebral microbleeds shown in MRI. CSVD cumulative score (0–4 scores): 0 score for normal, 1 score for each of the 4 MRI manifestations of lacunes of vascular origin, white matter hyperintensities, enlarged perivascular space > 10 mm, and cerebral microbleeds, and the scores were calculated cumulatively. Exclusion criteria: 1. History of stroke, 2. History of atrial fibrillation, 3. Severe hepatic and renal insufficiency, 4. Malignancy, 5. Death cases, 6. Poor MRI quality. The modified Rankin scale was used to measure the neurological recovery status of patients after stroke: 0 score for the patients who were completely asymptomatic; 1 score for the patients who can complete all daily work and life without obvious dysfunction despite symptoms; 2 score for the patients who were mildly disabled, unable to complete all activities before illness, but able to take care of their daily affairs without help; 3 score for the patients who were moderately disabled, requiring partial assistance but able to walk independently; 4 score for the patients who were moderately to severely disabled, unable to walk independently and requiring assistance in daily life; 5 score who were severely disabled, bedridden, incontinent and completely dependent on others in daily life. 0–2 score was classified as good neurological outcomes and 3–5 score as poor neurological outcomes.

Statistical analysis

SPSS 19.0 software was used for statistical analysis, and frequency, mean and median were used to represent categorical and continuous baseline variables. The one-way analysis of baseline variables was performed using Chi-square test, t-test or rank sum test. Variables with $p < 0.1$ in one-way analysis were included in logistic regression to determine the independent influencing factors of cerebral infarction recurrence or poor function. $p < 0.05$ was considered as a statistical difference.

Results

A total of 384 cases of type 2 diabetes cerebral infarction were reviewed, excluding 96 cases with a history of stroke, 12 cases of atrial fibrillation, 7 cases of severe hepatorenal insufficiency, 3 cases of malignant tumor, 3 cases of MRI quality inconsistent with the requirements. 263 cases were included in the follow-up, 19 cases died during hospitalization or within 3 months after discharge, 36 cases were lost to follow-up, and 208 cases were finally included in the statistical analysis. The flow chart of the case collection is shown in Fig. 1. The mean age was 65.2 ± 11.8 years old, of which 109 were males (52%) and 99 were females (48%). There were 109 cases with good functional outcome (mRS ≤ 2), and 22 cases with recurrent cerebral infarction within two years.

Univariate analysis showed that at 3 months after cerebral infarction, compared with the well-functioning group, the poorly functioning group was older, had higher NIHSS score at onset, had more MRI signs of white matter hyperintensities, lacunes, cerebral microbleeds, and had higher cumulative CSVD score ($p < 0.05$). In the univariate analysis of risk factors for recurrence, MRI manifestations of white matter hyperintensities, lacunes, cerebral microbleeds were statistically significant. The clinicopathological characteristics of the patients and the univariate analysis of functional outcome and recurrent cerebral infarction are shown in Table 1.

Variables with $p < 0.1$ in the univariate analysis were included in the logistic regression to determine the independent influencing factors of cerebral infarction recurrence or poor function. Multifactorial analysis showed that MRI with more white matter hyperintensities (OR = 1.84, 95%CI = 1.21–2.76, P = 0.004), lacunes (OR = 1.86, 95%CI = 1.25–2.74, $p < 0.001$), cerebral microbleeds (OR = 1.58,95%CI = 1.09–2.25, P = 0.002) signs, especially high cumulative CSVD scores (OR = 2.193,95%CI = 1.673–

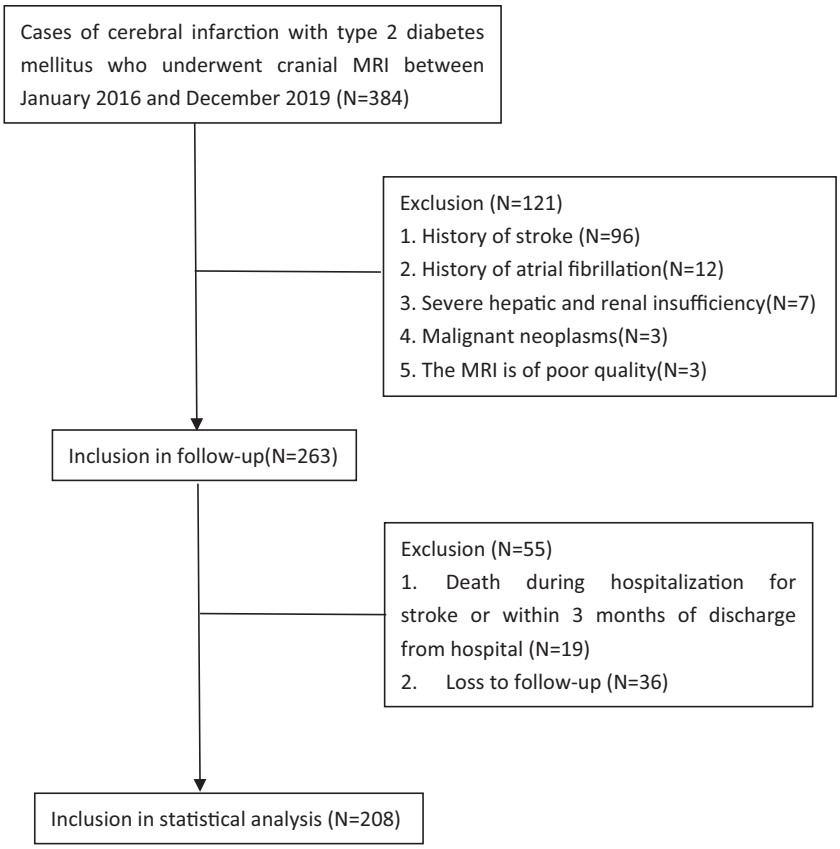

Fig.1 Flow chart of case collection

Table 1 Clinical characteristic of patients and univariate analysis of functional outcome and stroke recurrence

	The modified Rankin Scale (mRS)(3 months after cerebral infarction)			Recurrence (within 2 years)		
	≤2 (n = 109)	> 2 (n = 99)	*p*	No (n = 186)	Yes (n = 22)	P
Age (years)	60.5 ± 11.9	70.4 ± 9.3	< 0.001	64.9 ± 11.7	68.0 ± 12.9	0.243
Gender (Male)	68(62.4%)	61(61.7.3%)	0.909	116(62.3%)	13(59.1%)	0.765
Hypertension	77(70.6%)	80(80.8%)	0.089	144(77.4%)	13(59.1%)	0.069
Hyperlipemia	32(29.4%)	34(34.3%)	0.44	58(31.2%)	8(36.4%)	0.622
Smokers	53(48.6%)	46(46.5%)	0.755	86(46.2%)	13(59.1%)	0.254
Coronary heart disease	17(15.6%)	16(16.2%)	0.911	28(15.1%)	5(22.8%)	0.352
NIHSS	2.93 ± 0.988	5.47 ± 2.032	< 0.001	4.12 ± 2.011	4.27 ± 2.164	0.745
Cerebral infarction types			0.832			0.305
Large atherosclerotic type	50(45.9%)	43(43.4%)		85(45.7%)	8(36.4%)	
Small vessel occlusive type	23(21.1%)	26(26.3%)		45(24.2%)	4(18.2%)	
Cardiac embolism type	16(14.7%)	16(16.2%)		27(14.5%)	5(22.7%)	
Cerebral infarction with other definite causes	4(3.7%)	2(2.0%)		4(2.2%)	2(9.1%)	
Cerebral infarction with uncertain etiology	16(14.7%)	12(12.1%)		25(13.4%)	3(13.6%)	
white matter hyperintensities	19(17.4%)	79(79.8%)	< 0.001	82(44.1%)	16(72.7%)	0.011
lacunes	22(20.2%)	91(92.0%)	< 0.001	96(51.6%)	17(77.3%)	0.022
cerebral microbleeds	7(6.4%)	48(48.5%)	< 0.001	45(24.2%)	10(45.5%)	0.032
enlarged perivascular space	56(51.4%)	61(61.6%)	0.137	107(57.5%)	10(45.5%)	0.28
CSVD cumulative score	1.3 ± 1.143	1.77 ± 1.292	0.006	1.77 ± 1.490	2.41 ± 1.260	0.056

2.875, P = 0.004) were all independently associated with poor functional outcome at 3 months after cerebral infarction. Within 2 years after infarction, MRI with more lumens and a higher cumulative CSVD score was independently associated with recurrence Table 2.

Discussion

Microangiopathy is a unique chronic complication of diabetes and is more susceptible to various factors than large vessels, so the damage appears earlier and is more widespread, which is the basis for the occurrence of various diabetic complications. Diabetic cerebral microanglopathy is the pathological basis of brain complications, and early detection and diagnosis are of great significance to delay the course of the disease, prolong life and improve life quality [8–11]. On cerebral MRI imaging, small vessel injury can be manifested as: lacunes of vascular origin, white matter hyperintensities, enlarged perivascular space, and cerebral microbleeds [6]. Numerous clinical studies have found that these image features that can reflect the injury of small cerebral vessels are all risk factors for vascular diseases [12–14]. However, these imaging features often do not exist independently, and any one imaging feature cannot comprehensively measure the degree of cerebral small vessel injury, so some scholars have performed a semi-quantitative cumulative scoring of these imaging features to assess the degree of cerebral small vessel injury, which becomes the overall burden of cerebral small vessel disease. Clinical studies on its relationship with stroke, cognitive impairment, aging, etc. have also been conducted [15]. There are not yet any studies to apply this semi-quantitative assessment of cerebral small vessel disease in patients with diabetic cerebral infarction.

Our study showed that white matter hyperintensities, lacunes, and cerebral microbleeds were independently associated with functional outcomes in patients, which is consistent with the results of the current other studies. A study involving 5035 patients showed that patients with higher white matter hyperintensities signal load had worse mRS Scores at 3 months [16]. A recent meta-analysis showed that white matter hyperintensities, lacunes, cerebral microbleeds, and cerebral atrophy were associated with poor functional outcomes [17]. Our study did not find an association between enlarged perivascular space and functional outcome. More studies are needed to verify the relationship between enlarged perivascular space and functional outcome. Because imaging manifestations of cerebral small vessel disease often appear simultaneously in the same patient, and a single marker cannot fully reflect brain damage, scholars have proposed the concept of the overall burden of cerebral small vessel disease, and the method of CSVD cumulative score may be more beneficial to evaluate its clinical impact on patients. Our study found that the cumulative CSVD score was strongly associated with functional outcome in both univariate and multifactorial analyses, which is consistent with the current findings.

Patients with small cerebral vascular disease have a higher likelihood of recurrent cerebral infarction. Our study found that white matter hyperintensities, lacunes, and cerebral microbleeds were statistically significant in recurrent cerebral infarction in univariate analysis, while lacunes and CSVD accumulative scores were statistically significant in multivariate analysis. This may be caused by the error caused by the small sample size of our study. We speculated that patients with cerebral small vessel disease are more likely to have a recurrent stroke, and the higher the cumulative CSVD score, the more significant the predictive effect, which is consistent with the current research results. Xu et al. found that white matter hyperintensities, cerebral microbleeds and CSVD score are associated with recurrent stroke [13]. Cheng et al. found that factors associated with increased risk of recurrent stroke were white matter high signal, lacunae and CSVD score [17]. CSVD reflects severe brain damage that reduces the efficiency of neural connections and workings, leading to a slower recovery of patients after stroke, which is a possible mechanism that leads to an increased risk of recurrent stroke. Both Tian and Umeno's studies verified the role of CSVD score in predicting stroke recurrence [18, 19].

Table 2 Multivariate analysis of functional outcome and stroke recurrence

	The modified Rankin Scale (mRS) (3 months after cerebral infarction)			Recurrence (within 2 years)		
	OR	95% CI	*p*	OR	95% CI	*p*
White matter hyperintensities	1.84	1.21–2.76	0.004	2.14	0.83–5.34	0.203
Lacunes	1.86	1.25–2.74	< 0.001	2.07	1.19–4.86	0.020
Cerebral microbleeds	1.58	1.09–2.25	0.002	2.16	0.92–5.03	0.086
Enlarged perivascular space	1.07	0.75–1.68	0.47	1.06	0.47–2.83	0.87
CSVD cumulative score	2.193	1.673–2.875	0.004	2.715	1.363–2.979	0.023

Conclusion

Our study found that specific imaging markers of cerebral small vessel disease and cumulative CSVD scores in diabetic infarct patients could be used as predictors of functional outcome after infarction and were associated with recurrence of infarction in the short term. This study still has the following limitations: 1. Patients with severe stroke who underwent vascular recanalization therapy were not included in this study, because CSVD is associated with functional outcome in patients receiving thrombolysis and intravenous thrombolysis, and selection bias may affect the impact of cumulative CSVD score on functional outcome. 2. The data are from a single center, and the generalizability of the findings has certain limitations.

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Declarations

Ethics approval and consent to participate Ethical approvals were obtained from the Institutional Ethics Committees of Department of Neurosurgery, People's Hospital of Haimen District, Nantong City, Jiangsu Province, China. All the study participants provided written informedconsent.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

The effects of sodium-glucose cotransporters type 2 inhibitors on glycemic and extraglycemic laboratory parameters

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Abstract

Background An ideal glucose-lowering drug is expected to not only improve glycemic control, but also have positive effects on weight, blood pressure, dyslipidemia, and also cardiovascular and renal outcomes.

Objective To investigate and compare the impact of Sodium-glucose transport protein 2 (SGLT2) inhibitors on glycemic and extraglycemic laboratory parameters and the parameters which affect this impact.

Methods This retrospective study was conducted between January 2022 and December 2022. A total of 250 patients diagnosed with type 2 diabetes mellitus (T2DM) using SGLT2i were included in the study.

Results Patients had a mean age of 55.4 ± 9.6, and 53.6% ($n = 134$) were male. Among the patients, 19.6% ($n = 49$) used dapagliflozin and 80.4% ($n = 201$) used empagliflozin. Glucose, HbA1c, and triglyceride levels at 3 and 6 months showed significant reductions compared to baseline, while serum sodium and HDL-C levels showed significant increases ($p < 0.001$). Additionally, creatinine and serum potassium levels at 6 months were significantly higher than baseline, while LDL-C and urine albumin-to-creatinine ratio levels were significantly lower. Empagliflozin users exhibited significantly higher creatinine levels only at 3. months, higher serum sodium levels only at 6. months, and lower HbA1c levels only at 6. months compared to dapagliflozin users.

Conclusion While SGLT2i seem to provide positive effects on the lipid profile, as well as their well-recognized effects on glycemic parameters, there may be value in further evaluating renal safety and the long-term alterations in lipid profile.

Keywords Sodium-glucose cotransporters type 2 inhibitors · Empagliflozin · Dapagliflozin · Type 2 diabetes mellitus · Fasting glucose · Glycated hemoglobin · Lipid · Creatinine

Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease associated with conditions, such as obesity, cardiovascular diseases (CVDs), dyslipidemia and nephropathy [1]. Diabetes has also been shown to increase risks for CVDs and diabetic nephropathy [2, 3]. Therefore, an ideal glucose-lowering drug is expected to not only improve glycemic control, but also have positive effects on weight, blood pressure, dyslipidemia, and also cardiovascular and renal outcomes [4].

Sodium-glucose transport protein 2 (SGLT2) inhibitors (SGLT2i) are the most recent glucose-lowering drugs gaining widespread use in T2DM [5]. They block SGLT2 channels in the proximal renal tubule, thereby preventing reabsorption of glucose [6] and increasing urinary glucose excretion –which reduces plasma glucose regardless of the effects of insulin [4]. Studies have shown that SGLT2i use is associated with greater improvement in glycated hemoglobin (HbA1c) compared to placebo and oral antidiabetics [7]. Glycosuria also causes considerable calorie loss, potential weight loss, and a decrease in blood pressure [4]. Randomized controlled trials have shown that SGLT2i can reduce risks for major adverse cardiovascular events,

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heart failure, and poor renal outcomes [8–10]. It has even been reported that these effects may be partially independent of glucose-lowering activity [11, 12]. Based on these results, current guidelines recommend administration of SGLT2i in patients with T2DM and certain cardiovascular/renal comorbidities (or high risks for these) [2, 3, 13]. However, the mechanisms of cardiorenal protection conferred by SGLT2i still need to be clarified [14]. SGLT2i have also been associated with various adverse effects, including cardiovascular, renal and metabolic adverse consequences [5, 7, 15, 16]. The positive and negative consequences of these agents and their relationships with other factors, including concomitant medications, diabetes duration and timing of SGLT2i initiation, have not been adequately investigated.

The metabolic effects of SGLT2i may change over time and could be affected by other concomitant antidiabetic regimens, duration of diabetes, and SGLT2i initiation time. In this context, our aim was to investigate and compare the two SGLT2i medications marketed in our country (dapagliflozin and empagliflozin) by examining longitudinal changes (3 and 6 months) in glucose metabolism, lipid profile, renal functions and serum electrolyte levels. The relationship of these changes with concomitant antidiabetic regimens was also examined.

Materials and methods

Study design

A total of 250 patients diagnosed with type 2 diabetes mellitus, who applied to the diabetes outpatient clinic between January 2022 and December 2022 and had been started on SGLT2i between these dates, were included in the study. Examinations were planned to be performed at 3 and 6 months, and therefore, those using SGLT2i for less than 6 months at the end of the study period were excluded from the study. Additionally, patients using calcium channel blockers, diuretics or statins were excluded. The use of these drugs in baseline or during the 6-month period of the study was considered as exclusion criteria. Whether a statin indication occurred during SGLT2i treatment was evaluated according to the 2019 European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines [17]. Accordingly, patients with low risk according to the total cardiovascular risk score (SCORE) and low-density lipoprotein-cholesterol (LDL-C) < 116 mg/dL, patients with moderate risk and LDL-C < 100 mg/dL, patients with high risk and LDL-C < 70 mg/dL, and patients with very high risk and LDL-C < 55 mg/dL did not receive antihyperlipidemic therapy.

Data collection

This longitudinal retrospective study was carried out in the Department of Internal Medicine of Dr. Sadi Konuk Training and Research Hospital. All procedures agreed with the ethical standards of the institutional research committee and with the Helsinki declaration and its later amendments. The study plan and procedures were evaluated and approved by the Ethics Committee of Dr. Sadi Konuk Training and Research Hospital (Decision date: 21.02.2022, decision no: 2022–04-14).

Participants' data included in the study were as follows: age, sex and comorbidities, duration of T2DM, concomitant diabetes medication used with SGLT2i, time between onset of SGLT2i and diagnosis of T2DM, type of SGLT2i (dapagliflozin or empagliflozin), angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blocker (ARB) use, laboratory results (detailed below) and were retrieved from the computerized registry of the hospital and patient charts. The information about whether the patients used calcium channel blockers, diuretics or statins at the beginning or during the study was obtained from both the hospital records and the records by the Ministry of Health of the Republic of Turkey.

Laboratory analysis

Laboratory results immediately before SGLT2i initiation (baseline), and 3 months and 6 months after SGLT2i initiation, which were measured in the routine follow-up examination of T2DM patients, including blood fasting glucose, creatinine, urea, serum sodium, potassium and calcium, HbA1c, LDL-C, high density lipoprotein cholesterol (HDL-C), triglyceride levels and urine albumin-to-creatinine ratio (ACR) were examined. All samples were taken in accordance with international standards and measurements were performed in the certified local biochemistry laboratory with calibrated devices (Roche COBAS Integra 800; Roche Diagnostics Corporation, USA) and commercial test kits, according to manufacturer recommendations.

Patients management

Diabetes diagnoses were made and therapeutic decisions (indications and doses of SGLT2i, metformin and insulin) were based on the recommendations of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) [1, 3]. The patients were divided into 2 groups as those using dapagliflozin ($n = 49$) and empagliflozin ($n = 201$) and compared in terms of changes in laboratory parameters at baseline, 3 months and 6 months. Based

on concomitant therapies, patients were also divided into 4 groups: those not using any additional medication ($n=32$), those using metformin ($n=184$), those using metformin and basal insulin ($n=16$), and those using metformin, basal insulin, and bolus insulin ($n=18$) and these four groups were compared in terms of changes in laboratory parameters at baseline, 3 months, and 6 months later.

ACEi or ARB indications of the patients before and after SGLT2 initiation were determined according to the ESC and EAS guidelines [18, 19].

Statistical analysis

Statistical analyses, with significance denoted by $p<0.05$ values, were conducted using IBM SPSS, Version 21.0 (IBM, NY, USA). Continuous variables were evaluated for the absence of normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Mean ± standard deviation values were used to summarize continuous variables, while frequency (percentage) values were used for categorical variables. Repeated measurements were compared using Wilcoxon test or Friedman's test. Two-group comparisons utilized the Mann–Whitney U test, and comparisons involving more than two groups employed the Kruskal–Wallis test. Post-hoc analysis was adjusted with Bonferroni correction.

Table 1 Summary of patients' characteristics

Age (years)	55.4 ± 9.6
Sex	
Female	116 (46.4%)
Male	134 (53.6%)
Other comorbidities	
Hypertension	135 (54.0%)
Hyperlipidemia	128 (51.2%)
Coronary artery disease	58 (23.2%)
Congestive heart failure	7 (2.8%)
Chronic renal failure	6 (2.4%)
Duration of diabetes mellitus (years)	9.12 ± 6.35
SGLT2i start time after diagnosis (years)	7.38 ± 6.09
Type of SGLT2i	
Dapagliflozin	49 (19.6%)
Empagliflozin	201 (80.4%)
Other antidiabetics use	
None	32 (12.8%)
Metformin	184 (73.6%)
Metformin + Basal insulin	16 (6.4%)
Metformin + Basal insulin + Bolus insulin	18 (7.2%)
ACE inhibitors/ARB use	98 (39.2%)

Data are given as mean ± standard deviation for continuous variables and as frequency (percentage) for categorical variables

ACE Angiotensin converting enzyme inhibitor, *ARB* Angiotensin receptor blocker, *SGLT2i* Sodium-glucose cotransporters type 2 inhibitors

Results

The mean age of the patients was 55.4 ± 9.6, with 53.6% ($n=134$) being male. The mean duration of T2DM was 9.12 ± 6.35 years, while the mean time until SGLT2i initiation after T2DM diagnosis was 7.38 ± 6.09 years. Forty-nine (19.6%) patients used dapagliflozin and 201 (80.4%) used empagliflozin. Comorbidities and other drug uses are summarized in Table 1.

Laboratory parameter changes at 3 months and 6 months compared to baseline are summarized in Table 2. Glucose, HbA1c, and triglyceride levels measured 3 and 6 months after treatment were significantly lower than baseline, while serum sodium and HDL-C levels were significantly higher ($p<0.001$ for all). Levels of creatinine ($p=0.024$) and serum potassium ($p=0.028$) measured 6 months after treatment were significantly higher than baseline, while levels of LDL-C ($p<0.001$) and urine ACR ($p<0.001$) were significantly lower. Glucose, HbA1c, LDL-C, triglyceride and urinary ACR levels measured 6 months after treatment were significantly lower ($p<0.001$ for all), while serum potassium levels were significantly higher ($p=0.028$) compared to those 3 months after treatment. When compared based on SGLT2i type, patients using empagliflozin had significantly higher creatinine 3 months later ($p=0.048$) and serum sodium 6 months later ($p=0.020$), and significantly lower HbA1c levels 6 months later ($p=0.024$) than dapagliflozin users (Table 3).

Patients using metformin & basal insulin with SGLT2i had significantly higher baseline glucose levels (before metformin & basal insulin treatment) than those using metformin alone with SGLT2i (before metformin treatment) ($p=0.016$). Patients using metformin & basal insulin and those using metformin & basal insulin & bolus insulin with SGLT2i had significantly higher HbA1c levels at 6″ months ($p=0.003$) compared to those who were only receiving metformin as concomitant medication. HbA1c levels at 6 months were significantly lower in metformin & SGLT2i users compared to those receiving only SGLT2i ($p=0.003$). HbA1c values after 6 months decreased significantly in all 4 groups. Except for the Metformin + basal insulin + Bolus insulin group, there were significant decreases in glucose and LDL-C levels of the other 3 groups compared to baseline and 6 months later. A significant increase in creatinine ($p=0.023$), sodium ($p<0.001$) and potassium ($p=0.043$) levels was seen in the metformin group after 6 months. In the only SGLT2i group, urea levels decreased significantly after 6 months ($p=0.032$). There was a significant increase in HDL-C levels and a significant decrease in urinary ACR levels after 6 months in those using only SGLT2i ($p=0.043$)

Table 2 Summary of laboratory measurements of all patients with regard to time

	Time			
	Baseline	3rd month	6th month	*p*
Glucose (mg/dL)	215.60 ± 83.46	171.44 ± 63.49*	154.42 ± 56.06*#	**< 0.001**
Creatinine (mg/dL)	0.78 ± 0.23	0.79 ± 0.22	0.80 ± 0.25*	**0.024**
Urea (mg/dL)	31.21 ± 9.15	31.43 ± 9.87	31.68 ± 12.70	0.071
Serum sodium (mEq/L)	137.04 ± 4.26	137.87 ± 8.35*	138.53 ± 2.63*	**< 0.001**
Serum potassium (mEq/L)	4.52 ± 0.46	4.53 ± 0.38	4.59 ± 0.38*#	**0.028**
Serum calcium (mg/dL)	9.46 ± 0.55	9.42 ± 0.58	9.47 ± 0.56	0.104
HbA1c (%)	9.18 ± 1.87	8.05 ± 1.59*	7.69 ± 1.36*#	**< 0.001**
LDL-C (mg/dL)	121.79 ± 43.40	117.17 ± 39.01	104.96 ± 39.47*#	**< 0.001**
HDL-C (mg/dL)	45.29 ± 14.97	46.34 ± 18.52*	46.55 ± 13.69*	**0.001**
Triglyceride (mg/dL)	223.97 ± 152.10	184.47 ± 113.68*	165.69 ± 81.35*#	**< 0.001**
Urine ACR (mg/g)	98.53 ± 330.16	68.12 ± 238.00	53.23 ± 169.80*#	**< 0.001**

Data are given as mean ± standard deviation

*: Significantly different from Baseline, #: Significantly different from 3rd month

ACR Albumin-to-creatinine ratio, *HbA1c* Glycated hemoglobin, *HDL-C* High density lipoprotein cholesterol, *LDL-C* Low density lipoprotein cholesterol

and in those using metformin in addition to SGLT2i ($p = 0.004$). There was a significant decrease in triglyceride levels in the metformin ($p < 0.001$) and metformin + basal ($p = 0.006$) insulin groups after 6 months (Table 4).

Discussion

The main findings of this study demonstrate that, SGLT2i decreased glucose, HbA1c, LDL-C, triglyceride and urine ACR levels, and significantly increased creatinine, serum sodium and HDL-C levels over time. Compared to dapagliflozin, empagliflozin increased creatine at 3 months and serum sodium at 6 months more, and decreased HbA1c at 6 months more. Other diabetes treatment regimens used concomitantly with SGLT2i had no significant and reasonable differences on laboratory variables other than HbA1c.

Clinical studies have shown that SGLT2i improve both glucose and HbA1c levels in comparison to placebo and other oral antidiabetic drugs [7]. The present study did not include a placebo group or a comparison group (using only other antidiabetics), but it was observed that there was a significant decrease in glucose and HbA1c levels at 6 months after starting SGLT2i. HbA1c levels 6 months after starting empagliflozin were significantly lower compared to dapagliflozin recipients, although there was no difference at baseline. In a meta-analysis of 12 randomized controlled trials, it was demonstrated that SGLT2i use was associated with a greater reduction in HbA1c in comparison to oral antidiabetics. While there was no difference in HbA1c reduction between SGLT2i and metformin, SGLT2i have been shown to provide a greater HbA1c reduction effect compared to sulfonylurea and dipeptidyl peptidase 4 inhibitors. [7]. Although empagliflozin resulted in a significantly greater HbA1c reduction than dapagliflozin in the current study, some limitations should be taken into account, such as the relatively small sample size, retrospective design, significant number differences between groups, and the exclusion of the weight factor, which is an important factor that may affect HbA1c levels.

Diabetic nephropathy, a leading cause of end-stage renal disease, is associated with increased morbidity and mortality in diabetes and independently elevates the risk of adverse cardiac outcomes [20]. The basic pathophysiology of diabetic nephropathy includes inflammation and fibrosis caused by glomerular hyperfiltration [21, 22]. The CREDENCE ("The Canagliflozin and Renal Endpoints in Diabetes with Established Nephropathy Clinical Evaluation") trial included approximately 4500 patients with T2DM and chronic renal failure with proteinuria and was the first renal outcome trial of SGLT2i. It demonstrated renoprotection (30% reduction in adverse renal outcomes) and cardiovascular protection [8]. The superiority of SGLT2i over placebo in preventing renal deterioration was also demonstrated in the EMPA-REG OUTCOME ("Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients") study [23]. This study also demonstrated that empagliflozin reduced the risk of composite renal outcomes including progression to macroalbuminuria, doubling of serum creatinine, initiation of renal therapy, and death from kidney disease, by 39% [23]. The DECLARE-TIMI 58 ("Dapagliflozin Effect on CardiovascuLAR Events – Thrombolysis in Myocardial Infarction 58") trial reported lower rates of renal combined outcome by treatment with dapagliflozin compared to placebo [24]. Similar results were reported for canagliflozin in the CANVAS ("Canagliflozin Cardiovascular Assessment Study") [8,

Table 3 Summary of laboratory measurements with regard to time and type of SGLT2 inhibitor

	Type of SGLT2 inhibitor		
	Dapagliflozin ($n=49$)	Empagliflozin ($n=201$)	p
Glucose (mg/dL)			
Baseline	222.81 ± 84.95	213.85 ± 83.21	0.570
3rd month	194.80 ± 86.67	165.75 ± 55.22	0.057
6th month	161.69 ± 47.97	152.65 ± 57.83	0.094
Creatinine (mg/dL)			
Baseline	0.77 ± 0.29	0.78 ± 0.22	0.121
3rd month	0.74 ± 0.22	0.80 ± 0.23	**0.048**
6th month	0.77 ± 0.28	0.81 ± 0.24	0.114
Urea (mg/dL)			
Baseline	31.08 ± 8.83	31.29 ± 9.56	0.265
3rd month	31.99 ± 9.49	31.30 ± 9.97	0.636
6th month	32.46 ± 20.27	31.49 ± 10.08	0.453
Serum sodium (mEq/L)			
Baseline	136.57 ± 3.24	137.16 ± 4.47	0.105
3rd month	135.53 ± 18.17	138.44 ± 2.43	0.793
6th month	137.92 ± 2.34	138.68 ± 2.68	**0.020**
Serum potassium (mEq/L)			
Baseline	4.55 ± 0.45	4.51 ± 0.46	0.910
3rd month	4.54 ± 0.40	4.52 ± 0.37	0.764
6th month	4.55 ± 0.41	4.59 ± 0.38	0.249
Serum calcium (mg/dL)			
Baseline	9.54 ± 0.57	9.45 ± 0.54	0.490
3rd month	9.51 ± 0.44	9.40 ± 0.60	0.466
6th month	9.49 ± 0.48	9.46 ± 0.58	0.829
HbA1c (%)			
Baseline	9.40 ± 2.19	9.12 ± 1.79	0.583
3rd month	8.36 ± 2.05	7.97 ± 1.45	0.270
6th month	8.04 ± 1.38	7.61 ± 1.34	**0.024**
LDL-C (mg/dL)			
Baseline	122.23 ± 34.00	121.68 ± 45.47	0.434
3rd month	113.52 ± 36.12	118.06 ± 39.71	0.799
6th month	101.52 ± 40.53	105.80 ± 39.26	0.693
HDL-C (mg/dL)			
Baseline	43.08 ± 8.40	45.82 ± 16.12	0.399
3rd month	44.35 ± 8.62	46.83 ± 20.20	0.765
6th month	45.66 ± 9.92	46.77 ± 14.48	0.876
Triglyceride (mg/dL)			
Baseline	235.92 ± 160.79	221.05 ± 150.18	0.457
3rd month	195.16 ± 145.54	181.86 ± 104.74	0.669
6th month	171.49 ± 71.18	164.28 ± 83.74	0.265
Urine ACR (mg/g)			
Baseline	85.00 ± 240.92	101.83 ± 348.89	0.559
3rd month	61.17 ± 153.93	69.81 ± 254.60	0.827
6th month	53.31 ± 148.32	53.21 ± 174.97	0.623

Data are given as mean ± standard deviation

ACR Albumin-to-creatinine ratio, *HbA1c* Glycated hemoglobin, *HDL-C* High density lipoprotein cholesterol, *LDL-C* Low density lipoprotein cholesterol, *SGLT2* Sodium-glucose cotransporters type 2

10]. The DAPA-CKD ("The Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease") study investigating a larger population also demonstrated the renoprotective effects of dapagliflozin in chronic kidney disease patients with or without T2DM [25].

Some researchers have claimed that intensive glucose-lowering therapies can cause harm, resulting in questions surrounding the safety of SGLT2i [16, 26, 27]. Although the results of above mentioned trials do not show increased risks for acute kidney injury [8, 23, 24, 28] with SGLT2i use, there are few studies that have emphasized acute kidney injury [16], thereby demonstrating the need for further studies. Nonetheless, the long-term renoprotective effects of SGLT2i have been associated with reduced transglomerular pressure, similar to agents that block the renin–angiotensin–aldosterone axis [5, 16]. However, SGLT2i can sometimes cause a decline in kidney function and acute renal injury. In the present study, SGLT2i significantly increased serum sodium levels 3 months after baseline, and significantly decreased urinary ACR levels, as well as increased creatinine, serum sodium and serum potassium levels at 6 months relative to baseline. SGLT2i also significantly decreased urinary ACR levels and significantly increased serum potassium levels at 6 months compared to 3 months. The effect of empagliflozin on increasing creatinine at 3 months and serum sodium at 6 months was significantly higher than dapagliflozin. Based on the results of a systemic review, STLG2i were associated with a 0.60 μmol/L increase in creatinine compared to placebo [7]. In terms of creatinine change, similar results were found for SGLT2i and metformin or dipeptidyl peptidase 4 inhibitors [7]. The present study investigated the effect of SGLT2i on serum creatinine and electrolyte levels and how these effects changed over 3-month intervals, contributing to a lack of literature in this regard. We recommend checking serum creatinine, sodium and potassium levels at least every 3 months in patients using SGLT2i. It should be remembered that increases in creatinine and potassium levels may occur after 6 months of use.

Recently, the United States Food and Drug Administration reported cases of acute kidney injury in 101 patients treated with SGLT2i –all receiving dapagliflozin or canagliflozin. The fact that many of these patients required hospitalization and renal replacement therapy has raised concerns [23]. In another meta-analysis, both dapagliflozin and canagliflozin caused increased risk of compound renal events compared to the control group, whereas renoprotective outcomes were reported for empagliflozin. Both canagliflozin and dapagliflozin were associated with a tendency to increase the risk of acute renal failure compared to controls, but these results were not significant. Conversely, empagliflozin has also been associated with a significant reduction in the risk of acute renal failure [29]. Szalat et al. reported several cases of acute kidney injury that may be associated with initiation

Table 4 Summary of laboratory measurements with regard to time and other antidiabetics use

	Other antidiabetics use				
	None ($n=32$)	Metformin ($n=184$)	Metformin + Basal insulin ($n=16$)	Metformin + Basal insulin + Bolus insulin ($n=18$)	p
Glucose (mg/dL)					
Baseline	214.56 ± 73.80	208.44 ± 80.23	270.13 ± 105.45	242.19 ± 94.27	**0.016**
6th month	144.62 ± 37.33	151.60 ± 50.39	156.99 ± 63.04	198.42 ± 101.61	0.136
p	**< 0.001**	**< 0.001**	**0.001**	0.102	
Creatinine (mg/dL)					
Baseline	0.78 ± 0.27	0.78 ± 0.22	0.81 ± 0.24	0.77 ± 0.30	0.815
6th month	0.81 ± 0.31	0.81 ± 0.24	0.84 ± 0.27	0.72 ± 0.19	0.427
p	0.050	**0.023**	0.569	0.758	
Urea (mg/dL)					
Baseline	30.21 ± 8.95	31.11 ± 9.43	28.31 ± 8.18	27.98 ± 7.83	0.190
6th month	28.94 ± 10.54	32.15 ± 13.65	32.31 ± 7.41	31.24 ± 9.48	0.557
p	**0.032**	0.058	0.955	0.058	
Serum sodium (mEq/L)					
Baseline	137.83 ± 2.35	136.84 ± 4.68	136.98 ± 2.88	137.76 ± 3.29	0.637
6th month	138.85 ± 2.75	138.56 ± 2.49	137.90 ± 3.96	138.27 ± 2.62	0.760
p	0.075	**< 0.001**	0.096	0.678	
Serum potassium (mEq/L)					
Baseline	4.51 ± 0.37	4.54 ± 0.41	4.59 ± 0.24	4.24 ± 0.96	0.532
6th month	4.59 ± 0.38	4.60 ± 0.38	4.55 ± 0.33	4.41 ± 0.41	0.321
p	0.399	**0.043**	0.950	0.571	
Serum calcium (mg/dL)					
Baseline	9.41 ± 0.49	9.47 ± 0.55	9.45 ± 0.53	9.49 ± 0.66	0.853
6th month	9.46 ± 0.34	9.48 ± 0.61	9.53 ± 0.52	9.35 ± 0.48	0.443
p	0.673	0.361	0.552	0.552	
HbA1c (%)					
Baseline	9.31 ± 1.32	9.04 ± 1.97	9.75 ± 1.84	9.88 ± 1.49	0.051
6th month	8.08 ± 0.99	7.51 ± 1.26	8.31 ± 1.87	8.39 ± 1.95	**0.003**
p	**< 0.001**	**< 0.001**	**0.009**	**0.015**	
LDL-C (mg/dL)					
Baseline	115.93 ± 34.25	121.15 ± 45.87	132.85 ± 28.41	128.87 ± 42.91	0.419
6th month	96.91 ± 38.38	104.45 ± 40.00	103.33 ± 29.23	125.95 ± 39.52	0.100
p	**0.007**	**< 0.001**	**0.012**	0.557	
HDL-C (mg/dL)					
Baseline	46.62 ± 15.32	45.40 ± 15.63	41.89 ± 9.01	44.87 ± 11.75	0.845
6th month	51.31 ± 23.07	46.29 ± 11.87	43.61 ± 10.64	43.36 ± 9.77	0.494
p	**0.043**	**0.004**	0.754	0.256	
Triglyceride (mg/dL)					
Baseline	211.38 ± 164.87	222.38 ± 138.46	282.68 ± 277.07	210.33 ± 106.84	0.623
6th month	166.22 ± 72.22	163.77 ± 83.41	143.00 ± 38.57	204.56 ± 95.32	0.190
p	0.096	**< 0.001**	**0.006**	0.981	
Urine ACR (mg/g)					
Baseline	145.70 ± 505.44	89.04 ± 305.16	83.59 ± 98.92	125.04 ± 336.94	0.492
6th month	29.18 ± 63.15	51.11 ± 172.31	148.71 ± 307.52	32.75 ± 42.38	0.394
p	**0.001**	**< 0.001**	0.569	0.231	

Data are given as mean ± standard deviation

*: Significantly different from None group, #: Significantly different from Metformin group

ACR Albumin-to-creatinine ratio, *HbA1c* Glycated hemoglobin, *HDL-C* High density lipoprotein cholesterol, *LDL-C* Low density lipoprotein cholesterol

of SGLT2i therapy, including empagliflozin [16]. According to Szalat et al. [16], there were three possible causes of this damage: (i) effective volume depletion due to excessive diuresis; (ii) loss of trans-glomerular pressure in patients receiving renin–angiotensin–aldosterone blockade; (iii) renal medullary hypoxic injury. However, the role of these mechanisms has not been adequately explored due to the conflicting results of studies on acute kidney injury potentially caused by SGLT2i treatment. According to the results of the present study, the detrimental effect of SGLT2i on kidney and blood electrolytes seems to increase with time, and these effects seem to be more pronounced in empagliflozin. Also, these effects do not seem to be affected by other concomitant antidiabetic treatment regimens, duration of diabetes mellitus and SGLT2i initiation time after diagnosis. However, available data regarding the kidney safety of SGLT2i are conflicting and limited. Further information on the renal damage or protective properties of SGLT2i will be provided by clinical trials designed to evaluate outcomes in large populations.

The cardioprotective effects of SGLT2i have been demonstrated in many randomized controlled trials. In the EMPA-REG OUTCOME study of 7020 patients with T2DM and CVD, Zinman et al. showed that the composite incidence of major adverse cardiovascular events was lower in patients using empagliflozin compared to placebo [9]. The CANVAS trial compared the results of canagliflozin and placebo in 10,142 patients with T2DM and high cardiovascular risk. Patients treated with canagliflozin had a lower risk of cardiovascular events than those treated with placebo [10]. On the other hand, other meta-analyses showing no effects on cardiovascular events, death, and major safety outcomes have been published [7, 30]. These conflicting results may be due to the uncertain effects of SGLTi on lipid profile. In this study, we also investigated the temporal changes of SGLTi on lipid parameters. SGLT2i significantly decreased triglyceride levels and significantly increased HDL-C levels 3 months after baseline, and significantly decreased LDL-C and triglyceride levels and significantly increased HDL-C levels at 6 months. In a meta-analysis, SGLT2i were associated with increased HDL-C and LDL-C and decreased triglyceride compared with placebo. SGLT2i increased HDL-C and LDL-C compared to sulfonylurea treatment and dipeptidyl peptidase 4 inhibitors, but did not cause lower levels of triglycerides [7]. Other studies have also shown that SGLT2i increase HDL-C and LDL-C levels [9, 31, 32]. In a randomized placebo-controlled trial, dapagliflozin was not found to cause any alterations in lipid-related parameters, including HDL subfractions, cholesterol efflux, enzymes mediating the antioxidant role of HDL (PON1 and ARE), when compared to placebo [33]. In an experimental study using a mouse model to examine the effects of SGLT2 inhibition on plasma lipoprotein metabolism, SGLT2 inhibition was demonstrated to increase circulating LDL-C and reduce plasma triglycerides. Also, SGLT2 inhibition was shown to delay LDL turnover [15]. According to the current findings, SGLT2i seem to have increasing positive effects on lipid profile over time, and these effects seem to be independent of SGLT2i type, other medications, time with disease, and SGLT2i initiation time. Comprehensive studies are needed to demonstrate the precise effects of SGLT2i on lipid profile and to assess long-term effects.

Limitations

This was a single-center study and has relatively few participants compared to previously published comprehensive randomized controlled trials. This limits the generalizability of the results. As it is a retrospective study, the effect of SGLT2i on long-term outcomes and other laboratory parameters could not be investigated. In addition, due to the retrospective design, the weight factor, which is an important factor that may affect HbA1c levels, was not included in the study. A placebo control group or a comparison group using only other antidiabetics could not be created and included in the study. Differences in numbers between patients using dapagliflozin and empagliflozin may have affected the statistical results. The differences in these numbers were a natural consequence of following the recommendations of the ADA and the European Association for the Study of Diabetes. The sample size was not computed to compare the two types of inhibitors in the study, and the sample sizes in the two groups are unequal, caution should be exercised while drawing inferences from this comparison. Furthermore, as the study already has a retrospective nature, the probability of selection bias risk is very low. Finally, since canagliflozin was not marketed in Turkey at the time of the study, the results of this agent could not be included.

Conclusion

In conclusion, SGLT2i significantly decreased glucose and HbA1c over time, and this positive effect was more pronounced in empagliflozin. SGLT2i increased creatinine, serum sodium, and serum potassium over time and decreased urinary ACR levels, and the impact on sodium was more pronounced in empagliflozin recipients. SGLT2i significantly increased HDL-C levels, while significantly decreasing LDL-C and triglyceride levels over time. While SGLT2i seems to provide positive effects on the lipid profile in addition to its positive glycemic effects in patients with T2DM, it would be useful to review it in terms of renal safety and to perform further trials examining their effects on lipid profile. For this, more comprehensive studies are required.

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Declarations

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. The study was approved by the Ethics Committee of Dr. Sadi Konuk Training and Research Hospital (Decision date: 21.02.2022, decision no: 2022–04-14).

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Relationship between impaired sensitivity to thyroid hormones and MAFLD with elevated liver enzymes in the euthyroid population

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Abstract

Objective There is a delicate interplay between thyroid hormones and thyrotropin (TSH) and metabolic homeostasis. However, the role of thyroid hormone sensitivity in metabolic health, particularly in relation to metabolic dysfunction-associated fatty liver disease (MAFLD) and associated complications such as elevated liver enzymes and free fatty acid (FFAs) has not been elucidated in euthyroid populations.

Methods A total of 3929 euthyroid adults from the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University were included in this study. Thyroid hormone sensitivity indices were calculated by thyroid feedback quantile-based index (TFQI), TSH index (TSHI) and thyrotropin thyroxine resistance index (TT4RI). Associations between thyroid hormones sensitivities and risk of MAFLD, MAFLD with elevated liver enzymes, MAFLD with elevated FFAs were assessed with logistic regression.

Results After adjustment for multiple risk factors, adjusted odds ratio (AOR) of the fourth versus the first $TFQI_{FT4}$ quartile for MAFLD, MAFLD with elevated liver enzymes, and MAFLD with elevated FFAs were 1.778 (95% CI 1.378, 2.293), 1.466 (1.105, 1.945), and 1.936 (1.479, 2.534), respectively (all $p<0.001$). Per 1 SD in $TFQI_{FT4}$, ORs increased 2.27 (95% CI 1.74, 2.97) for MAFLD, 2.05 (1.51, 2.78) for MAFLD with elevated liver enzymes, and 2.43 (1.82, 3.24) for MAFLD with elevated FFAs. The other sensitivity to thyroid hormones indices showed similar associations for MAFLD and MAFLD with elevated liver enzymes.

Conclusions These findings have important implications for understanding the role of thyroid hormone sensitivity in metabolic health, particularly in relation to MAFLD and associated complications such as elevated liver enzymes and FFAs. $TFQI_{FT4}$, $TFQI_{FT3}$, TSHI and TT4RI can be used as new indicators for predicting MAFLD and MAFLD with elevated liver enzymes.

Keywords Sensitivity to thyroid hormones · Metabolic dysfunction-associated fatty liver disease · Free fatty acids · Elevated liver enzymes

Introduction

In 2020, an international panel of hepatologists proposed a novel terminology, metabolic dysfunction-associated fatty liver disease (MAFLD) [1]. The overall prevalence of MAFLD worldwide was estimated to be 38.77% [2]. MAFLD was proposed as a better definition of nonalcoholic fatty liver disease (NAFLD) to encompass the metabolic dysregulation associated with NAFLD. Metabolic comorbidities associated with MAFLD included obesity, type 2 diabetes (T2DM), hyperlipidemia, hypertension, and metabolic syndrome [3]. The pathogenesis of NAFLD is complex, involving insulin resistance, oxidative stress, lipid peroxidation, and mitochondrial dysfunction [4]. High levels of free fatty acids (FFAs) in hepatocytes result in an imbalanced fatty acid metabolism and can cause mitochondrial dysfunction increasing oxidative stress and steatosis [5]. Thyroid hormones stimulate lipolysis from fat stores in white adipose tissue and from dietary fat sources to generate circulating FFAs, which are the major source of lipids for the liver. hyperthyroidism increased triglyceride-derived fatty acid uptake in oxidative tissues such as liver and muscle, whereas hypothyroidism increased triglyceride-derived fatty acid uptake in white adipose tissue and decreased its uptake in liver [6].

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Overt hypothyroidism is significantly associated with NAFLD and is a risk factor that is independent from other known metabolic risk factors [7]. Of note, two studies from 2014 and 2016 demonstrate that serum levels of FT3 and FT4 are inversely associated and that TSH levels are associated with NAFLD in the general population, even among those within the reference range for euthyroid participants [7, 8]. However, the results from a large cohort study in euthyroid patients were inconsistent, higher serum FT3 and lower serum TSH levels were independently related to a higher incidence of MAFLD [9]. These metabolic mechanisms described in clinical thyroid diseases may not be enough to explain the associations found within the normothyroid range.

Laclaustra et al. found that thyroid feedback quantile-based index (TFQI), a new index of central sensitivity to thyroid hormones, was linked with many metabolic disorders in the general population [10]. So far, there has been some research on the association between sensitivity to thyroid hormone indices with the risk of T2DM, hyperlipidemia, hypertension, and metabolic syndrome. Nevertheless, the associations of thyroid hormones sensitivity with FFAs

Table 1 Baseline characteristics of participants with Non-MAFLD and MAFLD

	Overall (3929)	Non-MAFLD (2485)	MAFLD (1444)	*p*
Gender (Men/Women)	1906/2023	1139/1346	767/677	**< 0.001**
Age (years)	59.83 ± 13.49	59.88 ± 14.29	59.76 ± 11.99	0.78
BMI (kg/m^2)	23.84 ± 10.37	22.68 ± 12.49	25.83 ± 4.23	**< 0.001**
SBP (mmHg)	128.14 ± 20.00	125.49 ± 19.97	132.70 ± 19.23	**< 0.001**
DBP (mmHg)	74.76 ± 11.51	73.16 ± 11.57	77.52 ± 10.88	**< 0.001**
Diabetes (%)	1520 (38.7%)	748 (30.1%)	772 (53.5%)	**< 0.001**
Smoking (%)	707 (18.0%)	356 (14.3%)	351 (24.3%)	**< 0.001**
HbA1c (%)	5.9 (5.5, 6.8)	5.6 (5.4, 6.3)	6.2 (5.8, 7.9)	**< 0.001**
FFAs (mmol/L)	0.42 (0.29, 0.59)	0.38 (0.26, 0.56)	0.49 (0.36, 0.65)	**< 0.001**
TC (mmol/L)	4.64 (3.98, 5.38)	4.46 (3.80, 5.20)	4.91 (4.27, 5.68)	**< 0.001**
TG (mmol/L)	1.34 (0.96, 2.03)	1.17 (0.85, 1.67)	1.83 (1.25, 2.72)	**< 0.001**
HDL-C (mmol/L)	1.12 (0.93, 1.36)	1.18 (0.95, 1.42)	1.06 (0.89, 1.26)	**< 0.001**
LDL-C (mmol/L)	2.74 (2.16, 3.32)	2.57 (2.06, 3.15)	2.99 (2.43, 3.55)	**< 0.001**
UA (umol/L)	335.18 (271.56, 418.53)	323.28 (259.00, 402.72)	357.07 (290.99, 436.12)	**< 0.001**
Cr (umol/L)	67.60 (56.34, 81.71)	67.64 (56.52, 82.38)	67.51 (55.72, 80.97)	0.09
AST (U/L)	21.64 (17.59, 27.71)	21.38 (17.45, 27.46)	22.09 (17.84, 28.07)	0.01
ALT (U/L)	18.03 (12.85, 26.72)	15.87 (11.79, 23.54)	22.01 (16.00, 32.02)	**< 0.001**
ALP (U/L)	85.92 (70.49, 106.37)	85.10 (68.90, 105.33)	87.90 (72.50, 108.22)	**< 0.001**
GGT (U/L)	25.43 (17.30, 42.01)	22.60 (15.89, 37.11)	31.72 (21.10, 50.77)	**< 0.001**
T-BIL (umol/L)	12.49 (9.50, 16.76)	12.34 (9.31, 16.76)	12.75 (9.93, 16.79)	0.05
D-BIL (umol/L)	2.76 (1.97, 4.10)	2.73 (1.91, 4.73)	2.80 (2.04, 4.05)	0.10
I-BIL (umol/L)	9.72 (6.94, 13.18)	9.58 (6.90, 13.21)	10.05 (7.01, 13.17)	0.38
FT3 (pg/mL)	3.03 (2.78, 3.28)	2.98 (2.72, 3.23)	3.12 (2.87, 3.36)	**< 0.001**
FT4 (ng/dL)	1.25 (1.14, 1.38)	1.24(1.14, 1.37)	1.26 (1.15, 1.40)	**< 0.001**
TSH (mIU/L)	1.69 (1.14, 2.51)	165 (1.10, 2.44)	1.78 (1.22, 2.64)	**< 0.001**
$TFQI_{FT4}$	-0.08 (-0.26, 0.12)	-0.10 (-0.28, 0.09)	-0.03 (-0.23, 0.16)	**< 0.001**
$TFQI_{FT3}$	-0.06 (-0.23, 0.12)	-0.10 (-0.27, 0.07)	0.01 (-0.16, 0.18)	**< 0.001**
TSHI	2.73 (2.31, 3.12)	2.67 (2.25, 3.08)	2.80 (2.40, 3.20)	**< 0.001**
TT4RI	27.75 (18.51, 40.10)	26.73 (17.77, 38.56)	29.44 (19.88, 42.95)	**< 0.001**

Data were expressed as the mean ± SD or median (upper and lower quartiles) or number (%). *p* values are calculated by t-test and Mann–Whitney tests for continuous variables, Chi-square test for categorical variables. Bold indicates *p* value < 0.01. *MAFLD* metabolic dysfunction-associated fatty liver disease; *BMI* body mass index; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *HbA1c* glycated hemoglobin A1c; *FFAs* free fatty acids; *TG* triglyceride; *TC* total cholesterol; *HDL-C* high-density lipoprotein-cholesterol; *LDL-C* low-density lipoprotein-cholesterol; *UA* uric acid; *Cr* creatinine; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *GGT* gamma glutamyl transpeptidase; *ALP* alkaline phosphatase; *T-BIL* total bilirubin; *D-BIL* direct bilirubin; *I-BIL* indirect bilirubin; *FT3* free triiodothyronine; *FT4* free thyroxine; *TSH* thyroid stimulating hormone; $TFQI_{FT3}$ the thyroid feedback quantile-based index calculated by FT3; $TFQI_{FT4}$ the thyroid feedback quantile-based index calculated by FT4; *TSHI* TSH index; *TT4RI* thyrotropin thyroxine resistance index

and liver enzymes levels remains unclear. Therefore, in this study, we aimed to investigate the relationship between thyroid hormones sensitivity and MAFLD with elevated liver enzymes in the euthyroid population.

Materials and methods

Study design

This retrospective cohort study was approved by the Information Management Organization of the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University. The participants consisted of 3,929 adults (age ≥ 18 years old) who were hospitalised between January 2017 and October 2022. The exclusion criteria were age < 14 years old, missing data, type 1 diabetes mellitus, cancer, acute infection, autoimmune liver diseases, viral hepatitis, cirrhosis of liver, treatment with drugs associated with hepatic steatosis (including glucocorticoids, tamoxifen, amiodarone), thyroid dysfunction, replacement of thyroid hormones and anti-thyroid therapies.

Thyroxine, TSH, and thyroid hormone sensitivity indices

The reference ranges of FT3, FT4, and TSH were 2.3–4.2 pg/L, 0.89–1.8 ng/L, and 0.55–4.78 mU/L, respectively. Four different indices were calculated to evaluate resistance to thyroid hormones. Thyrotropin T4 resistance index (TT4RI) was calculated as FT4 (pmol/L) * TSH (mIU/L) [11]. TSH index (TSHI) was calculated as ln TSH (mIU/L) + 0.1345 * FT4 (pmol/L) [12]. $TFQI_{FT4}$ is achieved by the algorithm TFQI = cumulative distribution function (cdf FT4)—(1—cdf TSH) [10]. In order to investigate the role of FT3 in this index, FT4 in $TFQI_{FT4}$ formulas was replaced with FT3 to obtain $TFQI_{FT3}$. TFQI, TSHI, and TT4RI are central thyroid hormone sensitivity indicators, reflecting the response of the HPT axis to peripheral FT4 changes. The value of TFQI is between -1 and 1. Positivity indicates poor thyroid hormone sensitivity. A value of 0 indicates normal thyroid hormone sensitivity. Negative values indicate good thyroid hormone sensitivity. The higher the TSHI and TT4RI values, the lower the central sensitivity to thyroid hormone.

Other variables

Height and weight were measured by standard methods, and body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m). Blood pressure was measured using OMRON HEM-7130 blood pressure monitor with an appropriately sized cuff. Uric acid (UA), liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT)], and lipid profile measurements [free fatty acid (FFA), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG)] were measured using Automatic biochemical analyser (Cobas c800, Roche, Switzerland). Glycated haemoglobin (HbA1c) was measured using high-performance liquid chromatography (4500MD, AB SCIEX, USA). Elevated FFAs was defined as FFAs ≥ 0.4 mmol/L. Hyperuricemia was defined as a uric acid (UA) level ≥ 420 μmol/L in males and ≥ 360 μmol/L (6.0 mg/dL) in females [13]. Elevated liver enzymes were defined as one or more measurement of ALT or AST > 50 U/L in males and > 35 U/L in females or GGT > 60 U/L in males and > 40 U/L in females [14].

MAFLD Definition

Patients were diagnosed as MAFLD based on abdominal ultrasonography diagnosed hepatic steatosis and the presence of any one of the following three conditions: 1)

Table 2 AOR (95% CI) of MAFLD, elevated AST, elevated ALT, elevated GGT, MAFLD with elevated FFAs, and MAFLD with elevated liver enzymes for 1-SD increase of $TFQI_{FT3}$, $TFQI_{FT4}$, TSHI, and TT4RI

	$TFQI_{FT4}$ (+1 SD)	$TFQI_{FT3}$ (+1 SD)	TSHI (+1 SD)	TT4RI (+1 SD)
MAFLD	2.27 (1.74, 2.97)	3.72 (2.78, 4.98)	1.35 (1.21, 1.51)	1.01 (1.00, 1.01)
Elevated AST	2.97 (1.73, 5.12)	1.06 (0.58, 1.93)	1.37 (1.10, 1.71)	1.01 (1.00, 1.01)
Elevated ALT	2.43 (1.64, 3.60)	1.30 (0.85, 1.98)	1.24 (1.06, 1.46)	1.00 (1.00, 1.00)
Elevated GGT	3.28 (2.43, 4.44)	0.99 (0.72, 1.36)	1.47 (1.30, 1.67)	1.00 (1.00, 1.01)
MAFLD with elevated liver enzymes	2.05 (1.51, 2.78)	3.06 (2.20, 4.24)	1.31 (1.15, 1.48)	1.00 (1.00, 1.01)
MAFLD with elevated FFAs	2.43 (1.82, 3.24)	3.02 (2.21, 4.12)	1.33 (1.18, 1.50)	1.01 (1.00, 1.01)

AOR are estimated with binary logistic regression analysis models adjusted for age, sex, BMI, SBP, DBP, and smoking. *MAFLD* metabolic dysfunction-associated fatty liver disease; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *GGT* gamma glutamyl transpeptidase; *FFAs* free fatty acid; $TFQI_{FT3}$ the thyroid feedback quantile-based index calculated by FT3; $TFQI_{FT4}$ the thyroid feedback quantile-based index calculated by FT4; *TSHI* TSH index; *TT4RI* thyrotropin thyroxine resistance index

overweight or obesity (BMI ≥ 23 kg/m^2 in Asian populations); 2) presence of type 2 diabetes mellitus (T2DM); 3) presence of at least two metabolic abnormalities, including increased waist circumference, arterial hypertension, hypertriglyceridemia, low HDL-C, prediabetes, subclinical inflammation, and insulin resistance [2]. Incident T2DM was defined as a diagnosis of diabetes after discharge and HbA1c ≥ 6.5% or fasting blood glucose ≥ 7.0 mmol/L or random blood glucose ≥ 11.1 mmol/L, or have received insulin or oral hypoglycaemic drug treatment. Notably, waist circumference and insulin resistance were not assessed to identify metabolic dysfunction because of the scarcity of these data in the database of Second Affiliated Hospital of Nanchang University.

Statistical analyses

All analyses were performed using SPSS 26.0 (Chicago, IL, USA) and GraphPad Prism 9.0 (Inc, CA, USA). Normality was examined by the Kolmogorov–Smirnov test. Normally

Table 3 Association of TFQI $_{FT4}$ with MAFLD, elevated FFAs, hyperuricemia, elevated liver enzymes, and MAFLD with elevated liver enzymes in all participants

	TFQI $_{FT4}$				*p*
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
	≥ -1 and < -0.25	≥ -0.25 and < 0	≥ 0 and < 0.25	≥ 0.25 and ≤ 1	
T2DM					
Model 1	1.000 (Reference)	1.230 (1.033, 1.463)	1.421 (1.186, 1.703)	2.324 (1.848, 2.923)	**< 0.001**
MAFLD					
Model 1	1.000 (Reference)	1.271 (1.060, 1.524)	1.666 (1.383, 2.007)	2.069 (1.613, 2.655)	**< 0.001**
Model 2	1.000 (Reference)	1.222 (1.015, 1.470)	1.564 (1.293, 1.891)	1.778 (1.378, 2.293)	**< 0.001**
Elevated FFAs (mmol/L)	0.43	0.46	0.49	0.53	
Model 1	1.000 (Reference)	1.379 (1.172, 1.624)	1.589 (1.338, 1.887)	2.239 (1.782, 2.814)	**< 0.001**
Model 2	1.000 (Reference)	1.347 (1.142, 1.588)	1.520 (1.277, 1.808)	2.010 (1.594, 2.535)	**< 0.001**
Hyperuricemia (umol/L)	342.18	346.24	358.55	382.60	
Model 1	1.000 (Reference)	1.107 (0.922, 1.329)	1.362 (1.129, 1.643)	2.022 (1.605, 2.548)	**< 0.001**
Model 2	1.000 (Reference)	1.127 (0.938, 1.354)	1.406 (1.164, 1.697)	2.172 (1.718, 2.745)	**< 0.001**
Elevated AST (U/L)	26.51	24.89	31.32	30.51	
Model 1	1.000 (Reference)	0.705 (0.463, 1.073)	1.691 (1.158, 2.470)	1.927 (1.209, 3.070)	**< 0.001**
Model 2	1.000 (Reference)	0.697 (0.458, 1.062)	1.661 (1.136, 2.427)	1.831 (1.145, 2.928)	**< 0.001**
Elevated ALT (U/L)	22.98	23.49	30.08	31.71	
Model 1	1.000 (Reference)	0.877 (0.652, 1.179)	1.352 (1.016, 1.799)	1.828 (1.307, 2.556)	**< 0.001**
Model 2	1.000 (Reference)	0.873 (0.649, 1.174)	1.340 (1.007, 1.783)	1.802 (1.285, 2.529)	**< 0.001**
Elevated GGT (U/L)	33.47	37.19	41.30	57.17	
Model 1	1.000 (Reference)	1.153 (0.922, 1.441)	1.627 (1.303, 2.032)	2.420 (1.860, 3.150)	**< 0.001**
Model 2	1.000 (Reference)	1.151 (0.921, 1.439)	1.624 (1.301, 2.027)	2.396 (1.845, 3.112)	**< 0.001**
MAFLD with elevated FFAs					
Model 1	1.000 (Reference)	1.290 (1.049, 1.585)	1.664 (1.350, 2.052)	2.321 (1.786, 3.014)	**< 0.001**
Model 2	1.000 (Reference)	1.229 (0.995, 1.518)	1.539 (1.242, 1.908)	1.936 (1.479, 2.534)	**< 0.001**
Model 3	1.000 (Reference)	1.026 (0.805, 1.307)	1.259 (0.982, 1.614)	1.385 (1.017, 1.887)	0.06
MAFLD with elevated liver enzymes					
Model 1	1.000 (Reference)	1.018 (0.820, 1.265)	1.525 (1.225, 1.897)	1.699 (1.287, 2.242)	**< 0.001**
Model 2	1.000 (Reference)	0.976 (0.783, 1.215)	1.430 (1.147, 1.784)	1.466 (1.105, 1.945)	**< 0.001**
Model 3	1.000 (Reference)	0.930 (0.746, 1.160)	1.345 (1.077, 1.680)	1.322 (0.993, 1.761)	**< 0.01**

AOR are estimated with logistic models. *P* is calculated with the TFQI $_{FT4}$ quartile ordinal as a continuous variable. Bold values emphasized that $p<0.01$. *MAFLD* metabolic dysfunction-associated fatty liver disease; *T2DM* type 2 diabetes; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *GGT* gamma glutamyl transpeptidase; *FFAs* free fatty acid; *TFQI$_{FT4}$* the thyroid feedback quantile-based index calculated by FT4

Model 1: adjusted for sex, age, BMI, SBP, DBP, and smoking

Model 2: further adjusted for HbA1c and T2DM

Model 3: further adjusted for FFAs ≥ 0.4 mmol/L

distributed variables were shown as mean and standard deviation, while skewed-distribution variables were described as median and interquartile range. The difference between two groups was compared by the independent samples T test and the Mann–Whitney U test. Binary logistic regression analysis, adjusted for age, sex, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), and smoking, were used to examine the association of thyroid hormone sensitivity index with MAFLD, MAFLD with elevated liver enzymes. Odds ratios (ORs) for MAFLD, MAFLD with elevated liver enzymes criteria were calculated for 1-SD increase of sensitivity to thyroid hormones indices. We created population-based quartiles for $TFQI_{FT4}$, interquartile comparisons were performed for MAFLD, elevated FFAs, hyperuricemia, elevated AST, elevated ALT, elevated GGT, MAFLD with elevated FFAs, and MAFLD with elevated liver enzymes were modeled with logistic regression. Model 1adjusted for sex, age, BMI, SBP, DBP, and smoking. Model 2 further adjusted for HbA1c, and T2DM. Model 3 further adjusted for FFAs $\geq$ 0.4 mmol/L. To evaluate the performance of the thyroid hormone sensitivity indices, we examined the receiver operating characteristics curves (ROC), which plots sensitivity against 1-specificity, and calculated the cut-points from ROC results. All calculated *p* values were two-sided, and a *p* value <0.05 was considered statistically significant.

Results

Characteristics of the study population

Baseline characteristics of the study population with and without MAFLD are presented in Table 1. The proportion of MAFLD was 36.8%, higher in men than in women ($p<0.001$). Compared with the Non-MAFLD group, the BMI, SBP, DBP, HbA1c, FFAs, TC, TG, LDL-C, UA, AST, ALT, ALP, GGT, FT3, FT4, TSH, $TFQI_{FT4}$, $TFQI_{FT3}$, TSHI and TT4RI levels were significantly higher in the MAFLD group, while the HDL-C levels were significantly lower in the MAFLD group. In addition, participants in MAFLD groups were prone to have higher proportion of T2DM and smoking ($p<0.001$). No difference was found in age, creatinine, T-BIL, D-BIL, and I-BIL levels.

Association of sensitivity to thyroid hormones with MAFLD and MAFLD with elevated liver enzymes

The AOR of MAFLD and MAFLD with elevated liver enzymes for each one SD increase in $TFQI_{FT4}$ in the euthyroid population was presented in Table 2. Per 1 SD in $TFQI_{FT4}$, ORs increased 2.27 (95% CI 1.74, 2.97) for

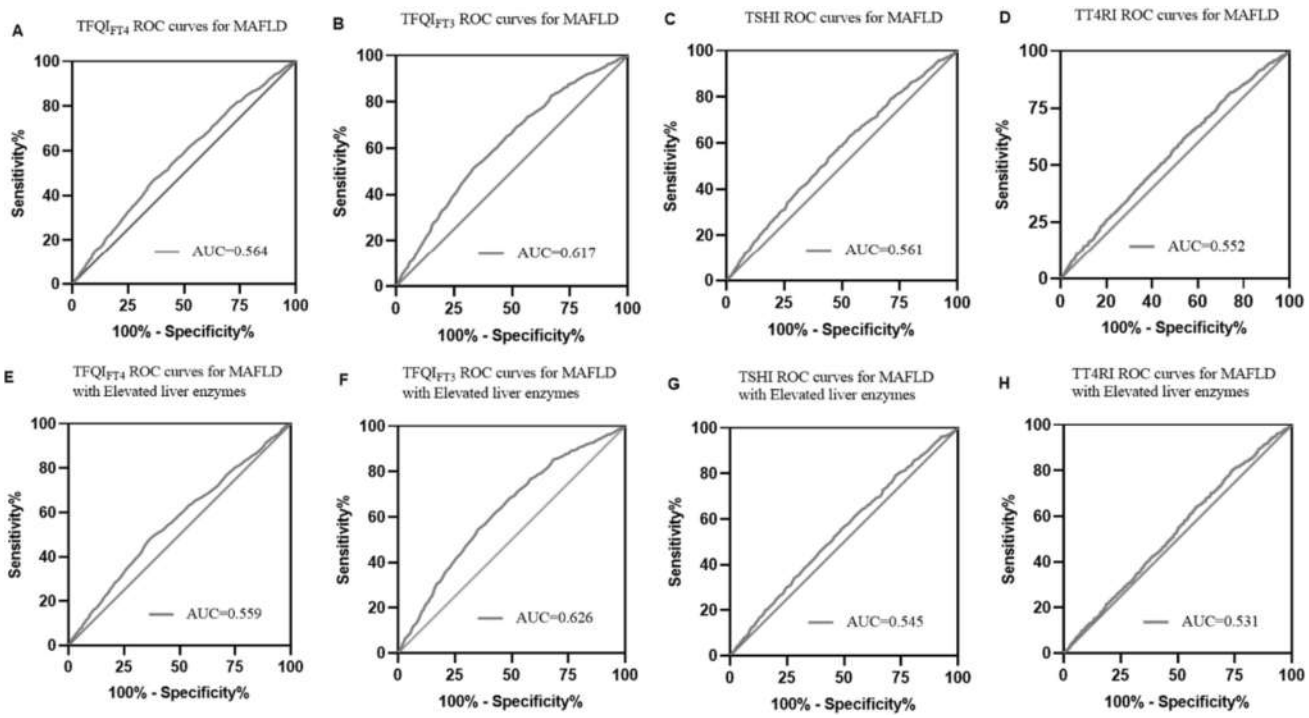

Fig. 1 ROC curves for optimal cut-points of $TFQI_{FT4}$, $TFQI_{FT3}$, TSHI, and TT4RI. (**A-D**) ROC curve for MAFLD from TFQIFT4, TFQIFT3, TSHI, and TT4RI. (**E–H**) ROC curve for MAFLD with elevated liver enzymes from TFQIFT4, TFQIFT3, TSHI, and TT4RI

MAFLD, 2.05 (1.51, 2.78) for MAFLD with elevated liver enzymes, and 2.43 (1.82, 3.24) for MAFLD with elevated FFAs. $TFQI_{FT3}$, TT4RI, and TSHI showed similar associations for MAFLD and MAFLD with elevated liver enzymes.

MAFLD prevalence, T2DM prevalence, MAFLD with elevated FFAs prevalence, MAFLD with elevated liver enzymes prevalence, elevated FFAs, hyperuricemia, elevated AST, elevated ALT, and elevated GGT were progressively higher across $TFQI_{FT4}$ quartiles after adjustment for sex, age, BMI, SBP, DBP, and smoking (all $p<0.001$). AOR of the fourth versus the first $TFQI_{FT4}$ quartile for MAFLD and MAFLD with elevated liver enzymes were 2.069 (95% CI 1.613, 2.655) and 1.699 (1.287, 2.242), respectively, and the association was independent of T2DM and HbA1c, given that after adjustment, AOR estimations were only partially reduced to 1.778 (1.378, 2.293) and 1.466 (1.105, 1.945), respectively (all $p<0.001$). For participants with MAFLD with elevated liver enzymes, the association with $TFQI_{FT4}$ remained statistically significant even after adjustment for FFAs ≥ 0.4 mmol/L ($p<0.01$) (Table 3). The results were similar in examination of $TFQI_{FT3}$, TT4RI, and TSHI instead of $TFQI_{FT4}$ (Supplementary Data).

ROC Curves for Optimal Cut-Points of thyroid hormone sensitivity indices

Figure 1 showed that $TFQI_{FT3}$ performed better than $TFQI_{FT4}$, TSHI, and TT4RI on ROC analyses for MAFLD prediction (area under ROC curve 0.617, $p<0.001$; 0.564, $p<0.001$; 0.561, $p<0.001$; 0.552, $p<0.001$, respectively). The optimal cut-points of $TFQI_{FT3}$ for MAFLD and MAFLD with elevated liver enzymes prediction were -0.002 and -0.079. Although $TFQI_{FT3}$ also performed better than $TFQI_{FT4}$, TSHI, and TT4RI on ROC analyses for MAFLD with elevated liver enzymes prediction, the area under ROC curve is relatively small (area under ROC curve 0.626, $p<0.001$; 0.559, $p<0.001$; 0.545, $p<0.001$; 0.531, $p<0.001$, respectively).

Discussion

As far as we are aware, we are the first to adopt MAFLD diagnostic criteria established by international expert consensus to evaluate the association between impaired sensitivity to thyroid hormones with the risk of MAFLD and MAFLD with elevated liver enzymes. MAFLD is not just a liver disease but rather one component of a multi-faceted, multi-organ collection of diseases driven by complex gene-environment interactions by a myriad of signals. Including chronic metabolic inflammation, endothelial dysfunction, intestinal dysbiosis, and hepatic or systemic insulin resistance. Other well described drivers include altered lipid metabolism; dysregulated production or secretion of adipokines, cytokines, and hepatokines; increased oxidative stress; platelet activation; and other processes that are associated with ageing [15]. Recent data robustly suggest the superior utility of MAFLD in identifying patients at high risk for metabolic dysfunction, the hepatic and extra-hepatic complications, including patients with concomitant liver diseases [16]. A cohort of 13,083 adults from the NHANES III (National Health and Nutrition Examination Surveys) database and showed that the MAFLD criteria are practical and more effective than the NAFLD criteria for identifying patients at high risk of fibrosis and metabolic dysfunction [17]. This change in name and criteria also appears to have improved disease awareness among patients and physicians.

We provide evidence of the association between indices measuring impaired sensitivity to thyroid hormones and prevalence of MAFLD, T2DM, hyperuricemia, elevated liver enzymes, elevated FFAs as well as between these indices and the risk of MAFLD with elevated liver enzymes in the euthyroid population, and these correlations remained significant even after adjusting for multiple risk factors.

The liver plays an essential physiological role in the regulation of thyroid hormones (THs), and vice versa, THs are essential for the metabolism of cholesterol, glucose, and hepatic lipids [18, 19]. THs act to increase cholesterol metabolism and simultaneously stimulates lipogenesis and fatty acid oxidation, with predominant action on oxidation [20]. THs can stimulate hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase, the key enzyme involved in cholesterol synthesis [21]. Moreover, triiodothyronine (T3) can stimulate hepatic de novo lipogenesis, and this process is also seen in hyperthyroid patients [22]. In addition, THs can stimulate FFAs delivery to the liver for re-esterification to TG by directly stimulating the fatty acids synthase and enzymes acetyl-CoA carboxylase, subsequently affecting fat accumulation in the liver [23, 24]. In hypothyroidism, the development and progression of MAFLD is enhanced by the decreased activity of hepatic lipase, which reduce the clearance of hepatic TG and promote hepatic TG accumulation [6, 25]. Furthermore, biologically, thyroxine (T4) must be converted into triiodothyronine (T3) in the body to exert its effects, multiple interdependent pathways involved in lipid metabolism are affected by higher FT3 levels, including increased the release of FFAs for TG synthesis in the liver, enhanced the delivery of nonesterified fatty acids to the liver [9, 22, 26]. However, hepatic fatty acids in MAFLD may impair nuclear thyroid hormone receptor activity [27]. Therefore, liver dysfunction might account for a major variation in the bioavailability of THs and clinical evidence also supports the different effects of various liver diseases on thyroid hormone metabolism [28, 29].

From a clinical point of view, the correlation between THs and MAFLD also remains disputable. Previous attempts to relate MAFLD with the thyroid axis either showed an

association with TSH but not with fT4 or yielded inconsistent results [30]. One cohort study revealed a negatively linear association between FT4 levels and incident MAFLD, but no significant association between TSH and incident MAFLD was observed [8]. On the other side, one study demonstrated that FT3 levels were positively associated with incidence of MAFLD, and that TSH levels were negatively associated with incident MAFLD, but there was no association between FT4 and incident MAFLD [9]. Previous studies have reported that the serum concentration of liver enzymes is frequently abnormal in patients with hypothyroidism, and hypothyroidism is associated with an increased risk of MAFLD [6, 18] And, it is worth noting that even in the euthyroid subjects, elevated TSH levels were reported to associate with MAFLD and advanced fibrosis [7, 31, 32]. Thus, we speculate that the contradictory results may reflect the close association between sensitivity to thyroid hormone with MAFLD. Our results offer an explanation for thyroid profiles commonly found in patients with MAFLD. That is, at the normal level, measurements of impaired sensitivity to thyroid hormones are cross-sectionally associated with MAFLD, independently of the degree of BMI, HbA1c, and FFAs, suggesting that there might be other underlying mechanisms linking MAFLD and impaired sensitivity to thyroid hormones.

The current study has some limitations. First, the cross-sectional design is insufficient to establish causality between sensitivity to thyroid hormone indices with risk of MAFLD and MAFLD with elevated liver enzymes. Second, liver biopsy was not used to accurately detect MAFLD, there was limited accuracy for detecting mild hepatic lipid accumulation. Finally, we did not evaluate thyroid-related antibodies, which might be potential confounding factors. Given that many factors may be associated with metabolic syndrome, it is not possible to adjust for unavailable variables and unknown factors, and the potential residual confounding must be considered when interpreting the study results. More randomized controlled trials are needed in the future to show beneficial effects of novel therapeutic approaches for MAFLD.

Conclusion

The study findings suggest that higher values in indices of sensitivity to thyroid hormone are associated with various metabolic conditions, including MAFLD, MAFLD with elevated liver enzymes, T2DM, hyperuricemia, and elevated FFAs in the euthyroid population. The evidence presented highlights the potential significance of thyroid hormones in their interactions with liver enzymes and fatty acid metabolism. These insights may have implications for the development of new therapies focused on energy expenditure. However, it's important to note that further research is needed to confirm these findings and elucidate the underlying mechanisms driving these associations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01308-y.

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Author contribution Haixia Zeng was responsible for the design, management, data collection, analysis, and writing of the study. Yuying Zhang was involved in the design of the study and data collection. Jianping Liu was involved in formal analysis, supervision, writing—review, and editing. All authors read and approved the final paper.

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

Declarations

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

Statement of ethics This retrospective cohort study was approved by the Information Management Organization of the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University.

Consent of patient Given that the study does not involve direct patient data or identifiable information, as well as enrolment of participants, the Research Ethics Committee of the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University deemed the research to not require informed consent.

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ORIGINAL ARTICLE

Effectiveness and safety of insulin glargine U-300 as compared to insulin glargine U-100 in oral antidiabetic (OAD) failure cases—record-based observational study

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Abstract

Background Type 2 diabetes is a significant public health concern that affects over 537 million adults worldwide. Oral antidiabetic (OAD) failure can be a complex management issue in patients with type 2 diabetes. Insulin glargine U-300 is a newer type of basal insulin with more consistent pharmacological effects than traditional insulin glargine.

Objective This study aimed to assess the safety and effectiveness of insulin glargine U-300 as compared to insulin glargine U-100 in Indian type 2 diabetes patients who had experienced OAD failure.

Methods This is a record-based observational study conducted on type 2 diabetes patients who had experienced OAD failure.

Results The study involved 389 cases (189 on insulin glargine U-300 and 200 on insulin glargine U-100). It was found that 56.6% and 58.1% of patients had reduced fasting glucose levels below 130 mg/dl after 1 month of treatment, and 78.8% and 76.1% had a reduction after 3 months following the use of insulin glargine U-300 and insulin glargine U-100, respectively. In patients on glargine U-300 and insulin glargine U-100, the mean fasting plasma glucose decreased from 241.05 ± 65.93 mg/dl at baseline to 142.61 ± 55.19 mg/dl ($p < 0.05$) and similarly from 250.68 ± 61.41 to 140.27 ± 48.29 mg/dl ($p < 0.05$) at the end of the first month, respectively. The incidence of hypoglycemia was comparatively fewer in patients using insulin glargine U-300 as compared to those using insulin glargine U-100 (8.1% vs. 6.7%, $p < 0.05$).

Conclusion The results suggest that insulin glargine U-300 is an effective and safer treatment option than insulin glargine U-100 for Indian patients with OAD failure. These findings have the potential to improve the management of type 2 diabetes patients with OAD failure globally.

Keywords Insulin · Oral antidiabetic failure · Type 2 diabetes · Insulin glargine · Oral antidiabetic · Metformin

Introduction

Type 2 diabetes is a chronic illness that affects over 537 million adults worldwide, making it a significant public health concern [1]. Managing high blood sugar levels in type 2 diabetes patients can be complex, and some patients may experience oral antidiabetic (OAD) failure [2]. Oral antidiabetic (OAD) failure is a term used to describe a clinical situation in which a patient experiences symptoms of insulinopenia and uncontrolled blood sugar levels despite receiving triple OAD therapy or when specific criteria are met. Such criteria include HbA1c levels remaining above the target range despite optimal use of three different OADs (including different classes—one being metformin and another sulphonylurea) and adherence to lifestyle modifications. Additionally, other conditions that could cause high

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blood sugar levels have to be excluded [3]. In the context of this complex management algorithm, a constant search for an efficacious and long-lasting therapy is in progress. Basal insulin therapy may be recommended in such cases, but the safety concerns associated with insulin use, such as hypoglycemia, need to be taken into consideration [4]. Introduction of insulin early in type 2 diabetes will provide good glycemic control and reduce the diabetic complications [5]. Insulin glargine U-300 is a newer type of basal insulin that has been shown to have a longer-lasting and more consistent pharmacological effect than traditional insulin glargine [6]. However, there is limited data on the safety and effectiveness of insulin glargine U-300 in patients with OAD failure, especially in Indian patients.

According to the International Diabetes Federation, India had an estimated 77 million adults with diabetes in 2019, making it one of the countries with the highest diabetes prevalence rates in the world. The high prevalence of type 2 diabetes in India has led to a significant burden on the healthcare system, and OAD failure is a common issue faced by healthcare providers in the country. In a study conducted by Patel et al., up to 41.9% of patients with type 2 diabetes in India experienced OAD failure within 5 years of diagnosis [7]. Therefore, investigating alternative treatment options for patients who experience OAD failure is crucial to improve diabetes management in India and globally. Glargine U300 has been reported to have a more stable pharmacokinetic and pharmacodynamic profile and a longer duration of action than glargine U100, leading to lower within-day variability and better day-to-day reproducibility.

In this record-based observational study, we aimed to assess the safety and effectiveness of insulin glargine U-300 as compared to insulin glargine U-100 in Indian patients who had experienced OAD failure. The results of this study could potentially inform clinical practice and improve the management of type 2 diabetes patients with OAD failure.

Materials and methods

Study design

This was a record-based observational study from a tertiary care hospital in Kolkata between January and July 2023 that analyzed data from electronic and non-electronic medical records of patients with type 2 diabetes who failed OADs and were prescribed insulin glargine U-300 or insulin glargine U-100.

Study population

The study population consisted of all patients attending the diabetes therapeutics clinics in Kolkata, with type 2 diabetes who failed OADs and were prescribed insulin glargine U-300 or insulin glargine U-100 during the study period. Insulin dose titration was done biweekly (±3 days) for the first 2 months and then monthly (±7 days).

The inclusion criteria were as follows:

- Patients with a diagnosis of type 2 diabetes
- Patients who failed OADs and were prescribed insulin glargine U-300 or insulin glargine U-100
- Patients with available records containing at least one FPG and PPPG measurement and HbA1c measurement at baseline and after 3 months of treatment

The exclusion criteria were as follows:

- Patients who were not prescribed insulin glargine U-300 or insulin glargine U-100during the study period
- Patients with a history of type 1 diabetes

Data sources

The study used data from diabetes therapeutics specialty clinics in India. The records contained demographic information, diagnosis codes, medication prescriptions, laboratory results, and other clinical data.

Variables

The primary outcomes of interest were changes in FPG and PPPG after 1 and 3 months of treatment, changes in HbA1c after 3 months of treatment, and adverse drug reactions, including hypoglycemic events. The pattern of prescribing other antidiabetic medications along with glargine U-300 or glargine U-100 was also evaluated.

Other variables included demographic information (age, sex, duration of diabetes), comorbidities (hypertension, dyslipidemia, cardiovascular disease), medication use (other diabetes medications, antihypertensive medications, lipid-lowering medications), and laboratory results (lipid panel, renal function tests).

Data analysis

Descriptive statistics were used to summarize patient characteristics, medication use, and clinical outcomes. Categorical variables were reported as frequencies and percentages, while continuous variables were reported as means and standard deviations or medians. Paired and unpaired *t*-tests were used for inter-group and intra-group comparisons, respectively.

Ethical considerations

The study was approved by the institutional review board of the diabetes therapeutics specialty clinics. As this was a retrospective observational study, informed consent was not required. The study was conducted in accordance with the applicable ethical guidelines.

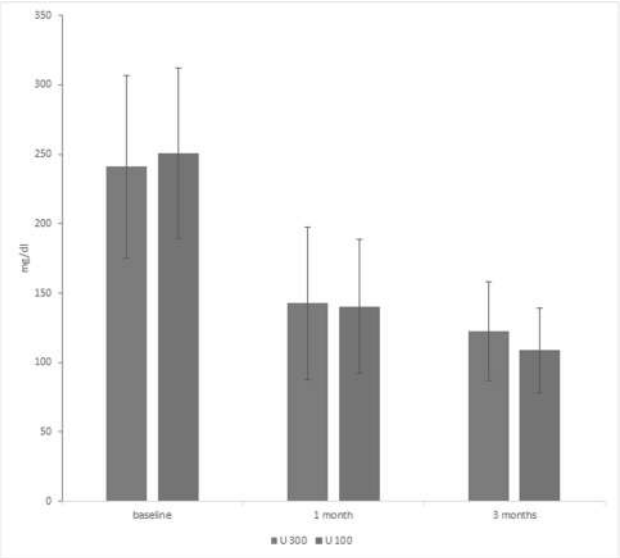


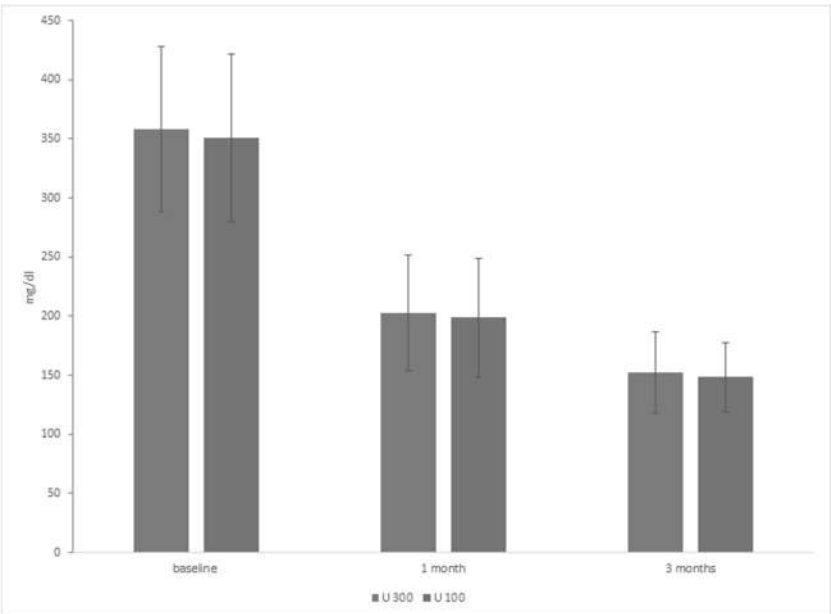
Fig. 1 Fasting plasma glucose before and after 1 and 3 months of treatment

Results

The study involved 389 cases (189 on insulin glargine U-300 and 200 on insulin glargine U-100). The glycemic control was similar with glargine U-300 and insulin glargine U-100. We showed that 56.6% and 58.1% of patients had a reduction in fasting glucose levels below 130 mg/dl after 1 month of treatment, and 78.8% and 76.31% had a reduction after 3 months following the use of insulin glargine U-300 and insulin glargine U-100, respectively. In patients on glargine U-300 and insulin glargine U-100, the mean fasting plasma glucose decreased from 241.05 ± 65.93 mg/dl at baseline to 142.61 ± 55.19 mg/dl ($p < 0.05$) at the end of the first month and to 122.44 ± 35.59 mg/dl ($p < 0.05$) at the end of the third month and similarly from 250.68 ± 61.41 to 140.27 ± 48.29 mg/dl ($p < 0.05$) at the end of the first month and to 108.69 ± 30.31 mg/dl ($p < 0.05$) at the end of the third month, respectively (Fig. 1). In patients on glargine U-300 and insulin glargine U-100, the mean post-prandial plasma glucose decreased from 358.02 ± 70.02 mg/dl at baseline to 202.87 ± 49.19 mg/dl ($p < 0.05$) at the end of the first month and to 152.44 ± 34.44 mg/dl ($p < 0.05$) at the end of the third month and similarly from 350.98 ± 71.10 to 198.87 ± 50.19 mg/dl ($p < 0.05$) at the end of the first month and to 148.69 ± 29.25 mg/dl ($p < 0.05$) at the end of the third month, respectively (Fig. 2).

The mean changes in HbA1C after 3 months of treatment were 4.34 ± 2.09% (12.3 ± 3.6 to 7.96 ± 2.91; 33.9% reduction) and 4.12 ± 1.99% (12.8 ± 3.1 to 8.68 ± 2.8; 32.18% reduction) among patients using insulin glargine U-300 and insulin glargine U-100, respectively. 70.2% and 69.8% of

Fig. 2 Postprandial plasma glucose before and after 1 and 3 months of treatment



patients showed a reduction in HbA1C levels of <7% after 3 months of treatment with insulin glargine U-300 and insulin glargine U-100, respectively. The incidence of hypoglycemia was comparatively fewer in patients using insulin glargine U-300 as compared to those using insulin glargine U-100 (8.1% vs. 6.7%, $p < 0.05$).

Among patients using insulin glargine U-300, at the beginning of the study, 97.3% of patients were taking metformin, 88.4% were taking SU, and 56.1% were taking DPP4 inhibitors. Additionally, 46.6% were taking SGLT2 inhibitors, 33.9% were taking alpha-glucosidase inhibitors, 29.7% were taking pioglitazone, and 22.0% presented with uncontrolled hyperglycemia accompanied by signs of insulinopenia. An evaluation of the prescription patterns after 3 months showed that 80.42% of the study cases were taking metformin, 41.2% were taking SU, and 91.3% were taking DPP4 inhibitors. In addition, 22.7% were taking SGLT2 inhibitors, 22.2% were taking alpha-glucosidase inhibitors, 19.05% were taking three bolus doses of short-acting insulin, 8.9% were taking repaglinide, 4.2% were taking GLP-1 analogs, and 2.6% were taking pioglitazone (Fig. 3). Furthermore, 16.1% of patients had their dosage reduced by the end of the first month, and 22.2% had their dosage reduced by the end of the third month.

Among patients using Insulin Glargine U-100, at the beginning of the study, 90.5% of patients were taking metformin, 90.2% were taking SU, and 49.9% were taking DPP4 inhibitors. Additionally, 40.8% were taking SGLT2 inhibitors, 35.5% were taking alpha-glucosidase inhibitors, 25.8% were taking pioglitazone, and 15.8% presented with uncontrolled hyperglycemia accompanied by signs of insulinopenia. An evaluation of the prescription patterns after three months showed that 78.2% of the study cases were taking metformin, 36.9% were taking SU, and 88.7% were taking DPP4 inhibitors. In addition, 23.5% were taking SGLT2 inhibitors, 19.7% were taking alpha-glucosidase inhibitors, 20.1% were taking three bolus doses of short-acting insulin, 9.0% were taking repaglinide, 5.6% were taking GLP-1 analogs, and 3.0% were taking pioglitazone (Fig. 3). Furthermore, 17.7% of patients had their dosage reduced by the end of the first month, and 20.9% had their dosage reduced by the end of the third month.

Discussion

The results of this study suggest that both insulins glargine 300 and glargine 100 are safe and effective in controlling glucose levels in patients with type 2 diabetes. The reduction in HbA1C levels observed after 3 months of treatment is consistent with previous studies that have shown the efficacy of insulin glargine 300 or glargine 100 in improving glycemic control [8, 9, 16, 20].

The use of insulin glargine in combination with other glucose-lowering medications such as metformin, SU, DPP4 inhibitors, and SGLT2 inhibitors observed in this study is consistent with current treatment guidelines [12, 13]. The use of short-acting insulin and GLP-1 analogs was observed

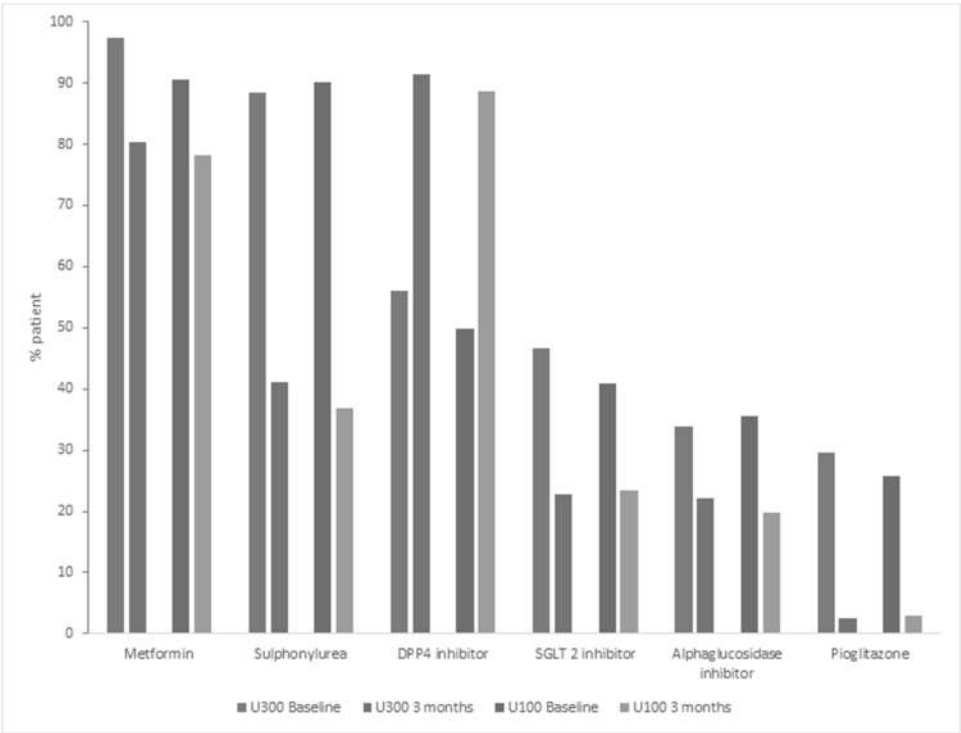


Fig. 3 Pattern of OAD use

in a small percentage of patients, which may reflect the need for additional therapy to achieve glycemic control in some patients.

In real-world practice, insulin dose was significantly higher in IGlar U300-treated than U100-treated patients at 6, 12, and 18 months, with similar reductions in HbA1c [15]. Hospital treatment with glargine U300 resulted in similar glycemic control compared with glargine U100 and may be associated with a lower incidence of clinically significant hypoglycemia. Glargine U300 is a good basal insulin alternative to treat T1DM, improving metabolic control in patients with HbA1c levels >7.5 and decreasing hypoglycemic events in patients with a history of hypoglycemia without increasing body weight [16]. The glycemic control goals achieved with Glar-300 were similar to those achieved with Glar-100, with a lower risk of hypoglycemia and less weight gain. These results suggest that Glar-300 may have a place as an alternative, long-acting basal insulin for patients with T1DM or T2DM [17]. Gla-100 and Gla-300 had comparable efficacy and safety profiles in both T1D and T2D populations. Gla-300 showed a lower risk of nocturnal and total hypoglycaemia, significant in insulin-experienced/exposed patients with T2D. Patients on Gla-300 required significantly more units of insulin daily than the Gla-100 group to achieve equivalent efficacy [18]. Our results are in agreement with the finding that Gla-300 has a better safety profile than Gla-100. The small percentage of patients experiencing hypoglycemic events is in line with the safety profile of insulin glargine 300, which has been shown to have a lower risk of hypoglycemia compared to other basal insulin analogs [10, 11]. The observation of dose reductions in some patients is consistent with the flexible dosing regimen of insulin glargine 300, which allows for individualized treatment. In the EDITION 3 trial, it was demonstrated that Gla-300 offered comparable blood sugar reduction to Gla-100 in insulin-naïve adult patients with type 2 diabetes over a span of 6–12 months, but with a lower risk of hypoglycemia, especially at the threshold of <3.0 mmoL/L [14, 19]. In the Toujeo trial conducted in Switzerland and Germany, the study participants were older, and the findings revealed that 50% of patients achieved their personalized HbA1c goal within a year of commencing Gla-300 therapy, while 61% achieved either the HbA1c or the FPG target [19]. In part, this finding is in agreement with our results too.

Conclusion

The results suggest that insulin glargine U-300 is an effective and safer treatment option than insulin glargine U-100 for Indian patients with OAD failure. These findings have the potential to improve the management of type 2 diabetes patients with OAD failure globally.

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Declarations

Ethics approval The study protocol was approved by the Institutional Ethics Committee.

Consent to participate Because of the retrospective study design, the requirement of informed consent was waived.

Competing interests The authors declare no competing interests.

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SHORT ARTICLE

Modeling of postprandial glycemic response by consecutive reaction kinetics model for precise glycemic control

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Abstract

Objective The dynamics of postprandial glycemic response are crucial for human health, while there is currently a lack of efficient models that can capture its fine features.

Methods To address this gap, a physiologically relevant model based on consecutive reaction kinetics (CRK) was developed in this study to describe human postprandial glycemic response dynamics.

Results The model yielded robust fittings for both simulated and experimental glycemic data (comprising 134 datasets), and demonstrated flexibility in capturing the fine features of glycemic responses to a wide range of real foods, such as blood glucose rising and dropping rates.

Conclusion The CRK model developed in this study should be applied in the future together with food and personal information to better understand the determinants of the variance of human postprandial glycemic response dynamics.

Keywords Postprandial glycemic response · Consecutive reaction kinetics model · Oral glucose tolerance test · Glycemic load

Introduction

Being able to precisely characterize and quantify the postprandial glycemic response is crucial in terms of identifying factors that are responsible for individual variation and optimizing diet recommendations to target broader improvements in cardiometabolic health [1]. Currently, fasting blood assays are applied in many clinical diagnoses, such as type 2 diabetes. However, most people are predominantly in their postprandial state during the waking hours. Postprandial hyperglycemia raises the risk of coronary heart disease, cardiovascular disease, and cardiovascular mortality, even in individuals with normal fasting glucose level, highlighting the relevance of diet and its metabolic consequences in cardiovascular risk [2].

Currently, the glycemic index (GI) is the widely used parameter for describing the postprandial glycemic response of carbohydrate-based foods [3]. GI is defined as the ratio of 2-h incremental area under the glycemic curve (iAUC) after consuming a carbohydrate-based food to that of a reference food by more than 10 healthy individuals (ISO method 26642:2010). Typically, white wheat bread and glucose are used as reference foods, with a GI value of 100. To consider the effects of consumed carbohydrate amount on the postprandial glycemic response, the concept of glycemic load (GL) was introduced [4]. GL is defined as the product of the amount of available carbohydrate (in a specified food consumption size) and GI, which is further divided by 100. Therefore, GL is of advantage compared to GI in terms of reflecting the actual postprandial glycemic response of foods. For instance, watermelon has a high GI value [4], but it is low in carbohydrate content (e.g., ~ 5 g carbohydrate per 100 g of watermelon). As a result, watermelon would have a small glycemic response.

Despite the widespread use of GI and GL, many criticisms exist regarding their methodology and applicability in improving human health. One of the fundamental issues is the high inter- and intra-individual variance of glycemic responses to foods with the same GI and GL values [5]. For instance, the postprandial glycemic response to the same food or mixed meals can differ substantially among different healthy individuals, possibly due to differences in lifestyle, degree of mastication, insulin sensitivity, and other physiological factors [5–8]. Furthermore, both GI and GL cannot capture the nuances of the postprandial glycemic response dynamics, such as the rate

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of glycemic rise and fall. Some physiology-based mathematical models have been proposed to fit the postprandial glycemic response dynamics and result in physiology-based parameters (e.g., [9]), but these models frequently involve many parameters and may suffer from overfitting issues.

Therefore, the objective of the current study was to develop a mathematical model depending on the consecutive reaction kinetics (CRK) that uses a few parameters to accurately describe the postprandial glycemic response dynamics of various foods. The CRK model was initially validated using manually generated data sets with added experimental noise. Once validated, the model was applied to analyze postprandial glycemic response curves for a broad range of oral glucose tolerance test data sets, as well as foods with different glycemic loads. By applying the newly developed CRK model to these postprandial glycemic response curves, new insights were gained that could be used to develop precise glycemic control strategies in the future.

Methods

Development of the consecutive reaction kinetics (CRK) model

Postprandial glycemic response is a kinetic process, generally consisting of two continuous steps as (1) food digestion in small intestine and glucose entrance to the blood vessel and (2) glucose absorption from blood vessel into tissue cells such as brain, liver, skeletal muscle, and adipose tissue (Fig. 1). Food (normally carbohydrate-based foods) digestion and the absorption of glucose into the blood vessel are the preliminary step, followed by the glucose absorption into tissue cells from the blood vessel [10]. To simplify the mathematical deduction process, each of these two steps was assumed to follow the first-order kinetics, with a characteristic rate constant of k_d (min^{-1}) and k_a (min^{-1}), respectively. Note, both k_d and k_a are defined as the average values for the overall food digestion (first step) and glucose absorption (second step) process instead of any specific process, as each process is consisted of many processes such as the oral mastication, gastric emptying, and small intestinal digestion, entrance to the hepatic cells from blood vessel. The following equations could then be deduced depending on the rate law.

For the available glucose concentration in the foods:

$$C_F(t) = (C(\infty)) \times \left(e^{-k_d t}\right) \quad (1)$$

For the glucose concentration in the blood vessel:

$$C_B(t) = \frac{k_d \times C(\infty)}{k_a - k_d} \times \left(e^{-k_d t} - e^{-k_a t}\right) \quad (2)$$

For the glucose concentration entering tissues:

$$C_T(t) = C(\infty) \times \left(1 - \frac{k_a \times e^{-k_d t} - k_d \times e^{-k_a t}}{k_a - k_d}\right) \quad (3)$$

In these equations, $C_F(t)$, $C_B(t)$, and $C_T(t)$ are the glucose concentration in food, blood, and tissues at time t (min), respectively, with a unit of mmol/L. $C(\infty)$ (mmol/L) is the maximum glucose concentration entering the tissues after an infinite time. These parameters were determined via the non-linear least squares refinement tool in Excel. The full deduction process of the Eqs. 1–3 was included in the supporting information.

Fitting to the manually produced glycemic data

A series of postprandial glycemic data with experimental noise and different fine features (e.g., peak rising and dropping rate) was manually generated to validate the developed model. The hypothesis is that if the model fitting can produce similar parameters to those applied to generate these artificial data, it suggests that the developed CRK model is a solid procedure to capture the fine details of human postprandial glycemic response dynamics. These parameters to generate the artificial postprandial glycemic data are summarized in Table 1, which were given here as an example and different parameters can also be tested. Twenty-five time points were generated in the range of 0 to 120 min in order to develop high-resolution postprandial glycemic response dynamics. The glucose concentration in the blood vessel

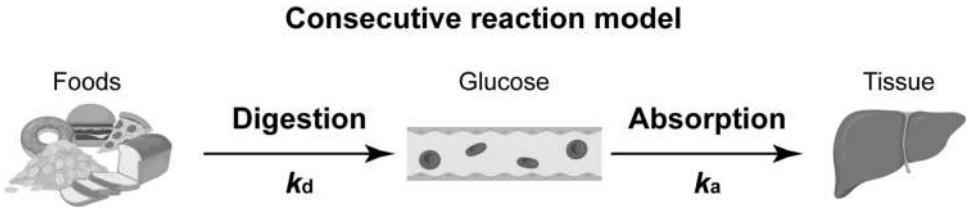

Fig. 1 Schematic diagram showing the consecutive steps of food digestion and glucose absorption. Digestion rate constant is related to many factors such as oral mastication efficiency, gastric emptying rate, and intestinal transit time. Similarly, glucose absorption rate constant (from blood vessel to tissues) is controlled by factors such as insulin resistance, activity of glycogen biosynthetic enzymes

Table 1 Artificial parameters applied to generate human postprandial glycemic response curves

Dataset	$C(\infty)$ (mmol/L)	k_a (min^{-1})	k_d (min^{-1})	G_f (mmoL)
1	10	0.05	0.04	5
2	20	0.05	0.04	5
3	10	0.1	0.04	5
4	10	0.05	0.1	5

$C(\infty)$ is the maximum glucose concentration entering the tissues after an infinite time. k_a and k_d are the rate constant for food digestion and glucose absorption, respectively. G_f is the fasting blood glucose concentration

was then produced following Eq. 2. To mimic experimental errors, a series of random numbers within the range of − 0.25 to 0.25 were generated via the "RAND" equation and added to the glycemic data in Excel. Each set of artificial data was generated in triplicates. These manually generated data were finally fitted with the developed CRK model via the nonlinear least squares refinement procedure in Excel.

Fitting to oral glucose tolerance test data and foods with a wide range of glycemic load

Oral glucose tolerance test (OGTT) data and postprandial glycemic data of different carbohydrate-containing foods (single or mixed meals) with a wide range of glycemic load from healthy subjects were obtained from the previous publication with permission [9], to further validate the developed CRK model. Generally, OGTT has a simpler glycemic kinetics compared to that for real foods. Datasets were only included when the subjects were identified as healthy and contained > 5 postprandial plasma glucose concentration measurements. Healthy subjects were those with (1) nonpregnant female, (2) stable body weight with no change in dietary habits (3 months prior to the measurements), (3) free of apparent diseases and regular medication, (4) no family history of diabetes and non-obese (i.e., BMI < 30 kg/m^2), (5) normal hemoglobin level with HbA_{1C} < 6.5%, (6) diastolic blood pressure < 80 mmHg and systemic blood pressure < 120 mmHg, and (7) normal glucose tolerance with fasting plasma glucose level < 5.6 mmol/L, 2-h postprandial plasma glucose level < 7.8 mmol/L, and postprandial plasma glucose peak < 11 mmol/L. Only the datasets within 2 h were collected when they only have the glucose rising and dropping period (i.e., without significant drop below the fasting glucose level and then a second rising), as it mainly involves the food digestion and glucose absorption process (other processes such as glycogen degradation are less significant during this period), as shown in Fig. 1. Detailed information for all these datasets is included in the supporting information.

Statistical analysis

The means and standard deviations were determined via Excel.

Results and discussion

Validation of CRK model

The manually generated glycemic data with the CRK model fitting results are shown in Fig. 2. It shows that CRK model generally gave satisfactory fittings, which could reproduce the whole manually generated glycemic data with $R^2 > 0.97$. Notably, the CRK model demonstrates the ability to capture the fine features of postprandial glycemic response dynamics by modifying the fitting parameters. For example, increasing the k_d from 0.04 to 0.1 resulted in a more rapid increase in glycemic response, while raising the k_a from 0.05 to 0.1 led to a sharper decrease in glycemic response. Additionally, increasing the $C(\infty)$ from 10 to 20 produced a marked increase in the area under the whole curve.

The CRK predicted parameters are given in Table 2. Consistent with Fig. 2, the CRK model produced parameters that were comparable to those employed in the creation of the simulated glycemic data (Tables 1 and 2). This further supports the notion that the developed CRK model was satisfactory in terms of fitting the postprandial glycemic data with the effects from the experimental noise (i.e., the noise introduced through the use of the "RAND" function).

OGTT data fitting

Thirty-four OGTT datasets were collected from thirty-one different publications (references [11–40]), and detailed information on these datasets can be found from the supporting information. Figure 3A illustrates the heterogeneity of the postprandial glycemic response dynamics for these OGTT datasets. Although these postprandial glycemic response profiles may have a comparable 2 h iAUC (i.e., GI = 100 and GL = 75 or 50), they all show distinct dynamics, such as peak height and width. It reinforces the idea, mentioned in the "Introduction" section, that GI or GL cannot fully capture the nuances of various postprandial glycemic response dynamics. In addition, GI and GL measurements are highly dependent on the postprandial glucose sampling interval.

The CRK model developed in this study was employed to fit all 34 OGTT datasets, and an example of such a fitting is shown in Fig. 3B. The fitting parameters for all datasets can be found in the supporting information. The R^2 values for the fittings were generally greater than 0.7, indicating a good agreement between the model and experimental data. As

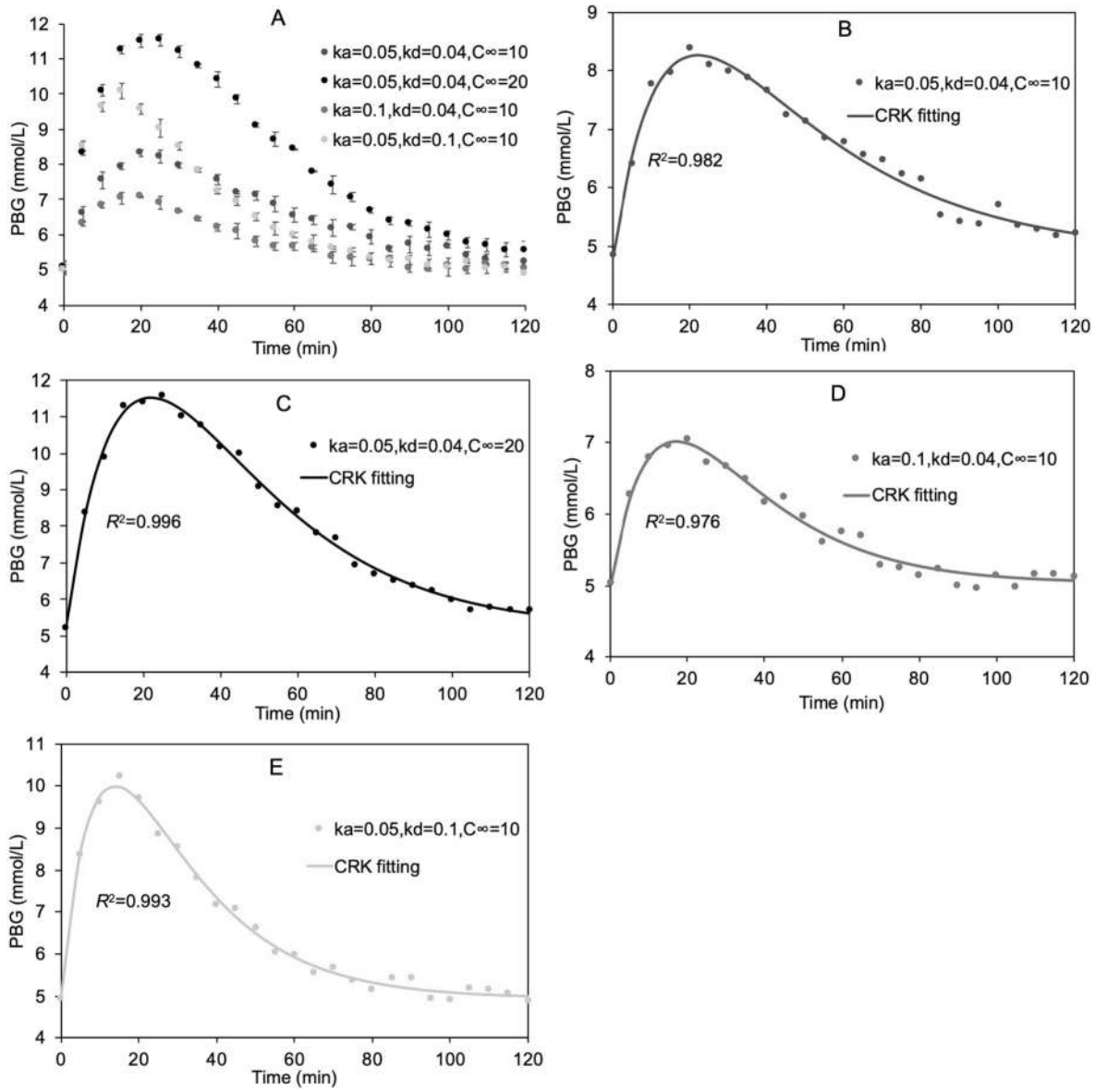

Fig. 2 CRK model fitting for different manually generated postprandial glycemic response dynamics data. These data were generated using Eq. 2 with the parameters summarized in Table 1. CRK is consecutive reaction kinetics. PBG is postprandial blood glucose

seen in Fig. 3A, there were no significant differences observed among the parameters for individuals who consumed 75 g glucose versus those who consumed 50 g glucose. This lack of significant difference could be attributed to the substantial physiological variability among individuals. The majority of k_a values were similar to k_d values, i.e., $k_a/k_d \approx 1$ (Fig. 3C). This outcome is reasonable as in healthy individuals, glucose entering and leaving blood vessels quickly balance out, resulting in an equilibrium (known as glucose homeostasis, which is the maintenance of stable blood glucose levels within a narrow range). This is why it is reasonable to observe that most of the values of k_a and k_d are similar, and their ratio is approximately 1. However, a few datasets exhibited abnormal k_a/k_d values. The comparison of one of these abnormal datasets to the dataset with $k_a/k_d \approx 1$ is shown in Fig. 3D. As depicted, when k_a was smaller than k_d, it resulted in a much slower decrease in blood glucose levels following the postprandial glycemic peak, suggesting that individuals with abnormal k_a/k_d values might suffer from reduced insulin sensitivity. It is thus proposed here that the k_a/k_d value may serve as an indicator of insulin resistance for clinical applications.

Fitting to foods with different glycemic load (GL)

Although the postprandial glycemic response for an OGTT test has a relatively simple kinetics, postprandial glycemic

Table 2 CRK predicted parameters for different simulated postprandial glycemic response curves

	$C(\infty)$ (mmol/L)	k_a (min^{-1})	k_d (min^{-1})	G_f (mmoL)	R^2
1	11.12 ± 3.84	0.057 ± 0.011	0.038 ± 0.010	5.07 ± 0.20	0.982 ± 0.003
2	18.74 ± 2.40	0.048 ± 0.004	0.043 ± 0.004	5.07 ± 0.16	0.996 ± 0.001
3	7.70 ± 3.97	0.077 ± 0.026	0.052 ± 0.017	5.01 ± 0.13	0.976 ± 0.003
4	9.87 ± 0.60	0.050 ± 0.002	0.102 ± 0.006	5.02 ± 0.07	0.993 ± 0.001

These values were calculated as mean ± SD of three replicates. $C(\infty)$ is the maximum glucose concentration entering the tissues after an infinite time. k_d and k_a are the rate constant for food digestion and glucose absorption, respectively. G_f is the fasting blood glucose concentration

response dynamics for real foods are much more complex. The primary biological factors that determine the food digestion process include oral salivation and mastication, gastric motility and emptying, small intestinal motility and enzymes, large intestinal food-microbiota interactions, and gut-brain feedback regulation [10]. Therefore, various foods with a wide range of GL (100 datasets from 23 publications) were further applied to confirm the validity of the developed CRK model [32–58]. Detailed information regarding these datasets can be found in the supporting information. Consistent with the OGTT data shown in Fig. 3A, a high heterogeneity in the postprandial glycemic response, such as peak height and width, was observed for these foods with different GL (Fig. 4A). Foods with similar GI or GL can also show distinct postprandial glycemic responses, indicating that the GI or GL fails to capture the fine features of the postprandial glycemic response dynamics.

An example of the CRK model fittings for different foods is shown in Fig. 4B, and all the fitting parameters are summarized in the supporting information. Although the CRK model for real foods showed less satisfactory fittings compared to that for OGTT data, most of the fittings can still reproduce the experimental data with an $R^2 > 0.7$. This is reasonable since the digestion of real foods is much more complex than glucose (as

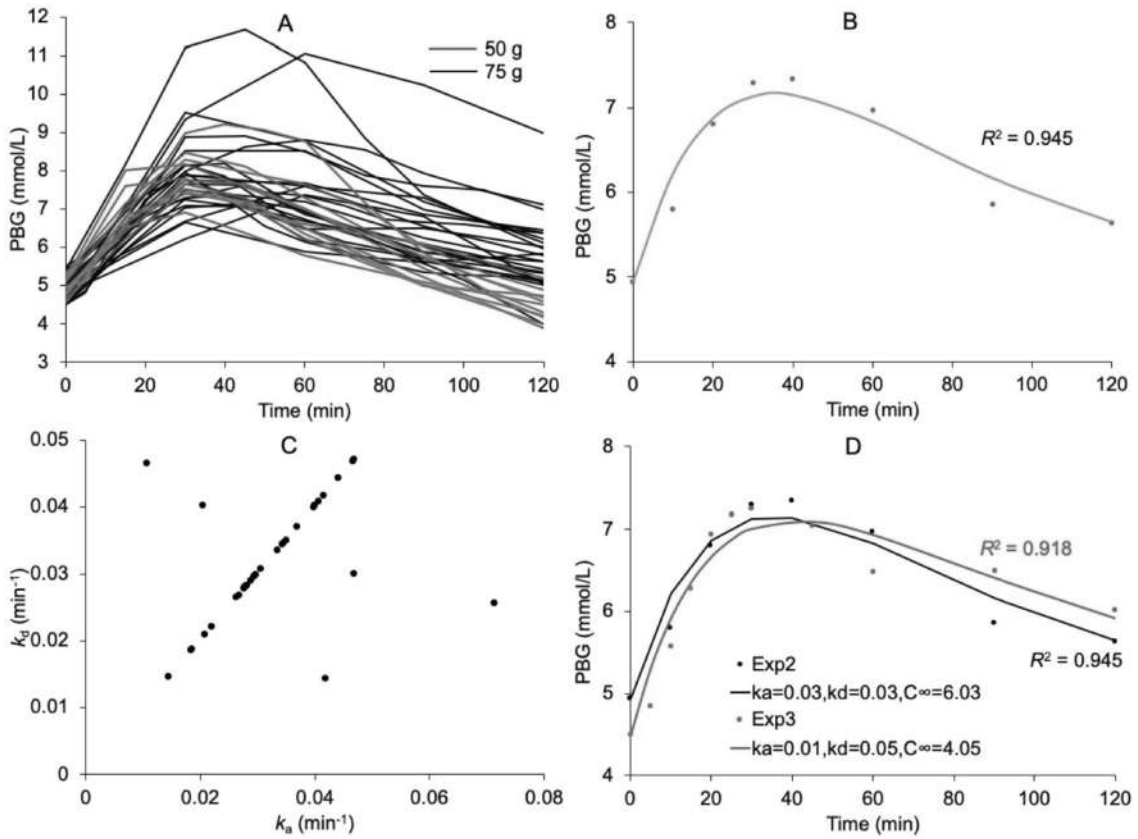

Fig. 3 OGTT datasets (**A**), an example of CRK model fitting to the OGTT data (**B**), comparison of k_a and k_d values (**C**), and comparison between datasets with abnormal k_a/k_d values and $k_a/k_d \approx 1$ (**D**). PBG is postprandial blood glucose. Exp2 and Exp3 are two datasets

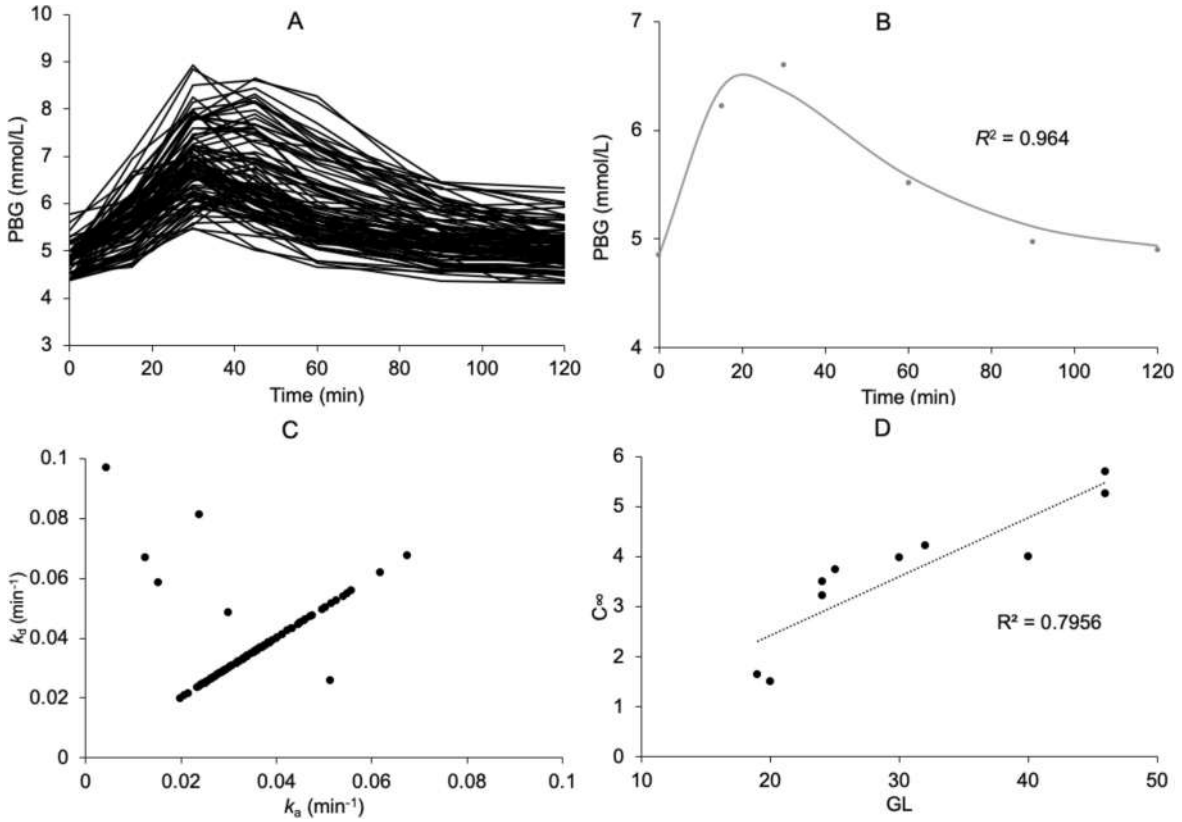

Fig. 4 Postprandial glycemic response dynamics for foods with different GL (**A**), an example of the CRK model fitting (**B**), comparison of k_a and k_d values for these foods (**C**), and relations between GL and $C(\infty)$ for these datasets collected from Ranawana et al. [37] (**D**). PBG is the postprandial blood glucose

mentioned above), which involves many factors from the oral, gastric, and intestinal digestion phase [10]. Therefore, more parameters inherent to the food digestion process might need to be included in the current CRK model in the future to better fit the postprandial glycemic response dynamics of real foods. These factors could include the type and amount of carbohydrates, fiber, fat, and protein, as well as individual differences in gut microbiota, metabolic rate, and insulin sensitivity [59, 60]. Nevertheless, the developed CRK model is generally flexible in fitting the postprandial glycemic response kinetics for a wide variety of real foods (i.e., $R^2 > 0.7$). Consistent with the OGTT data, most of the k_a/k_d values were close to 1 (Fig. 4C), supporting the validity of k_a/k_d value as an indicator of insulin resistance. It is often assumed that the GI of a food is determined solely by the food digestion rate or the glucose absorption rate into tissues. However, GI values from different foods did not show significant correlations with either k_a or k_d values, suggesting that GI of food does not solely depend on the food digestion rate or the glucose absorption rate into tissues. This finding highlights the need for a more comprehensive understanding of the factors that contribute to the GI of a food. Although the GL values showed less correlation with the $C(\infty)$ values for all the 100 datasets, their linear correlations were much clearer when plotted from the same study (e.g., Fig. 4D). It suggests that the GI and GL values obtained from different studies might not be suitable for comparison. On the other hand, it supports the validity of the developed CRK model, as a higher glucose load would result in a greater amount of glucose entering the tissues in healthy subjects.

Conclusions

In this study, a mathematical model depending on the consecutive reaction kinetics was developed to accurately describe and capture the complex dynamics of human postprandial glycemic response. Key findings from this study include as follows: (1) the developed CRK model was able to capture features such as the rising and falling rates of blood glucose, and was validated using both manually generated and previously published experimental data, although the model fit better to oral glucose tolerance test data compared to those for real foods; (2) by applying the CRK model to the actual experimental data, it suggested that the ratio of k_a/k_d could be an indicator of insulin resistance, with healthy individuals generally having equal k_a and k_d values.

This model has a wide range of potential applications in the field of nutrition and health. Personalized nutrition plans based on an individual's unique physiological characteristics can be developed by using the CRK model to gain a better understanding of the factors that impact postprandial glycemic response. In addition, food manufacturers can use the model to design products that have a lower glycemic response, which could be beneficial for people who are trying to control their blood sugar levels. In terms of clinical research, the model can be used to design and analyze clinical trials that investigate the effects of various interventions such as drugs, supplements, or lifestyle changes on postprandial glycemic response. Finally, this model could be used to help people with diabetes better manage their blood sugar levels by predicting how different foods and interventions will affect their glycemic response. Overall, the CRK model developed in this study has significant potential to improve our understanding of the complex dynamics of postprandial glycemic response and to inform interventions for the prevention and management of chronic diseases such as type 2 diabetes.

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Declarations

Conflict of interest The author declares no competing interests.

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LETTER TO THE EDITOR

Does benefits of rivaroxaban as add-on to aspirin apply to diabetes-related cardiovascular disease? Insights from the COMPASS and VOYAGER PAD trials

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Dear Editor.

The Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) trial and the Vascular Outcomes Study of ASA (acetylsalicylic acid) Along with Rivaroxaban in Endovascular or Surgical Limb Revascularization for PAD (peripheral artery disease) (VOYAGER PAD) trial have demonstrated that compared to aspirin alone, low-dose rivaroxaban and aspirin significantly improve major adverse limb events (MALE) and major adverse cardiovascular events (MACE) in patients with coronary artery disease (CAD) or peripheral artery disease (PAD) [1–3].

Diabetes mellitus is a major risk factor for cardiovascular diseases (CVD). Previous studies have shown that 20–30% of patients with PAD and nearly 20–50% of patients with CAD have diabetes [4]. As compared to non-diabetic patients, subjects with diabetes tend to have more diffused infrapopliteal arterial disease and poorer outcomes in terms of more amputations and higher mortality. Similarly, CAD patients with diabetes have more severe and diffuse angiographically documented coronary artery involvement compared to non-diabetics [5].

Considering the peculiarities of diabetes-related CVD, it is intriguing whether low-dose rivaroxaban would have similar beneficial effects in such subjects. To address the lacunae in the existing literature, we undertook a systematic review and meta-analysis to collate the effect of low-dose rivaroxaban with aspirin as compared to aspirin alone in patients with cardiovascular disease (CAD or PAD) and diabetes mellitus.

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The systematic review and meta-analysis were conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [6]. Two independent investigators (RP and MB) performed a systematic search of the literature across the PubMed/MEDLINE, Embase, and Web of Science databases from inception till October 31, 2022, using the MeSH/Emtree terms and/or appropriate keywords interposed with Boolean operators. The search strategy has been elaborated in the Supplementary Appendix. Only randomized controlled trials looking into the effect of low-dose rivaroxaban with aspirin vs. aspirin alone in patients with cardiovascular disease (CAD or PAD) and diabetes mellitus were considered eligible for selection.

Two investigators (MB and BM) independently scanned titles and/or abstracts to exclude duplicate studies and studies that failed to meet the aforementioned eligibility criteria. Potentially eligible studies were full-text assessed. Any discrepancies between the aforementioned investigators were solved by discussion, consensus, or arbitration by the other investigator (RP). The following data were extracted from the included studies: the study characteristics (first author, year of publication, country), number of patients with diabetes mellitus receiving low-dose rivaroxaban with aspirin vs. aspirin alone, median duration of follow-up, composite efficacy outcome reported in each of the included trials, and primary safety outcome reported in each of the trials. The risk-of-bias assessment was carried out independently by MB and BM using version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2).

After a scrupulous and meticulous literature search, two randomized controlled trials, namely the COMPASS and the VOYAGER PAD, were included in the systematic review and meta-analysis (Supplementary Fig. 1). The study characteristics have been summarized in Supplementary Table 1. In short, the COMPASS had included patients ($n = 18{,}278$) with CAD and/or PAD [3], while the VOYAGER PAD had

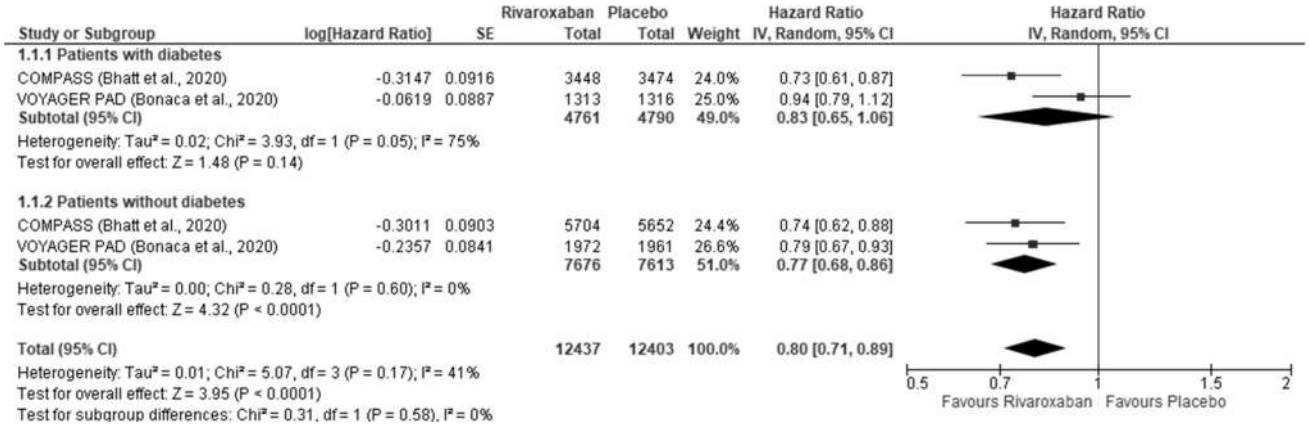

Fig. 1 Forest plot with subgroup analysis showing the effect of low-dose rivaroxaban and aspirin as compared to placebo and aspirin on efficacy outcome (composite of major adverse cardiovascular events and major adverse limb events and/or major amputations) in patients with cardiovascular disease

included only patients ($n = 6564$) with PAD who had undergone revascularization [2]. The proportion of participants having diabetes at baseline in the COMPASS and VOYAGER PAD was 37.8% and 40.0%, respectively [2, 3]. Both the studies had a low risk of bias.

The hazard ratios (HR) of the efficacy and safety outcome reported in the trials, namely the composite of MALE/MACE and major bleeding, respectively, were pooled together using the generic inverse variance method with fixed-effects/random-effects model. For each pooled analysis, we performed a subgroup analysis between patients with and without diabetes mellitus. Statistical heterogeneity among studies was assessed using I^2 statistics. A p value < 0.05 was considered to be statistically significant. Statistical analysis was performed using the RevMan 5.4 software.

Pooled analysis showed that low-dose rivaroxaban and aspirin did not result in a significant improvement in the composite outcome compared to aspirin alone in patients with CVD and diabetes (HR 0.83, 95% CI 0.65–1.06, $p = 0.14$, $I^2 = 75\%$, random-effects model); nevertheless, in patients without diabetes mellitus, the drug resulted in significant benefits (HR 0.77, 95% CI 0.68–0.86, $p < 0.001$, $I^2 = 0\%$, random-effects model) (Fig. 1). Even in patients with diabetes who had undergone revascularization at baseline, rivaroxaban was not found to be beneficial (HR 0.85, 95% CI 0.69–1.06, $p = 0.15$, $I^2 = 55\%$, random-effects model) (Supplementary Fig. 2).

With regard to the safety outcome, pooled analysis showed that the risk of major bleeding was increased in patients with diabetes mellitus receiving rivaroxaban (HR 1.82, 95% CI 1.38–2.40, $p < 0.001$, $I^2 = 0\%$, random-effects model). On the contrary, in patients with no history of diabetes mellitus at baseline, rivaroxaban did not increase such risk (HR 1.37, 95% CI 0.84–2.25, $p = 0.21$, $I^2 = 69\%$, random-effects model) (Supplementary Fig. 3).

The meta-analysis challenges the notion that low-dose rivaroxaban added to aspirin improves cardiovascular and limb outcomes in all patients with CVD and testifies that it might not offer any additional advantage in diabetes-related CVD. This apparent disparity seems unclear but can be explained based on more severe and diffuse arterial involvement in diabetes-related PAD/CAD than in those without diabetes [5, 7]. Besides, the high prevalence of polypharmacy and co-existing comorbidities seen in CVD patients with diabetes mellitus might also explain the poor prognosis and higher risk of bleeding with rivaroxaban use.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-024-01373-x.

Author contribution MB is the primary author. BM helped in data extraction and in editing the manuscript. RP is the corresponding author, performed the statistical analysis, and helped in editing the manuscript. All the authors approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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CASE REPORT

Skeletal fragility in type 1 diabetes mellitus: A rare case of avascular necrosis of talus

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Abstract

Background A divergent fracture pattern is seen in patients living with type 1 diabetes (T1D). These patients are at risk for fractures at unusual distal sites over and above the fractures occurring at major osteoporotic sites. Avascular necrosis (AVN) involving the talus has not been reported previously in T1D.

Case Presentation We hereby report an exceptional case of a 24-year old patient of T1D, who reported to us 3 months post-partum with swelling and pain over her right ankle. In the third trimester of her pregnancy, she encountered trivial trauma to her right ankle. One month after delivery, she developed fragility fractures over her left 2nd to 4th metatarsal heads. On further workup, she was found to have low bone mass and avascular necrosis of right talus on magnetic resonance imaging. On follow up 1 year later, she continued to have low bone mass although her bone mineral density (BMD) increased at the lumbar spine and hip. She was diagnosed with right talar AVN due to fragility fracture sustained in the third trimester with low bone mass consequent to T1D. A possible role of pregnancy- and lactation-related osteoporosis was considered in view of the chronological association with pregnancy.

Conclusion Our case highlights the fragile skeletal health of patients living with T1D making a case for greater scrutiny of declining bone health in these patients.

Keywords Avascular necrosis · Type 1 diabetes · T1D · Pregnancy- and lactation-related osteoporosis · Fragility fracture

Background

People living with type 1 diabetes (T1D) have been reported to have 22–37% lower bone mineral density with up to four times greater risk of fractures [1]. Multiple complex mechanisms are responsible for this increased skeletal fragility including insulin deficiency which has anabolic action on bone, oxidative stress, accumulation of advanced glycation end products, and associated autoimmune conditions like celiac disease.

Talar avascular necrosis (AVN) in T1D is an atypical finding unreported previously. Here, we detail an unusual case of AVN of talus in type 1 diabetes.

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Case presentation

A 24-year old primiparous woman, known case of T1D for the past 16 years, presented to us. She had a full-term normal vaginal delivery 3 months prior to presentation and was breastfeeding. At 6 months of pregnancy, she twisted her right ankle following which she developed swelling and pain, aggravated on weight bearing. No radiological imaging was done at the time. Few days after delivery, she slipped while getting out from her car and developed pain and swelling in her left foot. She had no history of any predisposing factor for AVN including alcohol abuse, steroid intake, systemic lupus erythematosus (SLE), and previous or any family history of thromboembolism. She did not have any prior history of fragility fractures.

On examination, she had pitting edema over her right ankle. She had no neuropathic symptoms, and examination revealed no sensory loss. She had bilateral non-proliferative diabetic retinopathy with left macular edema. The rest of the systemic examination was unremarkable.

Plain radiographs revealed fracture of the 2nd, 3rd, and 4th metatarsal heads in her left foot and complete

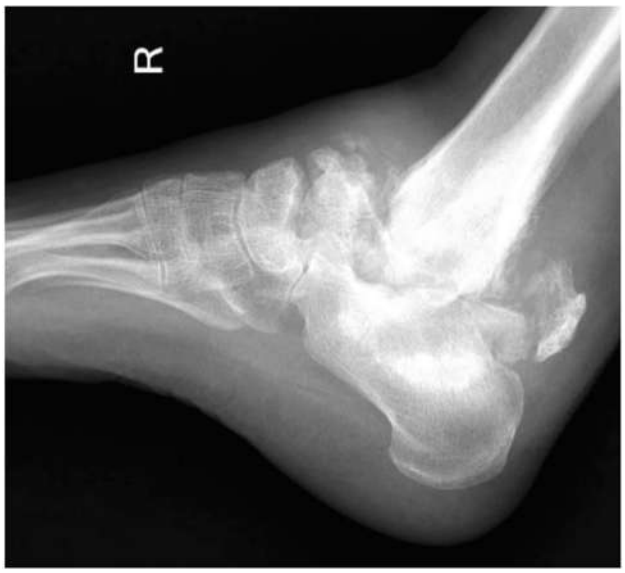

Fig. 1 Right ankle radiograph reveals collapse and fragmentation of the talus-Hawkins's type III fracture pattern with osteoid loose bodies in anterior and posterior joint recesses

collapse of talus in the right foot (Fig. 1). MRI of right ankle revealed complete collapse of talus secondary to AVN (Fig. 2). Laboratory parameters revealed high HbA1c and microalbuminuria (Table 1). She was worked up for secondary causes of AVN: Hemoglobin electrophoresis was normal, and deficiencies of anti-thrombin, protein C, protein S, autoimmune conditions, and hyperhomocysteinemia were ruled out. Dual-energy x-ray absorptiometry (DXA) scan revealed low bone mass with *Z* scores of − 3.1 at the femoral neck and − 2.6 at the lumbar spine (Table 1).

Left foot fracture was managed with casting and immobilization. She was put on a high calcium diet and vitamin D supplementation and was administered a 4-mg dose of intravenous zoledronic acid. She was also planned for surgical management for AVN of the right talus.

Fragility fracture of right talus was probably sustained in the third trimester leading to post traumatic AVN, although imaging was not done at the time. She also developed fragility fractures over the metatarsals of her left foot in the post-partum period. Her compromised skeletal strength was predominantly attributed to T1D, and in view of the temporal association with pregnancy, an additional possibility of pregnancy- and lactation-related osteoporosis (PLO) was considered.

A year later, her BMD improved by 8.2% at the femoral neck (above the least significant change) and by 2.07% at the lumbar spine (not significant), but low bone mass

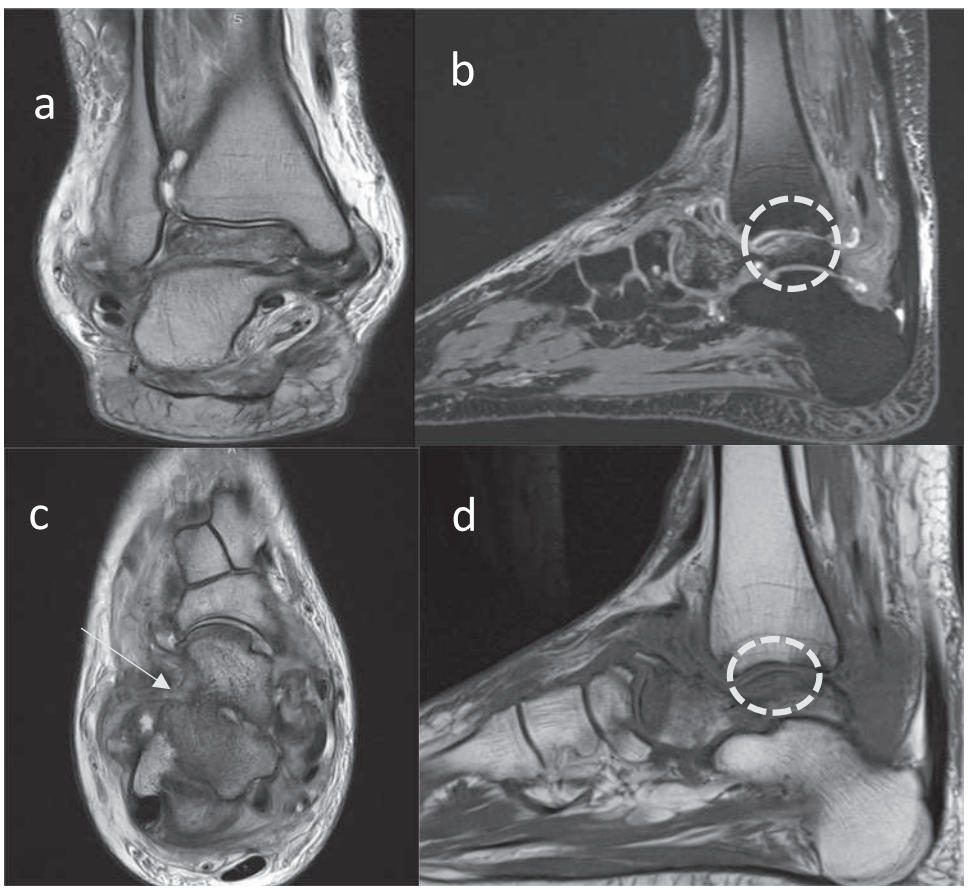

Fig. 2 Coronal T2W (**a**) images depicting near complete collapse of the talus with type III Hawkins fracture along the neck of the talus with fracture line better appreciated on axial T2W image (**c**) arrow. Sagittal GRE (**b**) and sagittal T1W (**d**) images reveal geographical areas of altered marrow signal (dashed circle) along the subchondral aspect of the tibiotalar joint and associated mild joint effusion, synovial thickening, and capsulitis

Table 1 Laboratory parameters of the patient

Parameter	Value	Reference range		
Hemoglobin, g/dL	11.2	11.5–16.5		
TLC, /cu mm	7220	4000–11000		
MCV, fL	87	82–98		
Platelet count, /cu mm	4.98×10^5	$1.5–4.0\times10^5$		
Creatinine, mg/dL	0.6	0.84–1.25		
LDL C, mg/dL	109	< 100		
HDL C, mg/dL	45	> 40		
Total cholesterol, mg/dL	169	< 200		
Triglyceride, mg/dL	94	< 150		
HbA1c,%	9.1	4–6.2		
Urine albumin to creatinine ratio, mg/g	113.5	< 30		
SGOT, IU/L	21	< 35		
SGPT, IU/L	26	< 35		
Bilirubin (T/D)	0.37/007	0.3–1.2/ < 0.2		
Total protein, mg/dL	7.22	6.6–8.3		
S. albumin, mg/dL	3.79	3.5–5.2		
TSH, mIU/L	3.08	0.5–3.5		
Anti TPO, IU/mL	110.4	1–16		
Anti-thrombin, %	108	80–120		
Protein C, %	69	70–140		
Protein S, %	77	70–140		
Homocysteine, µmol/L	5.47	3.7–13.9		
Hemoglobin HPLC	Normal electrophoretic pattern			
Calcium, mg/dL	9.0	8.2–10.2		
Phosphorus, mg/dL	4.9	2.5–4.5		
25(OH)D, ng/mL	29.7	< 20 deficiency		
iPTH, pg/mL	30.8	11.7–61.1		
ALP, IU/L	126	52–171		
BMD DXA (baseline)		g/cm^2	*T*	*Z*
	Spine	0.755	− 2.7	− 2.6
	Femur neck	0.508	− 3.1	− 3.1
BMD DXA (at 1 year)		g/cm^2	*T*	*Z*
	Spine	0.771	− 2.5	− 2.5
	Femur neck	0.550	− 2.7	− 2.7

persisted (*Z* scores of − 2.7 at the femoral neck and − 2.5 at the lumbar spine) (Table 1).

Discussion

AVN is a degenerative condition of bone occurring as a result of disruption of the vascular supply. AVN of talus is quite rare accounting for approximately 2% of all symptomatic cases of AVN [2]. Talus has a precarious blood supply which can be interrupted by trauma or various non-traumatic etiologies.

- Post-traumatic AVN: Around 75% of cases are accounted for by displaced fractures of the talus (neck and body). They typically occur secondary to high energy trauma, but even minor injuries can be causative in patients with risk factors [3]. Unilateral involvement is characteristic.
- Non-traumatic AVN: In the remaining cases, spontaneous AVN occurs without a traumatic history, and is usually associated with corticosteroids, alcoholism, hemoglobinopathies, hypercoagulable states or autoimmune conditions like SLE [4]. Bilateral involvement is seen in 54% of cases with multiple joints affected in 63% of cases [2].

Our patient had unilateral involvement and did not have any underlying risk factor for non-traumatic AVN reinforcing the post traumatic etiology for AVN. Pregnancy and diabetes, per se, have not been reported to increase the risk of talar AVN.

Pregnancy has been reported to be associated with AVN of hip. It is difficult to establish causation due to the small number of reported cases [5]. AVN at sites other than hip due to pregnancy alone has not been reported previously.

A possible association of diabetes mellitus with AVN was first considered among patients with jaw osteonecrosis related to bisphosphonate use. Fifteen to 70% of such patients have been reported to have diabetes [6]. However, in a systematic review and meta-analysis, the risk of AVN was non-significantly increased among patients with diabetes [7].

Although hip fractures are the most frequently studied in T1D, patients are also at increased risk of fractures at distal sites. A meta-analysis reported that as compared to controls, patients with diabetes were at increased risk of upper arm, hip, and ankle fractures [8]. In a population cohort study, it was found that lower limb fractures involving hip, femur, leg, ankle, and foot comprise a higher proportion of incident fractures in T1D as compared to controls [9].

PLO is a rare condition causing low trauma fractures during the third trimester or postpartum period. The underlying mechanism involves increased bone resorption occurring as a result of increased parathyroid hormone-related peptide

(PTHrP) secreted from the breast and placenta. The BMD prior to pregnancy was not known in our case, and therefore, the extent of bone loss occurring as a result of pregnancy cannot be specifically quantified. Substantial recovery in BMD is seen without any intervention following cessation of lactation [10]. Our patient had low bone mass in the postpartum period with only a modest improvement in BMD on follow up despite anti-osteoporotic treatment. Moreover, the commonly reported fractures in PLO are vertebral fractures with fractures at distal sites like foot and ankle reported less commonly [11]. The fracture site and the rare talar AVN in our patient relate to T1D. PLO may have additionally contributed although the pattern is more consistent with low bone mass associated with T1D.

Conclusion

Our patient had low bone mass secondary to long-standing T1D who developed talar AVN as a result of fragility fracture sustained in her right ankle during pregnancy with additional fragility fractures in her left foot. As far as we are aware, this is the first reported case of talar AVN in T1D. The unusual occurrence of talar AVN drew our attention to the more prevalent impaired bone health in T1D. Our case puts emphasis on the fragile bone health seen in T1D warranting higher surveillance in such patients.

Author contribution AR was involved in the writing of the manuscript with support from SS. MM critically reviewed and revised the document. All authors contributed to and endorsed the final submitted manuscript.

Data Availability All data pertaining to this case are available as part of the article and no additional source data is required.

Declarations

Ethical Approval Written informed consent was taken from the patient to collect data and publish findings.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Clinical reflections of diabetic nephropathy related pathological lesions

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Abstract

Background The Renal Pathology Society developed a universal pathological classification of diabetic nephropathy in 2010. Some research has been conducted to validate this classification's ability to predict the outcome. However, the clinical implications of these pathological abnormalities are still being investigated.

Objectives In this study, we aimed to demonstrate the clinical reflections of these lesions to better understand the underlying mechanisms.

Methods Data of 119 patients with biopsy proven diabetic nephropathy from a single center were included in the study. Pathology specimens were reclassified according to 2010 criteria suggested by the RPS.

Results Diabetic retinopathy was more frequently present in patients with advanced glomerular class, IFTA score, interstitial inflammation score, arteriolar hyalinosis score, arteriosclerosis score, and in patients with exudative lesions present ($p < 0.05$). The proteinuria levels of patients with advanced glomerular classes and exudative lesions were significantly higher, and serum albumin levels were lower ($p < 0.05$). Hematuria occurrence was more frequent in glomerular class III and IV patients and in patients with advanced arteriolar hyalinosis ($p < 0.05$).

Conclusion This large, single center, retrospective study reveals that diabetic retinopathy is associated with glomerular and arteriolar lesions but not with interstitial lesions. Proteinuria and hematuria were independent predictors of glomerular lesions, but not other renal lesions. Nevertheless, prospective studies which include all the confounding clinical factors are required to reach a conclusion on the relationship of hematuria and renal lesions.

Keywords Diabetic nephropathy · Renal biopsy · Chronic kidney disease · Renal glomerular lesions · Renal interstitial lesions

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Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease worldwide, making it a significant complication of diabetes. Numerous studies have investigated risk factors for the progression of clinically diagnosed diabetic nephropathy, but there is little research on biopsy-proven diabetic nephropathy populations.

Renal biopsy, a rather invasive procedure, is not commonly used to diagnose or monitor diabetic nephropathy. As a result, only atypical diabetic nephropathy presentations are considered for renal biopsy when other renal diseases are suspected. Although these patients may not represent the entire diabetic nephropathy population, the pathological data from their biopsies may help us better understand diabetic nephropathy pathophysiology.

The Renal Pathology Society (RPS) developed a universal pathological classification of diabetic nephropathy in 2010 [1]. Some research has been conducted to validate this classification's ability to predict the outcome [2–9]. However, the clinical implications of these pathological abnormalities are still being investigated. In this study, we collected data from patients with biopsy-proven diabetic nephropathy in our center between 2010 and 2019, reclassified their renal biopsies according to RPS classification, and analyzed each pathological lesion to demonstrate the clinical presentations of these lesions and determine their pre-biopsy predictors.

Methods

Study design

Data of 161 patients who had a renal biopsy in the past 10 years and were diagnosed with diabetic nephropathy in our center were collected. After excluding 42 patients with concomitant primary renal disorders or a history of immunosuppressive medication for autoimmune or malignant diseases, 119 patients were included in the study.

Clinical and laboratory data

The patients' clinical and laboratory data were obtained from our hospital's electronic records as well as the national health database (e-nabiz) if the patients gave consent upon registration.

The estimated glomerular filtration rate (eGFR) was calculated using the MDRD Eq. $(186 \times (\text{creatinine}/88.4) - 1.154 \times (\text{age}) - 0.203 \times (0.742 \text{ if female}) \times (1.210 \text{ if black}))$

Proteinuria was assessed using the protein to creatinine ratio from spot urine samples unless a 24-h urine test result was available. The presence of 5 or more red blood cells per microliter of urine sample was defined as hematuria. Urine samples after trauma (e.g., hours after renal biopsy or urinary catheter insertion) were excluded.

Renal biopsy and pathological classification

Specimens were obtained by experienced nephrologists using tru-cut biopsies and processed for light microscopy and immunofluorescence. During the study period, electron microscopy was not available at our center. Throughout the investigation, specimens were stained with hematoxylin and eosin, periodic acid-Schiff, crystal violet, or Masson's trichrome and examined by the same renal pathologist. The Renal Pathology Society's pathological classification of diabetic nephropathy, published in 2010 [1], was used to classify and score glomerular, vascular, and tubulointerstitial abnormalities.

In this classification, glomerular findings were categorized under four classes. In the absence of data suggesting class II, III, or IV, glomerular basement membrane (GBM) > 395 nm in females and > 430 nm in males was defined as class I. We did not have access to electron microscopy throughout the study period, thus we classified biopsies as class I in the presence of GBM thickness detectable with light microscopy and/or mild changes suggestive of diabetic nephropathy, as well as in the absence of higher class glomerular lesions. Class II was defined as the presence of mesangial expansion (formerly known as "diffuse diabetic glomerulosclerosis") but not meeting class III or IV criteria. They suggested identifying this class as IIA (mild) or IIB (severe). Because the number of class IIA or IIB patients in our population was very small, we preferred to analyze them as a single group for more robust statistical results. Unless it meets the criteria for class IV, the presence of at least one Kimmelstiel-Wilson lesion was classed as class III. Class IV, the most advanced form, was classified as global glomerulosclerosis in more than 50% of the glomeruli.

Interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation were the two types of interstitial lesions. The following was the IFTA scoring: no IFTA: 0, IFTA detected in less than 25% of interstitium: 1, 25 to 50% of the interstitium: 2, larger than 50%: 3. Interstitial inflammation was graded as follows: absent: 0, infiltration solely in relation to IFTA: 1, infiltration in non-IFTA areas: 2.

Arteriolar hyalinosis and arteriosclerosis were the two types of vascular abnormality. Arteriolar hyalinosis was rated as 0 when absent, 1 when only one area of arteriolar hyalinosis was identified, and 2 when more than one area of arteriolar hyalinosis was detected in the biopsy.

Arteriosclerosis was evaluated for the worst artery as following: 0 if no intimal thickening, 1 if intimal thickening less than thickness of media, 2 if intimal thickening greater than thickness of media.

Presence of an exudative lesion (capsular drop or hyaline cap) was also noted as present or absent.

Statistical analysis

Data were summarized as count and (percentage in the group) or as mean and (± standard deviation), as appropriate. Categorical variables were analyzed with the chi-squared test. Normal distributions of the continuous variables were assessed with Shapiro–Wilk's test. Continuous variables were compared using the *t*-test or ANOVA test, if distributed normally. Other continuous variables or ordinal variables were evaluated using Mann–Whitney *U* test or Kruskal–Wallis test, as appropriate. For post hoc analysis of the ANOVA and Kruskal–Wallis tests, Bonferroni correction was used. Univariate logistic regression analyses were performed for each renal lesion and relevant clinical variables ($p < 0.1$) were selected for multivariate logistic regression analyses. Relevant variables were included in multivariate analyses using enter method and presented as odds ratio (95% confidence interval). Two-tailed *p* value less than 0.05 was considered a statistically significant difference. Statistical analyses were performed with SPSS software (version 26.0, Chicago, IL, USA).

Results

Baseline characteristics

Baseline characteristics of study patients are presented in Table 1. The average age of the 119 patients in the study was 58.27 ± 12.14 (26–87) years. Females composed 52.1% of the study population. Only 3 patients were identified as type 1 diabetes, while the remainders were classified with type 2 diabetes. The average interval between diagnosis of diabetes and kidney biopsy was 8.61 ± 7.21 (0–30) years. Out of all the patients, 86.6% had hypertension, 42.9% had diabetic retinopathy (DRP), and 23.9% had coronary artery disease.

Nephrotic proteinuria with or without renal function loss was the primary reason for a renal biopsy in 44.5% ($n = 53$) of patients, acute kidney failure in 28.6% ($n = 34$), and chronic kidney failure with rapid progression in 26.9% ($n = 32$).

Associations between pathological lesions and clinical variables

Clinical and laboratory variables were compared between each level of pathological lesions. Diabetes mellitus duration, defined as the time between the diagnosis of diabetes and renal biopsy, was longer in patients with glomerular classes II and III compared to class I ($p = 0.018$, $p < 0.001$, respectively) (Table 1). Increasing severity of all lesions,

Table 1 Clinical and laboratory characteristics of patients with each glomerular class

	Glomerular class			
	I ($n = 47$)	II ($n = 16$)	III ($n = 39$)	IV ($n = 17$)
DM duration (years) ($n = 116$)	5.11 (± 5.24)	11.13 (± 7.04)[a]	11.23 (± 7.73)[a]	9.94 (± 7.37)
Diabetic retinopathy ($n = 112$)	4 (9.1%)	4 (30.8%)[a]	27 (71.1%)[a,b]	12 (70.6%)[a,b]
Systolic BP (mmHg) ($n = 94$)	137.7 (± 18.8)	133.5 (± 19.56)	133.45 (± 20.08)	150.58 (± 20.55)
Diastolic BP (mmHg) ($n = 94$)	85.41 (± 13.25)	79.43 (± 12.12)	78.55 (± 11.04)	87.75 (± 12.75)
HbA1c (%Hb) ($n = 115$)	7.16 (± 1.44)	7.87 (± 2.54)	8.64 (± 2.44)[a]	9.39 (± 3.52)
Urea (mg/dL) ($n = 118$)	45.94 (± 32.34)	72.38 (± 57.18)	78.16 (± 43.60)[a]	75.41 (± 35.58)[a]
Creatinine (mg/dL) ($n = 119$)	1.45 (± 1.86)	1.83 (± 1.75)	1.94 (± 1.58)[a]	2.53 (± 1.68)[a]
Estimated GFR (mL/min/1.73 m^2)	67.56 (± 29.31)[c,d]	51.93 (± 28.01)	48.24 (± 26.82)	36.54 (± 22.61)
Serum albumin (g/L) ($n = 119$)	38.1 (± 9)[c,d]	38.2 (± 6.1)[d]	31.9 (± 7.6)	29.6 (± 7)
Albuminuria (g/day) ($n = 65$)	1.99 (± 2.66)	1.64 (± 3.75)	3.11 (± 2.29)	3.31 (± 2.25)
Proteinuria (g/day) ($n = 118$)	2.77 (± 4.27)	1.81 (± 3.09)	5.04 (± 4.61)[a,b]	5.14 (± 3.34)[a,b]
Hematuria presence ($n = 116$)	5 (10.9%)	3 (18.8%)	12 (31.6%)[a]	7 (43.8%)[a]

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

[a]Greater than class I ($p < 0.05$)

[b]Greater than class II ($p < 0.05$)

[c]Greater than class III ($p < 0.05$)

[d]Greater than class IV ($p < 0.05$)

except arteriosclerosis, seems to be associated with longer duration of diabetes; however, the differences were not statistically significant.

Diabetic retinopathy was more frequently present in patients with glomerular class III compared to those with I and II ($p < 0.001$, $p = 0.01$, respectively), in class IV compared to class I and class II ($p < 0.001$, $p = 0.03$, respectively), and in class II compared to class I ($p = 0.048$); in those with IFTA score 3 compared to score 0, 1, and 2 ($p = 0.002$, $p = 0.01$, $p = 0.026$, respectively), interstitial inflammation score 2 compared to score 0 ($p = 0.015$), arteriolar hyalinosis score 1 and 2 compared to score 0 ($p = 0.01$, $p = 0.002$, respectively), arteriosclerosis score 1 and 2 compared to score 0 ($p = 0.011$, $p = 0.007$, respectively), and in patients with exudative lesions present ($p < 0.001$) (Tables 1, 2, 3, 4, 5 and 6). Degree of diabetic retinopathy (proliferative or non-proliferative) did not show any association with renal lesions.

Table 2 Clinical and laboratory characteristics of patients with each interstitial fibrosis and tubular atrophy score

	IFTA score			
	0 ($n = 24$)	1 ($n = 61$)	2 ($n = 20$)	3 ($n = 14$)
DM duration (years) ($n = 116$)	7.46 (±7.73)	7.86 (±6.85)	8.63 (±6.21)	13.71 (±7.59)
Diabetic retinopathy ($n = 112$)	6 (26.1%)	22 (40%)	8 (40%)	11 (78.6%)[a,b,c]
Systolic BP (mmHg) ($n = 94$)	145.42 (±23.54)	132.71 (±17.24)	133.2 (±12.16)	148.08 (±25.77)
Diastolic BP (mmHg) ($n = 94$)	87.11 (±13.05)	81.4 (±11.2)	78 (±12.07)	85.67 (±16.65)
HbA1c (%Hb) ($n = 115$)	7.28 (±1.39)	8.42 (±2.84)	7.73 (±1.96)	8.41 (±2.36)
Urea (mg/dL) ($n = 118$)	57.63 (±45.74)	60.58 (±41.39)	73.7 (±46.35)	76.93 (±37.34)
Creatinine (mg/dL) ($n = 119$)	1.72 (±2.53)	1.58 (±1.26)	2.24 (±1.96)	2.46 (±1.57)[a]
Estimated GFR (mL/min/1.73 m.^{2})	64.14 (±33.36)	58.49 (±28.68)	44.65 (±23.79)	36.34 (±22.29)
Serum albumin (g/L) ($n = 119$)	37 (±7.7)	35.2 (±9.3)	33.4 (±7.7)	32.1 (±8.1)
Albuminuria (g/day) ($n = 65$)	1.9 (±2.49)	2.47 (±2.45)	2.91 (±3.03)	3.63 (±2.8)
Proteinuria (g/day) ($n = 118$)	3 (±4.4)	3.74 (±4.49)	4.04 (±4)	4.41 (±3.82)
Hematuria presence ($n = 116$)	4 (16.7%)	13 (22%)	6 (30%)	4 (30.8%)

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

[a]Greater than no IFTA ($p < 0.05$)

[b]Greater than IFTA score 1 ($p < 0.05$)

[c]Greater than IFTA score 2 ($p < 0.05$)

[d]Greater than IFTA score 3 ($p < 0.05$)

Table 3 Clinical and laboratory characteristics of patients with each interstitial inflammation score

	Interstitial inflammation score		
	0 ($n = 27$)	1 ($n = 67$)	2 ($n = 25$)
DM duration (years) ($n = 116$)	6.12 (±6.85)	8.82 (±7.04)	10.56 (±7.55)
Diabetic retinopathy ($n = 112$)	7 (28.0%)	25 (39.7%)	15 (62.5%)[a]
Systolic BP (mmHg) ($n = 94$)	135.7 (±24.51)	135.11 (±17)	144.43 (±21.7)
Diastolic BP (mmHg) ($n = 94$)	82.5 (±13.33)	82.08 (±12.11)	83.81 (±13.96)
HbA1c (%Hb) ($n = 115$)	7.57 (±1.98)	8.31 (±2.57)	8 (±2.56)
Urea (mg/dL) ($n = 118$)	61.08 (±45.67)	55.69 (±41.18)	90 (±34.29)[a,b]
Creatinine (mg/dL) ($n = 119$)	1.81 (±2.42)	1.54 (±1.42)	2.59 (±1.5)[a,b]
Estimated GFR (mL/min/1.73 m^2)	59.32 (±30.77)[c]	61.85 (±28.33)[c]	30.52 (±15.45)
Serum albumin (g/L) ($n = 119$)	36.8 (±9)	34.7 (±9)	33.2 (±7)
Albuminuria (g/day) ($n = 65$)	1.98 (±2.28)	2.58 (±2.8)	3 (±2.42)
Proteinuria (g/day) ($n = 118$)	2.74 (±2.69)	3.61 (±4.58)	5.08 (±4.63)
Hematuria presence ($n = 116$)	5 (20.0%)	14 (21.2%)	8 (32.0%)

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

[a]Greater than no II ($p < 0.05$)

[b]Greater than II score 1 ($p < 0.05$)

[c]Greater than II score 2 ($p < 0.05$)

Table 4 Clinical and laboratory characteristics of patients with each arteriolar hyalinosis score

	Arteriolar hyalinosis score		
	0 ($n=28$)	1 ($n=39$)	2 ($n=52$)
DM duration (years) ($n=116$)	7.21 (±8.48)	8.25 (±6.56)	9.62 (±6.88)
Diabetic retinopathy ($n=112$)	4 (15.4%)	16 (47.1%)[a]	27 (51.9%)[a]
Systolic BP (mmHg) ($n=94$)	138.61 (±23.12)	139.9 (±19.67)	135.23 (±19.16)
Diastolic BP (mmHg) ($n=94$)	86.11 (±15.39)	85.41 (±13.15)	79.43 (±10.57)
HbA1c (%Hb) ($n=115$)	7.47 (±1.92)	7.81 (±2.3)	8.58 (±2.71)
Urea (mg/dL) ($n=118$)	49.11 (±36.73)	60.26 (±46.91)	74.87 (±40.26)[a,b]
Creatinine (mg/dL) ($n=119$)	1.42 (±1.63)	1.83 (±1.56)	2.02 (±1.92)[a]
Estimated GFR (mL/min/1.73 m^2)	69.54 (±33.37)[c]	55.23 (±29.14)	46.3 (±24.15)
Serum albumin (g/L) ($n=119$)	35.1 (±10.4)	35.1 (±8.3)	34.6 (±8)
Albuminuria (g/day) ($n=65$)	2.23 (±2.34)	2.56 (±2.91)	2.75 (±2.53)
Proteinuria (g/day) ($n=118$)	2.62 (±2.6)	4.33 (±5.48)	3.89 (±3.98)
Hematuria presence ($n=116$)	7 (25.9%)	3 (7.9%)	17 (33.3%)[b]

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

[a]Greater than no AH ($p<0.05$)

[b]Greater than AH score 1 ($p<0.05$)

[c]Greater than AH score 2 ($p<0.05$)

Table 5 Clinical and laboratory characteristics of patients with each arteriosclerosis score

	Arteriosclerosis score		
	0 ($n=25$)	1 ($n=42$)	2 ($n=52$)
Male sex	6 (24.0%)	24 (57.1%)[a]	27 (51.9%)[a]
DM duration (years) ($n=116$)	9.28 (±8.41)	6.54 (±6.11)	9.98 (±7.14)
Diabetic retinopathy ($n=112$)	4 (16.7%)	18 (48.6%)[a]	25 (49.0%)[a]
Systolic BP (mmHg) ($n=94$)	136.2 (±22.95)	139.97 (±21.3)	135.87 (±18.33)
Diastolic BP (mmHg) ($n=94$)	84 (±15.02)	83.75 (±14.54)	81.28 (±10.5)
HbA1c (%Hb) ($n=115$)	7.45 (±1.29)	8.31 (±2.83)	8.19 (±2.51)
Urea (mg/dL) ($n=118$)	55.72 (±47.18)	61.41 (±43.71)	70.35 (±39.63)
Creatinine (mg/dL) ($n=119$)	1.41 (±1.49)	1.99 (±2.2)	1.88 (±1.42)[a]
Estimated GFR (mL/min/1.73 m^2)	66.6 (±33.16)	54.75 (±29.67)	48.92 (±25.88)
Serum albumin (g/L) ($n=119$)	35.9 (±9)	35.2 (±8.8)	34.1 (±8.4)
Albuminuria (g/day) ($n=65$)	1.24 (±1.34)	2.69 (±2.55)	3.2 (±2.94)
Proteinuria (g/day) ($n=118$)	1.99 (±1.56)	3.88 (±4.71)	4.41 (±4.62)
Hematuria presence ($n=116$)	8 (32.0%)	8 (19.5%)	11 (22.0%)

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

[a]Greater than no AS ($p<0.05$)

Higher systolic BP was observed in glomerular class IV and interstitial inflammation score 2 patients; however, there was no statistically significant difference between groups in terms of systolic or diastolic BP (Tables 1 and 3).

Glycated hemoglobin (HbA1c) levels were significantly higher in patients with glomerular class III compared to those with class I ($p=0.032$) (Table 1). There was no other statistically significant difference in terms of HbA1c between subgroups of other lesions.

The proteinuria levels of patients with glomerular class IV were higher than those with class I and II ($p=0.01$, $p=0.005$, respectively). Glomerular class III patients had significantly higher proteinuria levels than class I and II patients ($p=0.007$, $p=0.007$, respectively). Also, the serum albumin levels of class IV patients were lower than those of class I and II ($p<0.001$, $p=0.032$, respectively), and class III than those of class I ($p<0.001$) (Table 1). In a similar fashion, the proteinuria levels of patients with exudative lesions were significantly higher ($p=0.04$) and serum albumin levels were lower ($p=0.033$) (Table 6).

Hematuria occurrence was more frequent in glomerular class III and IV patients compared to class I ($p=0.019$, $p=0.004$, respectively), and in patients

Table 6 Clinical and laboratory characteristics of patients with or without exudative lesions

	Exudative lesions	
	Absent ($n=103$)	Present ($n=16$)
DM duration (years) ($n=116$)	8.34 (±6.96)	10.47 (±8.7)
Diabetic retinopathy ($n=112$)	34 (35.4%)	13 (81.3%)*
Systolic BP (mmHg) ($n=94$)	136.6 (±19.49)	142.25 (±23.6)
Diastolic BP (mmHg) ($n=94$)	82.4 (±12.6)	83.58 (±13.67)
HbA1c (%Hb) ($n=115$)	8.06 (±2.41)	8.25 (±2.76)
Urea (mg/dL) ($n=118$)	61.95 (±42.74)	78.13 (±41.55)
Creatinine (mg/dL) ($n=119$)	1.73 (±1.74)	2.41 (±1.72)*
Estimated GFR (mL/min/1.73 m^2)	56.78 (±29.45)*	41.28 (±26.02)
Serum albumin (g/L) ($n=119$)	35.6 (±8.5)*	30.4 (±8.5)
Albuminuria (g/day) ($n=65$)	2.41 (±2.6)	3.41 (±2.55)
Proteinuria (g/day) ($n=118$)	3.38 (±4.05)	5.93 (±5.19)*
Hematuria presence ($n=116$)	22 (21.8%)	5 (33.3%)

DM diabetes mellitus, *BP* blood pressure, *HbA1c* glycated hemoglobin, *GFR* glomerular filtration rate

*$p<0.05$

with arteriolar hyalinosis score 2 compared to score 1 ($p=0.004$) (Tables 1 and 4).

Gender distribution was indifferent between subgroups of all lesions but arteriosclerosis. Male gender was more frequent in patients with arteriosclerosis score 1 and 2 compared to score 0 ($p=0.008$, $p=0.02$, respectively) (Table 5).

Univariate logistic regression analyses for each renal lesion performed with age, gender, diabetes duration, diabetic retinopathy, coronary artery disease, hypertension, hematocrit, HbA1c, eGFR, proteinuria, and hematuria. Multivariate logistic regression models were created using the relevant ($p<0.1$) variables found in univariate analyses for each renal lesion (Table 7).

Discussion

Diabetic retinopathy (DRP), which may lead to blindness, has been reported in 34.6% of general diabetic population [10]. Epidemiological studies have repeatedly demonstrated that DRP is a predictor of nephropathy development [11–13]. In our study population, all of whom had biopsy-proven nephropathy, DRP has been detected in only 42%. This rate was 65.3% in a similarly constructed study reported by Cao et al. [14] and 48% in the study population of Zhang et al. [9]. Because of the coexistence of two clinical entities,

Table 7 Multivariate regression models predicting each renal lesion

Variables*	Glomerular class III–IV	IFTA	Interstitial inflammation	Arteriolar hyalinosis	Arteriosclerosis	Exudative lesions
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	p value	*p* value	*p* value	*p* value	*p* value	*p* value
Male sex	–	–	–	–	4.87 (1.14–20.78) **0.033**	–
Hypertension	9.73 (1.49–63.6) **0.018**	0.83 (0.12–5.59) 0.845	–	1.77 (0.53–5.92) 0.357	–	–
DM duration (years)	1.01 (0.92–1.1) 0.873	–	1.02 (0.94–1.1) 0.705	–	–	–
Diabetic retinopathy	8.64 (2.56–29.1) **0.001**	0.9 (0.21–3.89) 0.888	1.17 (0.34–4.02) 0.803	3.81 (1.15–12.62) **0.029**	1.25 (0.23–6.91) 0.798	4.67 (1.05–20.81) **0.043**
HbA1c (%Hb)	–	1.42 (0.96–2.11) 0.081	–	–	1.37 (0.87–2.15) 0.174	–
Hematocrit (%)	0.92 (0.84–1.01) 0.071	–	0.9 (0.83–0.99) **0.031**	–	–	0.95 (0.85–1.07) 0.391
Estimated GFR (mL/min/1.73 m^2)	0.99 (0.97–1.01) 0.448	0.97 (0.94–0.99) **0.005**	–	0.98 (0.97–1) 0.069	0.96 (0.93–0.99) **0.007**	1 (0.97–1.02) 0.849
Proteinuria (g/day)	1.26 (1.07–1.48) **0.005**	–	–	–	1.35 (0.93–1.97) 0.117	1.08 (0.96–1.22) 0.192
Hematuria presence	4.94 (1.11–22.1) **0.036**	–	–	–	–	–

Glomerular class was grouped as I–II or III–IV, while other lesions were grouped as present or absent for these binary logistic regression analyses

*Only relevant variables ($p<0.1$ in univariate analysis) for each lesion were included in multivariate models

higher DRP rates may be expected in this group of clinically advanced diabetic nephropathy patients. However, because our study population was composed of patients who were chosen for a renal biopsy, due to their advanced renal disease inconsistent with their diabetes duration or other diabetic complications, patients without retinopathy predominated in our study. Among this special group of patients who underwent renal biopsy for various reasons, the diabetic retinopathy subgroup had more advanced glomerular class, IFTA, interstitial inflammation, arteriolar hyalinosis, arteriosclerosis, and more frequently had exudative lesions. Multivariate regression analyses revealed that diabetic retinopathy was an independent predictor of glomerular class III–IV, presence of arteriolar hyalinosis, and exudative lesions. In accordance with our findings, previous studies reported that DRP was in strong relationship with nodular glomerular lesions [15, 16]. Zhang et al. [9] also demonstrated that advanced glomerular lesion was an independent risk factor for DRP. Chavers et al. [17] found that mesangial expansion and arteriolar hyalinosis scores correlated with DRP scores in a group of type 1 DM patients who were candidates for pancreatic transplantation.

Proteinuria, along with renal function loss, is the most important finding of diabetic nephropathy. The degree of proteinuria is related to advanced glomerular lesions in diabetic nephropathy [18]. In our study, proteinuria levels appear to be higher in patients with more severe pathological lesions. Those with glomerular classes III and IV and exudative lesions exhibited significantly greater proteinuria levels. Higher proteinuria levels predicted glomerular class III–IV lesions, but not other lesions in multivariate analyses.

The role of hematuria in diabetic nephropathy is debatable. The occurrence of hematuria was significantly more frequent in patients with glomerular class III–IV or advanced arteriolar hyalinosis and was an independent predictor of glomerular class III–IV in our investigation, but not with other lesions. Matsumura et al. [19] reported that the patients with hematuria in their study had diffuse, nodular, and exudative glomerular lesions and interstitial lesions more frequent than those without. Wu et al. [20] found that patients with hematuria had more advanced interstitial inflammation lesions, and the number of red blood cells in urine sediment correlated with glomerular classes, IFTA scores, and interstitial inflammation scores. Okada et al. [3], on the other hand, found that hematuria was an independent predictor of arteriolar hyalinization but not of nodular glomerular lesions in their study, which only included individuals with overt proteinuria. The physiopathology of hematuria in diabetic nephropathy is still debated, and extensive research is needed to clarify it.

Although having a relatively large population of biopsy proven diabetic nephropathy patients, all of who were assessed by the same team of nephrologists and ophthalmologists, and whose biopsies were reclassified by the same experienced pathologist, our research has some limitations. First of all, our retrospective design prevents our findings to prove any casual relationships, and we had some missing information such as weight, BMI, history of smoking, and alcohol intake of the patients. Second, we were unable to access data prior to 2010, preventing us from verifying patient data such as duration of diabetes. Third, we did not have access to an electron microscope; thus, we were unable to measure the glomerular basement membrane widths.

Conclusions

In conclusion, this large, single center, retrospective study reveals that diabetic retinopathy is associated with glomerular and arteriolar lesions but not with interstitial lesions. Proteinuria and hematuria were independent predictors of glomerular lesions, but not other renal lesions. Nevertheless, prospective studies which include all the confounding clinical factors are required to reach a conclusion on the relationship of hematuria and renal lesions.

Data Availability All data will be made available upon request.

Declarations

Ethics approval This retrospective study was conducted with the approval of the 1st Ethics Committee of Ankara City Hospital (Date: 12.12.19, Decision No: E-1–19-187) and in compliance with the Declaration of Helsinki's ethical principles.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Cytokine gene polymorphism with type 2 diabetes and diabetic nephropathy in population from West India

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Abstract

Objective Genetic polymorphisms of the angiogenesis, inflammatory cascade, or apoptosis genes can influence chronic complications in diabetic patients. Cytokine gene polymorphisms are considered vital in diabetes and diabetic nephropathy (DN) susceptibility. The present study evaluated the role of cytokine gene polymorphism in type 2 diabetes and diabetic nephropathy.

Methods A total number of 648 participants comprising 180 healthy individuals, 164 type 2 diabetes mellitus patients without any complications, 148 individuals with diabetic nephropathy, and 156 with non-diabetic nephropathy were included in this study. The *IL-6* (-634 C/G), *IL-18* (-607 A/C), *IL-4* (-590C/T), and *IL-10* (-592 C/A) polymorphism were analyzed using the PCR-RFLP method, and their expression analysis was done using real-time PCR

Results We found a significant difference in the genotype frequency of *IL-6* and *IL-10* in the diabetic nephropathy group compared to the control, whereas no significant difference was found in *IL-18* and *IL-4*.

Conclusion The *IL-6* -634C/G and *IL-10*-592 C/A polymorphisms were found to be associated with diabetic nephropathy in the West Indian population. The higher transcript level of inflammatory cytokines in patient groups compared to the control group may suggest the essential role of inflammation in the pathogenesis of diabetes and diabetic nephropathy.

Keywords Diabetic nephropathy · Polymorphism · Cytokine expression · Genotype

Abbreviations

T2DM	Type 2 diabetes
DN	Diabetic nephropathy
NDN	Non-diabetic nephropathy
PCR	Polymerase chain reaction
RFLP	Restriction length polymorphism
IL	Interleukin
ADA	American diabetes association
GAPDH	Glyceraldehydes-3-phosphatedehydrogenase
HbA1c	Glycosylated hemoglobin
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
BMI	Body mass index
NF-AT	Nuclear factor of activated T-cell
GN	Glomerulonephritis
ESRD	End-stage renal disease
TNF-α	Tumor necrosis factor-alpha
AGE	Advanced glycosylated end product
PBMCs	Peripheral blood mononuclear cells
MS	Multiple sclerosis
SNPs	Single nucleotide polymorphism
IFN-γ	Interferon gamma

Introduction

Inflammation and the immune system have been reported to be implicated in diabetes and its complications. Type 2 diabetes (T2DM) is the most significant health challenge today. Diabetic nephropathy (DN) is a major complication of T2DM, which leads to end-stage renal disease (ESRD) [1]. Cytokines are crucial in elucidating inflammation by participating in diverse cytokine-associated signaling pathways [2]. The cytokine system is intricate, involving a series of gene activation and suppression [3]. The inflammatory

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cytokines *IL1*, *IL-6*, *IL-18*, and *TNFα* are known to play essential roles in developing T2DM and DN, with various actions and developing complications [4, 5].

Genetic and molecular studies have improved our understanding of the pathophysiology of T2DM and DN in recent years [6, 7]. Different methods can be used to identify genes linked to DN susceptibility. Many established methods exist for identifying candidate genes, including candidate gene approaches. No single gene with a significant effect has been identified at the movement, and only minor effects of various polymorphisms in several genes have been reported. Neither linkage analysis nor association studies are performed [8].

Anti-inflammatory and immune-suppressive factors primarily cause diabetic nephropathy. Consequently, diabetic nephropathy may be retarded by reducing the inflammatory response and making the patient more susceptible to infection. Serum and urinary levels of *IL-6* and *IL-18* have also been shown to be increased in patients suffering from DN [9]. Two polymorphisms, -137 G/C and -607 C/A, within the *IL-18* promoter region have been identified to increase the transcriptional activity of the promoter upon binding with its transcription factor [10]. Interleukin-10 (*IL-10*) and Interleukin-4 (*IL-4*) fulfill the criteria for an anti-inflammatory and immunosuppressive cytokine [11]. Three polymorphisms of the *IL-10* gene, -1082 G/A, -829C/T, and -592 C/A, were studied, which showed that these polymorphisms might have a significant impact on *IL-10* expression [12, 13].

Therefore, assessing gene polymorphisms of inflammatory cytokines may be a critical factor in developing microvascular diabetic complications, including nephropathy. In this context, the study aimed to examine the association of the promoter polymorphisms of *IL-6* -634 C/G (rs1800796), *IL-18* -607 A/C (rs1946518), *IL-4* -590 C/T (rs2243250), and *IL-10* -592 C/A (rs1800872) and expression level of cytokines in diabetes and diabetic nephropathy in West Indian population.

Materials and Methods

Study design

The study was conducted in 4 different groups based on diagnosis by a physician; they are (1) healthy control ($n = 180$), (2) type 2 diabetes ($n = 164$) without any complications, (3) T2DM with nephropathy ($n = 148$), and (4) non-diabetic nephropathy ($n = 156$), and the person with another disease were excluded from the study. An American Diabetes Association (ADA) recommendation led to diabetes mellitus being diagnosed. Diagnosis of diabetes begins at the time the patient is diagnosed. Nephropathy was diagnosed by persistent microalbuminuria (20–200 mg/24 h) or proteinuria (> 200 mg/24 h) in diabetic and non-diabetic participants by a consulting physician. The participants were recruited based on their availability and willingness to participate.

The ethical approval was obtained from Muljibhai Patel Urological Hospital Nadiad, Gujarat, India (EC/236/2013). Detailed medical and clinical demography were obtained after getting informed concerns from each study participant.

Isolation of DNA and genotyping of cytokine genes

Genomic DNA was extracted from peripheral blood by the phenol: chloroform method [14]. Genotyping of the cytokine genes was checked by the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). The *IL-4* -590 C/T [15], *IL-6* -634 C/G [16], *IL-10* -592 C/A [17], and *IL-18* -607 A/C [18] polymorphisms were analyzed using previously reported primers.

Determination of cytokine expression

RNA isolation and cDNA preparation

The Trizol method was used to isolate total RNA from blood samples. The cDNA was synthesized by an oligo-dT18TNV primer and a reverse transcriptase [19]. The quality and quantity of total RNA were checked by agarose gel electrophoresis and spectrophotometrically at 260/280 nm, respectively.

Quantitative real-time-PCR

The QuantiTect SYBR Green technology (QIAGEN) was used for real-time PCR. The reactions were performed by the Rotor gene 6000 (Corbett research). The purity of the amplified PCR products was verified by melting curves. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as the housekeeping gene to normalize the values. The primers used in this study were selected based on a previous report [19].

Statistical analysis

Statistical analysis was done by SPSS version 22.0. The Hardy–Weinberg equilibrium was analyzed by the chi-square method. The $\chi 2$ independence test with 3×2 and 2×2 contingency tables and z-statistics was used to find allele positivity, allele frequencies, and genotype distribution. Fisher's exact test performed allele frequencies and allele positivity in all the studied groups. The statistical *t*-test was considered significant with a *p*-value of 0.05 or less.

Results

Clinical characteristics

The individuals were grouped: (1) healthy control ($n = 180$), (2) type 2 diabetes ($n = 164$) without any complications, (3)

T2DM with nephropathy ($n=148$), and (4) non-diabetic nephropathy ($n=156$), and person with another disease were excluded from the study. A total of 648 individuals were successfully genotyped. The statistical analysis was done with various statistical tools. The clinical and biochemical characteristics of all the studied groups are summarized in (Table 1).

Effects of polymorphisms on T2DM, DN, and NDN risk

IL-4 polymorphism

Table 2 shows the genotypic distribution of all the studied groups. The wild-type C allele and CC genotype were dominant in all the studied groups, whereas the altered allele (T) was dominant in T2DM. The altered genotype (-590 C/T) was higher in the T2DM group (Table 2) than the other three. We found a significant difference between the T2DM and DN groups ($p=0.001$) in the C/T genotype (Table 3). The transcription level of *IL-4* was upregulated in patients compared to controls (Table 4). We found a higher level of *IL-4* (-590 C/T) in DN compared to controls and T2DM patients. The *IL-4* (-590 C/T) genotypes show a different level of mRNA transcription in the CC genotype, which showed a higher expression level in all studied groups (Table 4).

Table 2 Genotype and allelic frequency of *IL-4* (-590 C/T), *IL-6* (-634 C/G), *IL-10* (-592 C/A), and *IL-18* (-607 A/C)

Gene polymorphism	Genotype and allele frequency	Control	T2DM	DN	NDN
IL-4 (-590 C/T)	CC	77.62	67.96	87.25	70.37
	CT	19.58	30.09	12.74	28.70
	TT	2.79	1.39	0.00	0.92
	C	88.65	83.00	93.62	84.72
	T	11.34	16.99	6.37	15.27
IL-6 (-634 C/G)	CC	67.13	68.93	58.82	60.18
	CG	25.17	25.24	25.49	24.07
	GG	7.69	5.84	15.68	15.74
	C	79.72	81.55	71.56	72.22
	G	20.27	18.44	28.43	27.77
IL-10 (-592 C/A)	CC	22.37	25.24	52.94	45.37
	AC	58.04	53.39	45.09	45.37
	AA	19.58	21.35	1.96	9.25
	C	51.39	51.94	75.49	68.05
	A	48.60	48.05	24.50	31.94
IL-18 (-607 A/C)	CC	45.45	36.89	45.09	39.81
	AC	28.67	20.38	23.52	43.51
	AA	25.87	42.71	31.37	16.66
	C	59.16	58.25	60.78	49.09
	A	40.83	41.74	39.21	50.90

T2DM type 2 diabetes mellitus, *DN* diabetic nephropathy, *NDN* non-diabetic nephropathy

IL-6 polymorphism

The genotype frequency of CC was higher than CG and GG in patients and controls. The allelic frequency of the C was more elevated in T2DM compared to DN and NDN patients (Table 2). The genotype frequency was significantly similar in the T2DM patients compared to DN ($p=0.02$) and NDN ($p=0.02$) patients (Table 3). In RT-PCR, we found higher levels of *IL-6* in patient groups compared to the control, which showed that inflammation in T2DM and its complications increases the *IL-6* levels (Table 4). When *IL-6* mRNA level was estimated for promoter genotype, we found that CC homozygote had the highest expression level compared to other genotypes in all the study groups. Notably, among all the studied groups, the DN group had a maximum level of *IL-6* with CC genotype (Table 4). The reduction in the GG genotype frequency in the DN group suggests that it grants a defensive effect against the progression of diabetic nephropathy.

IL-10 polymorphism

The RFLP study on polymorphisms of the *IL-10* gene showed a significant difference in genotype frequency of

Table 1 Clinical and biochemical characteristics of studied participants

Variables	Control	T2DM	DN	NDN
Number (*n*)	180	164	148	156
Age (years)	44 ± 11.2	52 ± 7.3	58 ± 12.5	51 ± 12.3
BMI (kg/m^2)	20.7 ± 2.5	23.5 ± 2.5	23.6 ± 2.4	21.8 ± 1.5
SBP (mm Hg)	138.5 ± 8.1	147.3 ± 11.3	149.25 ± 12.5	140.35 ± 10.9
DBP (mm Hg)	91.33 ± 5.3	95.54 ± 7.3	96.2 ± 8.1	92.01 ± 7.1
Creatinine (mg/dl)	0.56 ± 0.6	0.83 ± 0.6	4.23 ± 0.4	3.87 ± 0.8
HbAC1	4.3	7.1	8.6	5.2

Data are expressed as mean ± SD

BMI body mass index, *HbAC1* hemoglobin A1C, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *DM* diabetes mellitus, *DN* diabetic nephropathy, *NDN* non-diabetic nephropathy

Table 3 Strength of association (OR, 95%CI) of various studied groups for all gene polymorphisms

Gene polymorphism	C vs T2DM[a]	C vs DN[b]	C vs NDN[c]	T2DM vs DN[d]	T2DM vs NDN[e]
IL-4 (-590 C/T)	1.59 (0.92–2.76)	0.53 (0.25–1.08)	1.40 (0.81–2.45)	0.33 (0.16–0.67)	0.88 (0.50–1.52)
IL-6 (-634 C/G)	0.88 (0.55–1.43)	1.56 (1.00–2.42)	1.51 (0.97–2.33)	1.75 (1.07–2.87)	1.70 (1.04–2.76)
IL-10 (-592 C/A)	0.97 (0.67–1.42)	0.34 (0.22–0.51)	0.49 (0.33–0.72)	0.35 (0.22–0.54)	0.50 (0.33–0.70)
IL-18 (-607 A/C)	1.039 (0.71–1.51)	0.93 (0.63–1.37)	1.50 (1.03–2.17)	0.90 (0.59–1.36)	1.44 (0.96–2.10)

C control group, *T2DM* type 2 diabetes mellitus, *DN* diabetic nephropathy, *NDN* non-diabetic nephropathy, *OR* odds ratio, *CI* confidence interval

[a,b,c]For strength of association study, reference is control group

[d,e]For strength of association study, reference is T2DM group

Table 4 Quantitative profile of mRNA level and genotype distribution of polymorphisms in different groups

Gene SNP	Geno type	Control		T2DM		DN		NDN	
		Average ΔΔCt value	Fold	Average ΔΔCt value	Fold	Average ΔΔCt value	Fold	Average ΔΔCt value	Fold
IL-4 (-590 C/T)	CC	3.46	11	3.700	13	4.169	18	3.807	14
	CT	1.585	3	2.807	7	3.321	10	3.46	11
	TT	0	1	1	2	3	8	2.584	6
IL-6 (-634 C/G)	CC	0.847	1.8	2	4	3	8	2	4
	CG	0.137	1.1	0	1	0.584	1.5	−1	0.5
	GG	−3.32	0.1	0	1	−1	0.5	1	2
IL-10 (-592 C/A)	CC	0.263	1.2	2.321	5	3.472	11.1	2	4
	AC	1.070	2.1	2.807	7	4.153	17.8	4.392	21
	AA	−0.51	0.7	2	4	1.584	3	2.807	7
IL-18 (-607 A/C)	CC	1.956	3.88	2.807	7	3.817	14.1	3.807	14
	AC	−1.556	0.34	1.157	2.23	2.584	6	1.226	2.34
	AA	1.263	2.4	1.584	3	3	8	2.321	5

T2DM type 2 diabetes mellitus, *DN* diabetic nephropathy, *NDN* non-diabetic nephropathy

NDN ($p = 0.01$) and DN ($p = 0.01$) patients compared to the control. The C allele frequency was higher in DN and NDN groups compared to the T2DM and control (Table 2). According to statistical analysis, the genotypes and allele frequency of the T2DM group were not different compared to the control group (Table 3). The transcriptional expression level of the *IL-10* was higher in the patients' groups than in the control group. The low-grade systemic inflammation in type 2 diabetes activates the *IL-10* expression (Table 4).

IL-18 polymorphism

In NDN ($p = 0.02$), we found a significant difference in the genotype frequency compared to the control at a significance level of $p \leq 0.05$. We found a substantial difference in the NDN group compared to the DN group ($p = 0.06$). No significant association was found in the genotype frequency of DM and DN patients compared to the control. All the studied groups had a higher C frequency than the A allele. The mRNA profile showed that the *IL-18* level was higher in the patient groups than in the control group because of the inflammation observed in T2DM (Table 4). The CC genotype was highly expressed compared to other genotypes of the *IL-18* cytokine. Therefore, it can suggest that the C allele influences *IL-18* expression in DN and NDN.

Discussion

The major pathological cause of T2DM and its inflammatory complications like nephropathy is unclear. It was reported that inflammatory cytokines could contribute to the pathogenesis of T2DM and its complications [20]. Several allelic polymorphisms of cytokine genes that regulate gene transcription had demonstrable clinical significance. The gene transcription and cytokine secretion may be influenced by polymorphisms in cytokine genes leading to renal disease [21]. In the present work, we analyzed the association of four gene polymorphisms (*IL-4*, *IL-6*, *IL-10*, and *IL-18*) in patients with T2DM, DN, and NDN.

We did not find a significant association of *IL-4 (*-590 C/T) polymorphism with DM and DN groups in the West Indian population. In contrast, a study on north Indian people showed a significant association of *IL-4* gene polymorphism with T2DM [22]. Another study supports our results, which verified that there was no significant difference found in the distribution of *IL-4* genotypes between the T1DM (Type 1 Diabetes Mellitus) group and the control group [23]. A study in the Indian and Japanese populations found that *IL-4* polymorphism is linked with renal disease [24, 25].

IL-4 expression has not been determined in diabetes or diabetic nephropathy. In this study, we got the *IL-4* expression for polymorphism rs2243250 and found an increased level of *IL-4* in DN compared to DM and control. Ballardie et al. showed an upregulation of *IL-4* in IgA nephropathy [26].

Kitamura et al. reported that the GG genotype and G-allele of *IL-6* C/G polymorphism could be used to predict the progression of T2DM and complications like DN [16]. Similar to the above study, we found a significant positive association of the G/G homozygous allele with DN in the West Indian population. In the expression study of *IL-6* for SNP -634 C/G, we found higher levels of *IL-6* in patient groups compared to the control group. It was given that inflammation in T2DM and its complications increase IL-6 levels. Aso et al. studied the relationship between inflammation and DN, finding that the *IL-6* level was significantly higher in the T2DM group than in the control group [27]. An *IL-6* -634 C/G polymorphism has been reported to increase the secretion of *IL-6* by peripheral blood mononuclear cells (PBMCs) [9]. It has been demonstrated that *IL-6* promoter polymorphisms are vital regulators of the *IL-6* gene and downstream protein levels in vitro and in vivo [28]. Therefore, *IL-6* polymorphism may be a predisposing factor for developing DN in the individual.

The present study indicates a significant difference in genotypes and alleles of the -592 region of the *IL-10* gene between T2DM patients with and without nephropathy compared to the control group. We did not find a significant difference between the T2DM and the control groups. Our data is well in accordance with results observed by Scarpelli et al., who reported that *IL-10* gene polymorphism is not associated with T2DM [29]. On the other hand, Chang et al. reported a significant relationship between T2DM without nephropathy and healthy controls regarding the -592 region of the *IL-10* gene [30]. Similar to the results of Kolla et al. and Mtiraoui et al., we also found the association of *IL-10* -592 A/C gene polymorphism with DN and NDN groups in the West Indian population [31, 32].

Van Exel et al. found that metabolic syndrome and type 2 diabetes are associated with lower levels of *IL-10* production [33]. It was noted by Karjalainen et al. that lower expression of *IL-10* is related to the C allele [34]. Due to the findings in patients from the southeastern region of Iran, higher frequencies of the C allele and the C/C genotype in patients with T2DM, multiple sclerosis (MS), and asthma may result in decreased *IL-10* expression through immune cells [35]. Similar to this study, we also found lower expression of *IL-10* in the CC genotype.

Several studies have shown conflicting results regarding the SNPs of the *IL-18* gene promoter polymorphism in people with autoimmune diabetes. The prevalence of the AA genotype decreased [36], likely due to the lower incidence of T1DM in the Japanese population. We also found a reduced frequency of the AA genotype in control, DM, and DN groups relative to CC and AC genotypes. Similar results have been reported in the Brazilian population, in which there was no association of *IL-18* -607 A/C (rs1946518) gene polymorphisms with T1DM [37].

Similar to our results, Moriwaki and his colleagues reported a higher level of *IL-18* in the T2DM group than in the control group [38]. Besides, *IL-18* was also increased in T2DM patients with the development of urinary albumin excretion. Nakamura et al. observed that urinary albumin excretion rates among people with diabetes were associated with urinary and serum *IL-18* levels [9]. According to Ide et al., the higher promoter activity of haplotype -137G/-607C of the *IL-18* gene might lead to increased expression of *IL-18*, resulting in enhanced IFN-γ-producing T-cells [36]. Similar to this study, we report that *IL-18* -607 A/C polymorphism can increase *IL-18* expression levels in the patient's group. The CC genotype was found to have the highest *IL-18* expression compared to other genotypes. Therefore, it is suggested that the C allele can influence *IL-18* expression in DN and NDN.

Conclusion

Our study indicates that IL-6 and IL-10 gene polymorphisms may be involved in the pathogenesis of type 2 diabetes and diabetic nephropathy. Moreover, we observed increased expression of pro-inflammatory cytokines IL-6 and IL-18, as well as anti-inflammatory cytokines IL-4 and IL-10 in diabetic and nephropathic patients, suggesting an association between inflammation and the development of these conditions. Our findings highlight the need for further investigation into the mechanisms underlying cytokine gene polymorphisms and their impact on inflammation in diabetes and diabetic nephropathy, with larger sample sizes to improve the generalizability of the results.

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Declarations

Ethics approval This study was approved by the institutional ethical committee of Muljibhai Patel Urological Hospital, Nadiad, Gujarat, India (EC/236/2013).

Consent to participate After an explanation of the purpose of this study, signed informed consent forms were obtained from the participants incorporated in this study.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

The value of contrast-enhanced ultrasound in the diagnosis of microcirculatory perfusion abnormalities in diabetic foot

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Abstract

Background Diabetic foot is one of the most serious complications of type 2 diabetes mellitus (T2DM), and its incidence is increasing in China. Early detection of abnormal microcirculation in the foot is very important for the prevention and treatment of diabetic foot.

Objective To investigate the value of contrast-enhanced ultrasound (CEUS) in diagnosing microcirculatory alterations in the dorsum of the foot for patients with type 2 diabetes mellitus (T2DM).

Methods Eighty-eight T2DM patients were included, among them 30 patients sustained diabetes mellitus without complications (group A), 28 with lesions in the dorsum of the foot (no acute infection) that can be classified as Wagner grade 0 ~ 1 (group B), and 30 with lesions in the dorsum of the foot that can be classified as Wagner grade 2–5 (group C). Another 30 healthy adults were included as the control group. All subjects underwent CEUS to examine the dorsalis pedis arteries and blood perfusion to the underlying soft tissues. Parameters of the time-intensity curve (TIC), including rise time (RT), ascending slope (AS), time to peak (TTP), peak intensity (PI), area under the curve (AUC), and half of drop time (DT/2) were analyzed.

Results The analysis of TIC data of the dorsalis pedis arteries showed that group C had decreased AS, PI, and AUC and increased TTP, RT, and DT/2 compared with groups A, B, and the control group; the differences were statistically significant ($p < 0.05$). The analysis of TIC data of the perfusion to the underlying soft tissues showed that AS, PI, and AUC decreased from the control group through group A, B, and then C; the differences were all statistically significant ($p < 0.05$). The TIC data were correlated with the severity of microcirculatory impairment in the dorsum of the foot and among them the AUC, PI, and AS had higher predictive value.

Conclusions Microcirculatory impairment in the dorsum of the foot in T2DM patients presents itself as "delayed wash-in, delayed wash-out, and weak enhancement" on CEUS images. CEUS can provide quantification of the microcirculatory changes in the soft tissues in the dorsum of the foot and reflect the differences of microcirculatory perfusion across different grades of lesions.

Keywords Contrast-enhanced ultrasound · Diabetic foot · Microcirculatory perfusion

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Introduction

Diabetic foot is one of the serious chronic complications of type 2 diabetes mellitus (T2DM) with an increasing morbidity and high disability and mortality rates [1–5]. Microcirculatory dysfunction is an important pathological basis for its pathogenesis and an important factor in determining its prognosis [6–9]. Therefore, early detection and accurate assessment of microcirculatory perfusion abnormalities in the foot are essential for the prevention and treatment of diabetic foot. Contrast-enhanced ultrasound (CEUS) can provide quantitative assessment of the microcirculatory perfusion in tissues [10, 11] and has been widely used to characterize liver and kidney lesions [12–16]. However, few studies using CEUS to assess microcirculatory perfusion in diabetic foot have been reported. The study applied CEUS to assess soft tissue perfusion in the dorsum of the foot in order to provide more knowledge on the varied microcirculatory impairment across different grades of lesions in diabetic foot and to investigate the value of CEUS in diagnosing microcirculatory perfusion abnormalities.

Materials and methods

Eighty-eight patients with T2DM admitted to the Department of Endocrinology of the Hospital of Chengdu University of TCM from October 2021 to March 2023 were included, all of whom met the diagnostic criteria of diabetes mellitus by the American Diabetes Association (2021 edition). Among them, 30 patients sustained T2DM alone without peripheral vasculopathy and neuropathy (group A); 28 patients were with lesions in the dorsum of the foot that were classified as Wagner grade 0–1 (group B); 30 patients were with lesions in the dorsum of the foot that were classified as Wagner grade 2–5 (group C) (see Table 1 for the composition); 30 adults without T2DM and with normal body mass index (BMI) were included in the control group. The included patients with diabetic ulcer all have the ulcer located on areas that are supplied by the dorsalis pedis artery. Patients with lower extremity vascular diseases from other etiologies, malignant tumors, severe heart conditions, acute diabetic foot infections, severe stenosis and/or occlusion of the posterior tibial and peroneal arteries, and patients with contraindications for CEUS were excluded. All subjects received echocardiography, lower limb arterial color Doppler ultrasound, and transcutaneous partial pressure of oxygen ($TcPO_2$) tests. During $TcPO_2$ tests, electrodes were all placed on the same dorsal areas of the foot as examined on CEUS. The general data and laboratory findings of the four groups are shown in Table 2. CEUS was performed on the dorsal area unilaterally, and for those with bilateral lesions, the more severe side was selected. All patients signed informed consent form for this procedure. Time intensity curves (TIC) of the ROIs were obtained, and the correlation between TIC parameters and foot microcirculation perfusion abnormalities was analyzed. Then, the optimal cut-off value of the parameters such as AUC, PI, and AS for diagnosing foot microcirculation perfusion abnormalities was determined with receiver operating characteristic curve (ROC) and Youden's index.

Table 1 Composition of the cohort

Grade	0	1	2	3	4	5
Case	14	16	8	8	9	5

Instrument and contrast agent

A Philips EPIQ7C ultrasound diagnostic instrument equipped with quantitative ultrasonography analysis software and its L12-3 probe were used. The contrast agent was SonoVue (Bracco, Italy). Each vial of the contrast agent contained 59 mg of phospholipid-coated sulfur hexafluoride lyophilized powder, which was shaken for 30 s in 5 ml

Table 2 Comparison of general data of the three groups to the control group

	Group A/30 cases	Group B/28 cases	Group C/30cases	Control group/30 cases	*p*
Male/female	18/12	16/12	19/11	15/15	0.186
Age (years)	61.45 ± 10.80	63.05 ± 6.30	69.88 ± 11.70	63.51 ± 11.08	0.698
LVEF (%)	64.68 ± 7.02	67.43 ± 5.24	66.38 ± 3.29	66.68 ± 5.12	0.804
Disease course (years)	3.8 ± 1.12	13.91 ± 6.23*	20.05 ± 9.68$^{*\&}$	/	< 0.001
HbA1c (%)	7.11 ± 1.4$^{\#}$	8.81 ± 2.2$^{\#*}$	9.81 ± 1.64$^{\#*\&}$	5.24 ± 0.73	0.001
FBG (mmol/l)	9.8 ± 1.57$^{\#}$	11.28 ± 2.20$^{\#*}$	12.40 ± 4.29$^{\#*\&}$	5.0 ± 0.93	< 0.001

Note: $^{\#}p < 0.05$ when the experiment group was compared with the control group

$^{*}p < 0.05$ when the group was compared with group A

$^{\&}p < 0.05$ when the group was compared with group B

FBG fasting blood glucose

of normal saline to form a microbubble suspension with phospholipid as the shell enveloping the sulfur hexafluoride bubble inside.

The CEUS procedure

The patient lay supine with the knee at 90 degrees of flexion and kept the lower extremity stationary and fully relaxed. The probe was placed in the ankle fossa to show both the dorsalis pedis artery and the underlying soft tissues that were directly in front of the ankle capsule, then CEUS was performed with mechanical index (MI) set at 0.07. As shown in Fig. 1, 2.0 ml of contrast agent was injected through the median cubital vein and flushed with 5 ml of normal saline; upon this, a 4-min real-time observation of the dorsalis pedis artery and its underlying soft tissues was performed and the images recorded. The sample gate was set as 4 mm × 2 mm and placed on the dorsalis pedis artery and its underlying soft tissues, respectively. TIC were obtained and the CEUS perfusion data of this area analyzed. A goodness of fit index (GFI) > 0.75 was used as the criterion for successful quantitative analysis. The main parameters included the ascending slope (AS), time to peak (TTP), peak intensity (PI), area under the curve (AUC), rise time (RT), and half of drop time (DT/2).

Qualitative and statistical analyses

SPSS 26.0 (the statistical Package for Social Sciences, version 26; IBM Corp., Armonk, NY, USA) was used. Numerical variables were expressed as ($\overline{x} \pm s$), *t*-test was used for comparison between two groups, one-way ANOVA was used for multiple group comparisons, and Tukey's multiple comparisons test was used for further two-by-two comparisons. Count data were expressed as (*n*%), and Fisher's exact test was used for comparison between rates. Correlation analysis was performed using Spearman's analysis. The predictive value of each parameter on abnormal microcirculatory perfusion in the soft tissues of the foot dorsum was analyzed with the receiver operating characteristic curve (ROC), and the values of the area under the ROC curve (AUROC) were obtained. The value of AUROC above 0.7 indicates a fair predictive performance of a variable and above 0.9, an excellent predictive performance. In our study, the criteria used to determine the best cut-off value of each parameter was Youden's index (*J*):

$$J = \text{sensitivity} + \text{specificity} - 1$$

The index indicates the overall diagnostic capability of a variable in a test and the best cut-off value can be determined by the maximum value of *J*. [17, 18] $p < 0.05$ was considered a statistically significant difference.

Results

Comparison of general data and laboratory test results

There were no significant differences in terms of age, gender, and left ventricle ejection fraction (LVEF) when the three experimental groups were compared with the control group respectively. The years of disease course, glycated hemoglobin (HbA1c), and fasting blood sugar (FBS) levels were compared, and they were on a decreasing trend from Group

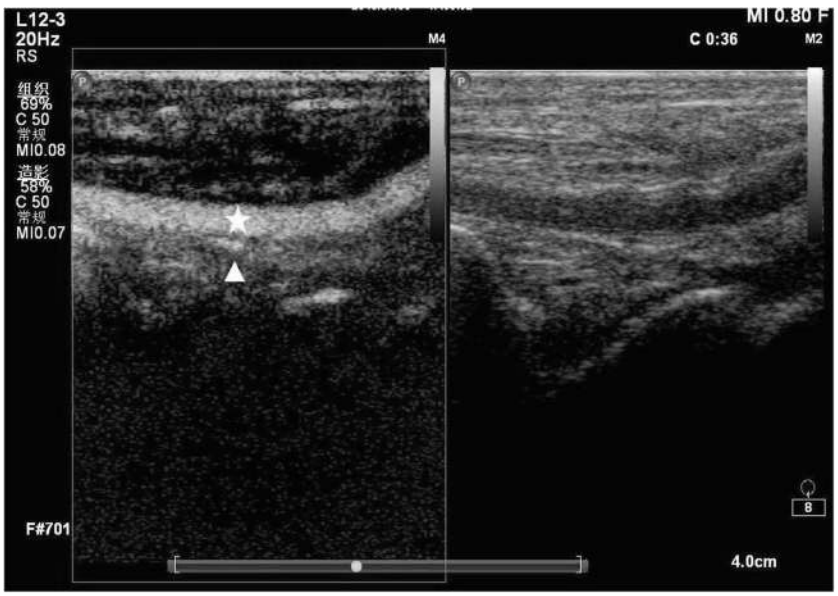

Fig. 1 ☆ indicates the dorsalis pedis artery and △ the underlying tissues

C, to Group B, and then Group A. The differences were all statistically significant ($p < 0.05$), see Table 2.

Three patients from group C showed bimodal TICs for the dorsalis pedis artery and the soft tissues and their results were excluded before curve fitting. The rest of the patients all showed unimodal parabolic TICs.

Comparison of TIC data of the dorsalis pedis artery

There were no statistically significant differences in each parameter between the control group and groups A and B ($p > 0.05$). Group C showed reduced AS, PI, and AUC and prolonged TTP, RT, and DT/2 compared with the other three groups; the differences were statistically significant ($p < 0.05$), see Table 3 and Fig. 2.

Comparison of TIC data of the underlying soft tissues

AS, AUC, and PI were on a decreasing trend from the control group, to Group A, to Group B, and then Group C; the differences were all statistically significant ($p < 0.05$).

Comparison among the groups A, B, and C demonstrated an increasing trend of TTP, RT, and DT/2 with no statistically significant differences between groups A and B (TTP 32.68 ± 4.38 vs. 38.31 ± 3.76, $p = 0.294$; RT 8.64 ± 4.32 vs. 9.41 ± 3.46, $p = 0.374$; DT/2 21.88 ± 2.43 vs. 22.17 ± 2.74; $p = 0.608$) and statistically significant difference between groups B and C was (TTP 38.31 ± 3.76 vs. 56.00 ± 3.20, $p = 0.004$, RT 9.41 ± 3.46 vs. 19.09 ± 1.73, $p = 0.001$, DT/2 22.17 ± 2.74 vs. 33.68 ± 3.88, $p = 0.002$), see Table 4 and Fig. 2.

Diagnostic value of CEUS for microcirculatory perfusion abnormalities in the dorsum of a diabetic foot

Spearman correlation analysis showed a correlation between the severity of the abnormalities and the data obtained for each parameter, with r values of AS, PI, AUC, TTP, DT/2, and RT being − 0.784, − 0.897, − 0.8 77, 0.518, 0.476, and 0.105, respectively. Further analysis with ROC on the predictive value of each parameter of TIC for microcirculatory perfusion abnormalities in the dorsum of the foot found that AUC, PI, and AS of

Table 3 Comparison of TIC data of the dorsalis pedis artery

	AS (dB/s)	TTP (s)	PI (dB)	AUC (dB*s)	RT (s)	DT/2 (s)
Group A	7.21 ± 1.89	33.12 ± 3.02	37.16 ± 3.41	1198.26 ± 143.52	7.96 ± 0.84	18.21 ± 2.64
Group B	6.98 ± 0.16	35.50 ± 1.60	36.62 ± 2.89	1100.95 ± 110.97	8.06 ± 0.54	18.4 ± 3.07
Group C	$1.14 \pm 0.11^{\#*\&}$	$48.29 \pm 2.36^{\#*\&}$	$20.65 \pm 2.37^{\#*\&}$	$423.37 \pm 94.56^{\#*\&}$	$10.44 \pm 0.88^{\#*\&}$	$26.7 \pm 4.97^{\#*\&}$
Control	7.33 ± 1.26	32.00 ± 2.1	37.32 ± 5.88	1314.52 ± 159.34	8.09 ± 0.77	17.33 ± 4.64
p (control vs. group C)	< 0.001	0.001	< 0.001	< 0.001	0.001	0.005

Note: $^{\#}p < 0.05$ when the experiment group was compared with the control group

$^{*}p < 0.05$ when the group was compared with group A

$^{\&}p < 0.05$ when the group was compared with group B

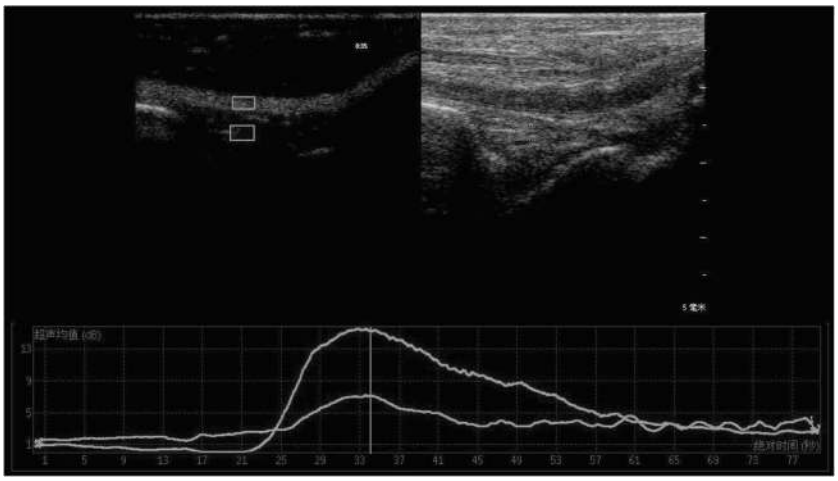

Fig. 2 The blue curve represents the TIC for the dorsalis pedis artery; the yellow is for the underlying soft tissues

Table 4 Comparison of TIC data of the underlying soft tissues in the foot

	AS (dB/s)	TTP (s)	PI (dB)	AUC (dB*s)	RT (s)	DT/2 (s)
Group A	$1.26 \pm 0.39^{\#}$	32.68 ± 4.38	$8.66 \pm 2.02^{\#}$	$243.92 \pm 46.57^{\#}$	8.64 ± 4.32	21.88 ± 2.43
Group B	$0.85 \pm 0.24^{\#*}$	38.31 ± 3.76	$6.38 \pm 1.95^{\#*}$	$180.49 \pm 51.96^{\#*}$	$9.41 \pm 3.46^{\#}$	22.17 ± 2.74
Group C	$0.35 \pm 0.08^{\#*\&}$	$56 \pm 3.20^{\#*\&}$	$4.53 \pm 1.01^{\#*\&}$	$79.31 \pm 24.27^{\#*\&}$	$19.09 \pm 1.73^{\#*\&}$	$33.68 \pm 3.88^{\#*\&}$
Control	1.32 ± 0.06	34.34 ± 2.58	12 ± 1.85	348.57 ± 50.81	7.27 ± 3.28	21.76 ± 1.82
p (Control vs Group C)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note: $^{\#}$ indicates $p < 0.05$ when the experiment group was compared with the control group

* indicates $p < 0.05$ when the group was compared with group A

$^{\&}$ indicates $p < 0.05$ when the group was compared with group B

the TIC had higher predictive value for microcirculatory perfusion abnormalities (AUROC > 0.7). The best cut-off values of the three parameters of the TIC determined with Youden's index were as follows: the best cut-off value of AUC of the TIC, 290.23 (AUROC, 0.984; sensitivity 93.8%; specificity 89.9%); the best cut-off value of PI of the TIC, 10.17 (AUROC, 0.911; sensitivity 87.5%, specificity 86.9%); the best cut-off value of AS of the TIC, 1.05 (AUROC, 0.954; sensitivity 87.5%, specificity 92.3%) as shown in Table 5 and Fig. 3.

Comparison of the performance of CEUS and $TcPO_2$ in detecting microcirculatory impairment in the dorsum of a diabetic foot

Generally, a foot can be diagnosed with microcirculatory impairment if the oxygen tension is < 40 mmHg in a $TcPO_2$ test [19] and if two or more of the TIC parameters, i.e., AS, PI, and AUC, are abnormal on CEUS.

For group A, the positive rate was 33.33% with CEUS and 10% with $TcPO_2$; the difference was statistically significant ($p < 0.05$). For group B, the positive rate was 100% with CEUS and 75% with $TcPO_2$; the difference was statistically significant ($p < 0.05$). For group C, both CEUS and $TcPO_2$ registered a positive rate of 100%, see Table 6.

Discussion

That microcirculatory dysfunction causes ischemia and hypoxia in local tissues plays a critical role in the pathogenesis of diabetic foot [20], hence the importance of an accurate evaluation of microcirculatory function. Currently, various techniques can be used to evaluate the microcirculatory perfusion in diabetic foot. Among them, percutaneous partial pressure of oxygen monitoring, dynamic capillary microscopy, laser Doppler perfusion imaging, etc. can only evaluate the microcirculation of the capillaries in the skin due to their limited penetration capabilities; iontophoresis measures red blood cell flow by nourishing the subpapillary vascular plexus, but only indirectly reflects microcirculation to the tissue [21–23]. Magnetic resonance imaging (MRI) can offer quantitative analysis of perfusion to the underlying soft tissue, but being time-consuming and expensive with various contraindication have made it unfeasible to be a routine diagnostic work-up [24–26].

CEUS has been widely used to evaluate microcirculatory perfusion in the heart and kidneys of patients with T2DM [27, 28]. And in the peripheral vasculature, CEUS has been reported to be mainly used in the evaluation of the arterial patency in the lower extremities, the stability of arterial plaque, the extent of ischemia in foot ulcers, and the microcirculatory function in the calf muscle [29–31], whereas studies on its usage in evaluating microcirculatory perfusion

Table 5 The best cut-offs of TIC parameters

TIC parameters	AUROC	95% CI	Best cut-off	Sensitivity	Specificity	Maximum J
AUC	0.984	0.959–1.000	290.23	93.8%	89.9%	0.837
PI	0.911	0.834–0.988	10.17	87.5%	86.9%	0.744
AS	0.954	0.899–1.000	1.05	87.5%	92.3%	0.798

AUROC the area under the ROC curve; AUROC value > 0.9, excellent test; 0.8–0.9, good test; 0.7–0.8, fair test

CI confidence interval;

J the Youden index = sensitivity + specificity − 1

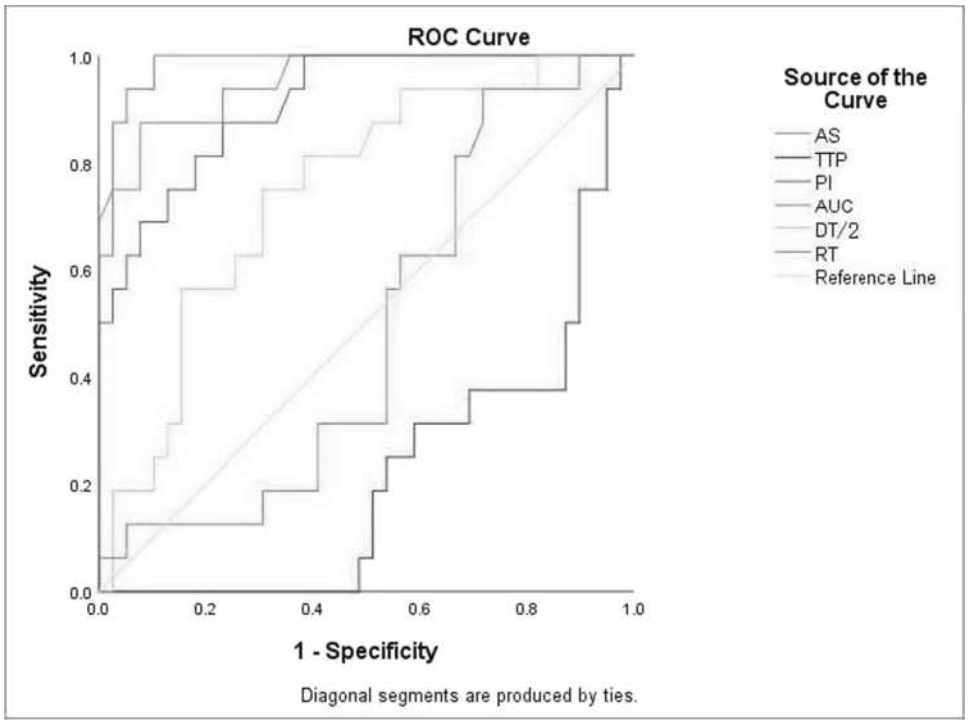

Fig. 3 ROCs of different TIC parameters

Table 6 performance of CEUS and $TcPO_2$ in detecting microcirculatory impairment in the dorsum of a diabetic foot

Group	CEUS Positive rate (%)	$TcPO_2$ Positive rate (%)	*p* value
Group A	33.33	10	0.029
Group B	100	75	0.005
Group C	100	100	/

Positive rate number of patients with microcirculatory perfusion impairment/total number of patients

in diabetic foot are still lacking. Li et al. [32] reported that CEUS could distinguish the differences of microcirculatory perfusion to the phalangeal area in the foot between patients with impaired glucose tolerance (IGT) and patients with diabetes (without complications), but to date no studies addressing diabetic foot microcirculatory perfusion in female patients and across different disease courses have been reported. Therefore, this study included both male and female patients with diabetic foot in equal proportions across Wagner's 0–5 grades to investigate the value of CEUS in evaluating microcirculatory perfusion to the foot of T2DM patients in a wider patient population.

In this study, group C with the most severe conditions of diabetic foot had the longest disease course and the highest HbAlc and blood sugar, indicating that the length of disease course; the fluctuation range of blood sugar were directly proportional to the severity of impairment in the foot of diabetic patients; the longer the disease course and the poorer the blood sugar control, the severe the impairment in the foot.

This study compared the TIC data of the dorsalis pedis artery obtained from CEUS for all groups. PI and AUC values reflected the volume of blood flow in the region of interest and larger values indicated better perfusion, while RT, AS, TTP, and DT/2 values reflected the blood flow velocity in the region of interest and would indicate a fast or slow perfusion. The results showed that the differences of the TIC data of the dorsalis pedis artery were statistically significant between the control group and group C only, which might indicate that long-term abnormal glucose metabolism had led to the severest atherosclerosis in group C where the narrowed or even occluded lumen impeded the passage of the contrast agent, resulting in a lowered blood flow velocity at the distal end of the stenosis, a delayed enhancement of the dorsalis pedis artery, and a reduced amount of contrast agent to the artery, whereas there were no statistically significant difference in the TIC data between the other two experimental groups and the control group, which might indicate that less severe atherosclerosis in the other groups allowed for a smoother passage of the contrast agent.

The intergroup comparison of TIC data of the underlying soft tissue in the dorsal region of the foot of all groups showed that as the condition of diabetic foot worsened, parameters reflecting the rapidity of perfusion such as TTP, RT, and DT/2 prolonged and AS decreased; among them, TTP, RT, and AS reflected the rapidity of wash-in while

DT/2 reflected the rapidity of the return of blood flow; and parameters reflecting the intensity of perfusion such as PI and AUC decreased. These signs demonstrated an enhancement pattern of "delayed wash-in, delayed wash-out and weak enhancement" for microcirculatory impairment in the dorsum of the foot on CEUS. This may be due to the thickening of microvascular basement membrane, the microvascular distortion, and the narrowing or even occlusion of the lumen following microvascular impairment under the influence of hyperglycemia, which resulted in the prolonged passage time and the decreased passed amount of the contrast agent. Meanwhile, due to the insufficient perfusion, the distal venules were in a diastolic state, which slowed down the return of the contrast agent, further reducing effective perfusion and aggravating ischemia, hypoxia, and undermining the vitality of local tissues, thereby resulting in local "weak enhancement."

The comparison of TIC data of the underlying soft tissue showed that compared with the control group, AUC and PI decreased and AS increased in group A, and the difference was statistically significant, indicating the existence of microcirculatory perfusion abnormalities in the dorsum of the foot of patients with diabetes mellitus alone (without diabetic foot). The intergroup comparison among the three experimental groups showed that AUC and PI decreased and AS increased from group A to group B and then group C, suggesting the differentiated microcirculatory impairment between different grades of diabetes. Moreover, as the condition of diabetic foot worsened, the soft tissue perfusion intensity decreased, the perfusion time extended, and the microcirculation became worse. AS, RT, and AUC of groups A and B were statistically different from the control group, whereas DT/2 reflecting the rapidity of the return of blood flow between them was without statistically significant difference, suggesting that the microcirculatory impairment mainly involve the arterioles and metarterioles and relatively less impact the venules.

From the results of correlation analysis and ROC curve analysis, TIC parameters were found to be correlated with microcirculatory perfusion abnormalities in the dorsal area of a diabetic foot and could be used to predict the severity of lesions, particularly, AUC, PI, and AS of the TIC had higher diagnostic efficacy for microvascular lesions in the dorsal area of a diabetic foot. Further, the study used Youden's index to determine the best cut-off values of the three parameters. By this way, the obtained best cut-off values of AUC, PI, and AS of the TIC for diagnosing microcirculatory perfusion impairment in diabetic feet were 290.23, 1.05, and 10.17, respectively. The presence of anomalies of at least two of the three parameters was used as the criteria for diagnosing diabetic foot microvascular impairment, and the results were compared with that of $TcPO_2$. The results showed that CEUS and $TcPO_2$ had the same efficacy in detecting microcirculatory perfusion impairment for patients in group C with severe diabetic foot, while for patients with milder microcirculatory perfusion impairment in group A and group B, the positive rate with CEUS was significantly higher than that with $TcPO_2$. This indicated a higher sensitivity of CEUS in detecting milder microcirculatory perfusion impairment than $TcPO_2$. It might be due to the limited performance of electrodes which were deployed on the skin surface to detect oxygen partial pressure on in $TcPO_2$ and cannot detect microcirculatory perfusion in deep underlying soft tissues. And since its results are influenced by various factors such as environmental temperature, skin thickness, edema degree, and patient preparation, the $TcPO_2$ test is often considered for screening. In contrast, CEUS utilizes a contrast agent that has a similar diameter to red blood cells and can enter the microvasculature to directly display the microcirculation status of the region of interest (ROI), without being impeded by the ROI's depth. Meanwhile, the TIC quantitative assessment with CEUS better overcomes the aforementioned interference and provide more scientific details of the microcirculation status of soft tissue. It is arguable that CEUS may be reliable and superior to $TcPO_2$ in detecting microcirculatory perfusion impairment in the dorsal area of the foot.

In this study, three patients from group C showed bimodal changes in sync in their TIC curves of the dorsalis pedis artery and the underlying soft tissue, with the first peak higher than the second. The medical records of the three patients showed that they all received digital subtraction angiography (DSA), and all were confirmed to have severe stenosis of the anterior tibial artery with collateral angiogenesis. The collateral vessels resulted by atherosclerosis now first allowed the majority of the contrast agent to pass and reach the dorsalis pedis artery to form the first main peak of the TIC curve, while the rest small portion of the contrast agent reached the artery through the narrowed lower extremity arteries and its delay resulted a secondary peak lower than the previous one. The above perfusion patterns suggested that collateral circulation in the lower extremity of diabetic foot might be assessed and quantified through TIC curves obtained from CEUS.

Atherosclerosis of all pedal vessels, either the dorsalis pedis artery or its distal extensions and arterioles, affect the microcirculatory perfusion in the soft tissues of the foot. However, an effective tool to detect calcification in the distal arterioles are still absent. The study chose the dorsalis pedis artery because it is superficially located and relatively larger in diameter, making it easily detectable. And since the superficial soft tissue above the dorsalis pedis artery is located in the near field of ultrasound which has a poorer image resolution, this study chose the deep underlying soft tissue as the ROI. All patients were examined with the same instrument by the same radiologist, the size and location

of the ROI were kept the same for quantitative analysis to ensure the reliability of the acquired data. The limitation to our study is that it is a single-center study, so additional centers and patients are needed for further study.

Conclusion

Microcirculatory impairment in the dorsum of a diabetic foot presents a characteristic enhancement pattern of "delayed wash-in, delayed wash-out and weak enhancement" on CEUS. With the capability to quantify microcirculatory alterations in the soft tissues of the foot and reflect the variability of microcirculatory perfusion across different grades of diabetic foot lesions, CEUS can be expected to be an effective tool for assessing microvascular lesions in diabetic foot.

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Author contribution LFH, HYY, and JK conceived the original ideas, designed the study, and drafted the manuscript. YY analyzed the data and revise the manuscript. YK and DL supervised the drafting of the manuscript. All authors reviewed and approved the final manuscript.

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Data availability Data supporting the finding of this study are available within the article text and tables.

Declarations

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Informed consent Written informed consent was obtained from all the patients who participated in the study.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

The correlation between serum 1, 5-anhydroglucitol and β-cell function in Chinese adults with different glucose metabolism statuses

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Abstract

Objective Serum 1, 5-AG is a glycaemic marker reflecting control and fluctuations of short-term glucose. To reveal the relationship between 1, 5-AG with β-cell function, we investigated a certain number of Chinese adults with different glucose metabolisms.

Methods In clinical cross-sectional study, 2184 subjects with no prior diabetes history are included and underwent an OGTT. HOMA-IR and HOMA-β were calculated. Correlations between 1, 5-AG and HOMA-IR or HOMA-β were observed, correcting for interference factors, and independent factors for HOMA-β and HOMA-IR were analysed. Subjects were divided into three groups according to OGTT results, and 1, 5-AG levels differed significantly between them.

Results A significant positive correlation existed between 1, 5-AG and HOMA-β only in the diabetes mellitus group ($r = 0.265$, $p < 0.001$). Above phenomenon remained after adjusting for indicators, however, disappeared after considering serum uric acid. Both 1, 5-AG and HbA1c were independent factors for HOMA-β (1, 5-AG: $\beta = 0.772$, $p = 0.023$; HbA1c: $\beta = -7.52$, $p = 0.003$). Conclusion: 1, 5-AG remained a factor for HOMA-β only for those with NUA. 1, 5-AG reflects different glucose metabolism statuses and is an auxiliary observation reflecting the secretory function of β cells in NUA patients.

Keywords 1, 5-anhydroglucitol · β-cell function · Diabetes · Glucose metabolism status

Abbreviations

1, 5-AG	Serum 1, 5-anhydroglucitol
OGTT	Oral glucose tolerance test
HOMA-IR	Homeostasis model assessments for insulin resistance
HOMA-β	Homeostasis model assessments for β cells
NUA	Normal serum uric
T2DM	Type 2 diabetes mellitus
OGIRT	Oral glucose insulin release test
HbA1c	Glycosylated hemoglobin
GA	Glycated albumin
FPG	Fasting blood glucose
2hPG	2-Hour postprandial plasma glucose
TC	Total cholesterol
TG	Triglycerides
HDL-c	High-density lipoprotein cholesterol
LDL-c	Low-density lipoprotein cholesterol
BUN	Blood urea nitrogen
SCr	Serum creatinine
SUA	Serum uric acid
eGFR	Estimated glomerular filtration rate
NGT	Normal glucose tolerance
IGR	Impaired glucose regulation
HUA	High uric acid

Introduction

According to statistics for 2021 from the International Diabetes Federation (IDF), China is ranked first in the world for number of diabetes mellitus patients [1]. It is well known that islet β-cell dysfunction and insulin resistance are important pathological factors in T2DM [2]. Furthermore, Chinese subjects with T2DM are characterized by a worse secretory function of β cells and a more serious early phase secretion defect [3]. Therefore, a timely understanding of

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the functional status of islets in diabetic patients is helpful for adjusting treatment plans and determining prognosis. Although an OGIRT and hyperinsulinaemic-euglycaemic clamp can be used to reflect insulin function, their strict operational methods limit random and flexible application. Therefore, a simple and easy way to estimate the status of β-cell function in diabetic patients is needed. Recently, a new glucose indicator, serum 1,5-AG, has gradually become known to the public [4]. It is a naturally occurring 1-deoxy form of glucose that can reflect dynamic changes in blood glucose through the mechanism of renal reabsorption [5–7]. A considerable number of studies in the literature have confirmed its superiority in monitoring short-term glycaemic levels (3–7 days) and glycaemic variability [8–12]. However, few studies in China or abroad have reported on the relationship between 1, 5-AG and the pathophysiological mechanisms of diabetes [13, 14]. So far, no study has been performed on the relationship between 1, 5-AG and β-cell function among people with different glucose metabolism levels, especially among people with normal glucose metabolism.

Therefore, a natural adult population without a history of prior diabetes was assembled from Jiangsu Province, China as research subjects for this study. Each subject underwent an OGTT and OGIRT to explore the relationship between 1, 5-AG and β-cell function in those with differing glucose metabolism levels and to see possible influencing factors.

Methods

Study design

The multi-center cross-sectional study was conducted in six cities in Jiangsu Province over a 1 year period (November 2015 to June 2016).

Selection of subjects

2500 individuals (18 to 65 years) were selected using a multistage, stratified sampling method. Patients with known diabetes/on treatment, liver dysfunction, or those with chronic kidney disease stage 4–5 were excluded. Non-resident population, pregnant subjects and those with incomplete data were also excluded. Ultimately, 2184 subjects were included in the final analysis.

Anthropometry and biochemical measurements

Each subject underwent OGTT, physical examination (height, weight, waist circumference, hip circumference), and biochemical examination (liver function, renal function, blood lipids). HbA1C was determined by high-performance liquid chromatography using a D-10 HbA1C analyser (Bio-Rad, USA); GA was measured using a liquid enzymatic method with a Glamour 2000 biochemical automatic analyser (Lucica GA-L, Tokyo, Japan); Serum insulin levels were determined by electrochemiluminescence immunoassay using a Gobas e411 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The plasma concentration of FPG, 2hPG, TC, TG, HDL-c, LDL-c, BUN, SCr and SUA levels were all measured. The calculation of the eGFR was based on a revised formula for Chinese subjects [15]. We collected blood samples from patients after overnight fasting, which were stored at − 80 °C prior to the further measurement of 1, 5-AG. Serum 1, 5-AG levels were measured with a GlycoMark assay using a Hitachi 917 analyser (Roche Diagnostics, USA).

Definition of β-cell function

B-cell function was assessed by the calculation of insulin resistance index and secretion index. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = FINS (in mU/L) * FPG (in mmol/L)/22.5. The homeostasis model assessment for β-cell function (HOMA-β) was calculated as follows: HOMA-β = 20 *FINS [mU/L]/(FPG [mmol/L] – 3.5) [16].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 22.0 (International Business Machines Corporation, USA). With the normality test, all variables were divided into normally distributed data (presented as mean ± SD) and skewed data (presented as quartile P50 [P25, P75]). A one-way ANOVA was used to analyse differences among the three groups of the study. A Pearson or Spearman's simple correlation analysis was used to analyse the correlations between 1, 5-AG and the other indicators. A partial correlation analysis was used to adjust the other confounding variables. Finally, a stepwise linear regression analysis was used to find the independent factors for HOMA-β. A two-tailed p value of < 0.05 was considered to be statistically significant.

Results

Clinical characteristics of the three groups

The 2184 subjects in the study were divided into three groups according to the WHO diabetes diagnostic criteria from 1999 [17]. 307 subjects were diagnosed as belonging in the T2DM group, 685 in the IGR group, and 1192 in the NGT group, accounting for 14.06%, 31.36%, and 54.58%, respectively. 1, 5-AG levels gradually decreased from the NGT group to the IGR group to the T2DM group (26.99 ± 7.23 μg/mL,

21.58 ± 8.29 μg/mL, and 15.74 ± 9.6 μg/mL, respectively, all $p < 0.001$). HOMA-IR gradually increased (all $p < 0.001$) and the T2DM group showed the most serious insulin resistance. HOMA-β was the highest in NGT group and the lowest in T2DM groupT2DM (all $p < 0.001$), so insulin secretion function was the worst in the T2DM group. The other indicators, including gender, age, glycaemic indexes (FPG, 2hPG, HbA1c, GA), blood pressure, lipid profile, blood urea nitrogen (BUN), serum uric acid (SUA), BMI, and W/P, had significant differences among the three groups. SCr and eGFR had no significant differences among the groups, as shown in Table 1. T2DM.

The correlations between 1, 5-AG and both HOMA-IR and HOMA-β in the three groups and their interference factors

A Spearman's simple correlation analysis was used to find the correlations between 1, 5-AG and both HOMA-IR and HOMA-β in the three groups. A significant positive correlation was shown between 1, 5-AG and HOMA-β in the T2DM group (r = 0.265, $p < 0.001$), which still existed after adjusting for gender, age, blood pressure, BMI, W/P, blood lipid profile (HDL, LDL, TG, TC), glycaemic indexes (HbA1c, GA, FPG, 2hPG), as show in Table 2. Meanwhile, 1, 5-AG and HOMA-IR showed a weak negative correlation in the T2DM group (r = -0.119, p = 0.038), but this negative

Table 2 Spearman correlation analysis between serum 1, 5-AG and HOMA-IR, HOMA-β in three groups

correlation		NGT	IGR	T2DM
HOMA-β	r	-0.041	-0.078	0.265
	P	0.159	0.041*	< 0.001*
HOMA-IR	r	0.05	0.056	-0.119
	P	0.086	0.145	0.038*

* means $p < 0.05$

Table 1 Demographic and Clinical Characteristics

Characteristics	NGT	IGR	T2DM	*P*-value
N (%)	1192(54.58%)	685(31.36%)	307(14.06%)	
1, 5-AG, μg/mL	26.99 ± 7.23	21.58 ± 8.29*	15.74 ± 9.6*#	< 0.001
Gender, M/F	456/736	308/377*	162/145#	< 0.001
Age, years	46(34, 53)	49(42, 56)*	51(46, 57)#	< 0.001
FPG, mmol/L	5.45 ± 0.41	5.93 ± 0.57*	7.83 ± 2.07*#	< 0.001
2 h-PG, mmol/L	5.97 ± 0.93	8.10 ± 1.47*	13.24 ± 4.11*#	< 0.001
HbA1C, %	5.58 ± 0.32	5.81 ± 0.42*	6.98 ± 1.29*#	< 0.001
GA, %	12.57 ± 1.75	12.93 ± 1.96*	16.72 ± 5.26*#	< 0.001
HOMA-IR	1.39(0.88, 2.08)	1.79(1.17, 2.55)*	2.30(1.43, 3.67)*#	< 0.001
HOMA-β, %	59.82(40.38, 88.15)	57.97(36.02, 86.16)*	34.31(20.78, 59.52)*#	< 0.001
SBP, mmHg	127.53 ± 18.33	135.80 ± 19.91*	141.52 ± 18.92*#	< 0.001
DBP, mmHg	79.29 ± 11.34	83.58 ± 11.85*	86.33 ± 10.47*#	< 0.001
TG, mmol/L	1.17(0.83, 1.72)	1.42(0.97, 2.06)*	1.80(1.30, 2.67)*#	< 0.001
TC, mmol/L	4.72 ± 0.97	4.94 ± 0.94*	5.12 ± 1.00*#	< 0.001
HDL, mmol/L	1.41 ± 0.51	1.39 ± 0.39	1.31 ± 0.33*#	0.004
LDL, mmol/L	2.63 ± 0.74	2.82 ± 0.78*	2.89 ± 0.78*#	< 0.001
BUN, mmol/L	5.08 ± 1.43	5.25 ± 1.49	5.32 ± 1.48#	0.009
SCr, μmol/L	70.60 ± 15.85	71.27 ± 15.84	71.93 ± 17.09	0.376
SUA, μmol/L	302.75 ± 84.74	327.95 ± 91.83*	332.45 ± 91.44*	< 0.001
eGFR, mL/min/1.73 m^2	126.67 ± 31.29	125.46 ± 32.33	126.96 ± 35.49	0.78
BMI, Kg/m^2	24.99 ± 3.74	26.24 ± 3.60*	27.38 ± 3.97*#	< 0.001
W/P	0.87 ± 0.06	0.90 ± 0.06*	0.92 ± 0.06*#	< 0.001

Data were expressed as mean ± SD for normal distribution variables or as the median (P25, P75) for skewed distribution variables. * means $p < 0.05$ compared with NGT; # means $p < 0.05$ compared with IGR

NGT, normal glucose tolerance; IGR, impaired glucose regulation; T2DM, diabetes mellitus; 1,5-AG, 1,5-anhydroglucitol; M, male; F, female; FPG, fasting plasma glucose; 2 h-PG, 2-h postprandial glucose; HbA1c, glycated hemoglobin; GA, glycated albumin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BUN, urea nitrogen; SCr, serum creatinine; SUA, serum uric acid; eGFR, estimated glomerular filtration rate; BMI, body mass index; W/P, waist-to-hip ratio

correlation was lost after adjusting for other factors. In addition, we did not find a significant correlation between 1, 5-AG and HOMA-IR or HOMA-β in the NGT and IGR groups. Table 3 presents partial correlation analyses of factors correlated with the association between serum 1, 5-AG and HOMA-β, HOMA-IR in the T2DM group. In Table 3, Mode 1 indicates adjustment for age and gender. Model 2 represents model 1 plus SBP, DBP, BMI, W/P, HDL, LDL, TC and TG. Model 3 represents model 2 plus HbA1c, GA, FPG and 2H PG. Model 4 represents model 3 plus eGFR, Cr and BUN. Model 5 means model 4 plus UA.

Independent factors for HOMA-β for different SUA levels in the T2DM group

In the T2DM group, a multiple stepwise linear regression analysis was used by employing HOMA-β as a dependent variable. We found that both 1, 5-AG and HbA1c were independent factors for HOMA-β (1, 5-AG: $\beta = 0.772$, $p = 0.023$; HbA1c: $\beta = -7.52$, $p = 0.003$) after adjusting for age, blood pressure, BMI, W/P, lipid profile (TG, TC, LDL, HDL), glycaemic indexes (FPG, 2hPG, HbA1c, GA, 1, 5-AG), and renal function indicators (BUN, Cr, SUA, eGFR). As shown by the above results, SUA may be considered an interference factor between 1, 5-AG and HOMA-β. To study this further, the T2DM group was further divided into a NUA group and HUA (SUA male ≥ 420 μmol/L, female ≥ 360 μmol/L) [18, 19] group. In sub-group analysis, 1, 5-AG was observed to be an independent predictor in NUA group($\beta = 0.677$, $p = 0.017$) but not HUA group. Furthermore, we found that there was no positive correlation between 1, 5-AG and HOMA-β in the HUA group. However, there was still a significant positive correlation between 1, 5-AG and HOMA-β in the NUA group, which was stronger in males than females. Figure 1 shows pearson's correlation coefficient between levels of serum 1, 5-AG and HOMA-β.

Discussion

Maintaining β-cell function and improving insulin resistance are considered to be the most effective measures for preventing diabetes and its progression [20] as they are both part of the pathological mechanisms of the development of T2DM. Studies have shown that once hyperglycaemia has obviously occurred, islet β-cell dysfunction is clearly manifest [21, 22]. Even in high-risk populations still in the NGT stage, β-cell function has been impaired [23, 24]. Therefore, the early evaluation and protection of β-cell function plays a key role in the prevention and treatment of diabetes. In addition to the invasive glucose clamp test, the monitoring of previous β-cell function can also be indirectly estimated by an insulin release test and some glycaemic indicators such as HbA1c, GA, and GA/A1c [25].

Serum 1, 5-AG, a new glycaemic marker, has received increasing attention since it was first described in 1972 [26]. It is a naturally occurring 1-deoxy form of glucose that undergoes glomerular filtration and tubular reabsorption and has a closed pyran ring structure that confers metabolic stability [27]. When glucose fluctuates beyond the renal threshold of glucosuria, the reabsorption of 1, 5-AG in the renal tubules is competitively inhibited by high levels of glucose, resulting in a decrease in serum 1, 5-AG levels [28]. Previous articles have confirmed that 1, 5-AG have a good correlation with 2hPG even in prediabetes [29]. Moreover, one study in China has confirmed that on the cellular level, 1, 5-AG is a good glycaemic marker [30]. In that study, the Michaelis constant and maximum velocity were determined to measure the affinity of glucose oxidase and hexokinase for 1, 5-AG and glucose. The authors concluded that 1, 5-AG is difficult to metabolize in vivo, and its transport is influenced by an acute glucose load in the hepatocytes. There have been few recent studies on the relationship between 1, 5-AG and β-cell function. A Korean study of a small sample in 2009 showed that low levels of 1, 5-AG were closely related to an elevation of 2hPG and a decline in islet secretion function in subjects with pre-diabetes and well-controlled T2DM [31]. In addition, a report on a small sample in China from 2015 showed that 1, 5-AG was closely associated with early-phase insulin secretion in newly diagnosed T2DM patients [32]. Our study took a natural population from Jiangsu Province, China that had no prior history of diabetes as a subject of research and explored for the first time the relationship between 1, 5-AG and β-cell function in people with different glucose metabolism statuses [33].

In the natural population of Jiangsu Province, 1, 5-AG, HOMA-IR, and HOMA-β had significant differences among the NGT, IGR, and T2DM groups [34]. In addition, 1, 5-AG

Table 3 Partial correlation analyses of factors correlated with the association between serum 1, 5-AG and HOMA-β, HOMA-IR in the T2DM group

correlation		Model 1	Model 2	Model 3	Model 4	Model 5
HOMA-β	r	0.216	0.252	0.132	0.129	0.107
	P	< 0.001*	< 0.001*	0.024*	0.028*	0.071
HOMA-IR	r	0.014	0.046	0.086	0.087	0.062
	P	0.811	0.425	0.14	0.141	0.293

* means $p < 0.05$

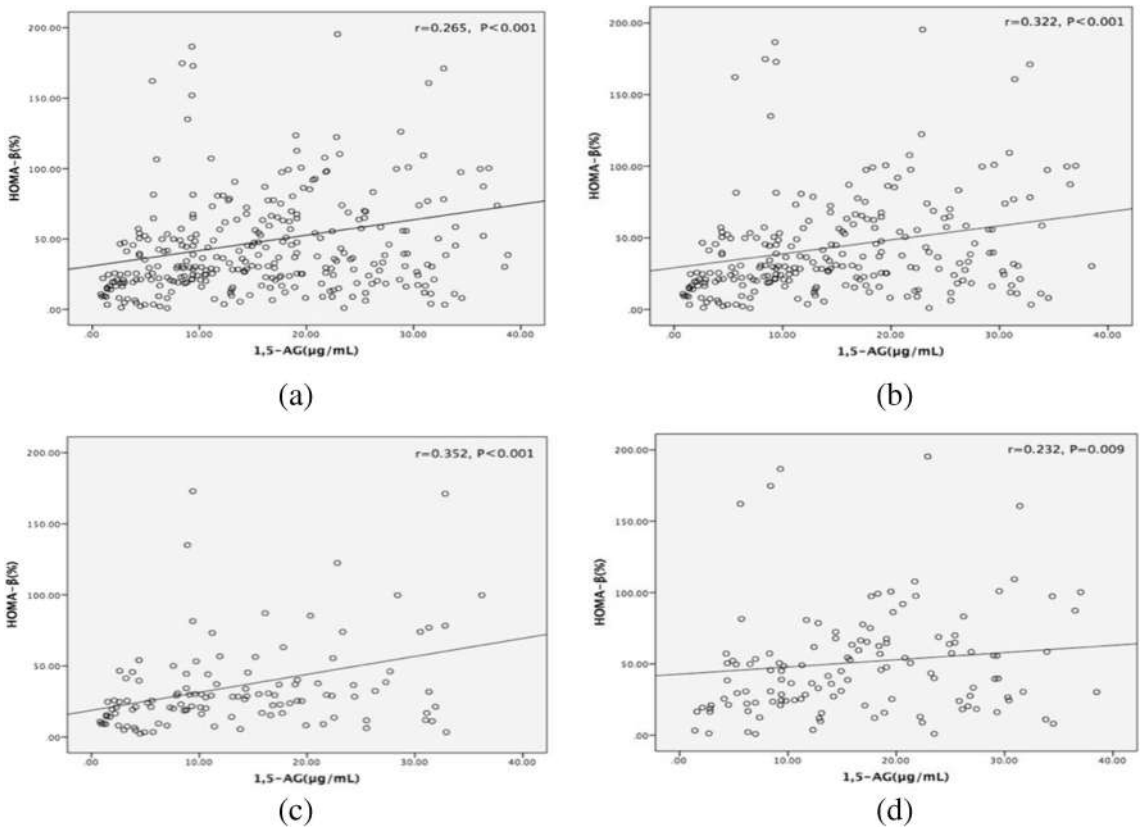

Fig. 1 Pearson's correlation coefficient between levels of serum 1,5-AG and HOMA-β: (**a**) n T2DM group(r = 0.265, $p < 0.001$); (**b**) n NUA of T2DM group(r = 0.322, $p < 0.001$); (**c**) n male of NUA group(r = 0.352, $p < 0.001$); (**d**) n female of NUA group (r = 0.232, $p = 0.009$)

was able to reflect different glucose metabolism statuses. With the gradual aggravation of glucose metabolism disorders, 1, 5-AG and HOMA-β showed gradually decreasing trends while HOMA-IR showed the opposite [35, 36]. It can be found that 1, 5-AG and HOMA-β maintained good positive correlations in the T2DM group as a whole and in the NUA group within the T2DM group (r = 0.265, 0.322, $p < 0.001$). Meanwhile, 1, 5-AG was an independent factor for HOMA-β (T2DM: β = 0.882, p = 0.023; NUA: β = 0.677, p = 0.017), proving that 1, 5-AG can reflect the secretory function of β cells. However, this correlation was lost in the HUA group. In the NUA group, 1, 5-AG could be used as an auxiliary observation index reflecting the secretory function of β cells.

SUA is a product of the metabolic breakdown of purine nucleotides and is excreted and absorbed via the kidney. Under physiological conditions, SUA levels in males are usually higher than in females [37]. In the three of groups of our study, men had higher SUA levels than women, and an independent sample t-test also proved that there were significant differences between males and females (all $p < 0.001$), which was consistent with previous observations. One possible explanation for this result is that oestrogens may promote renal clearance of SUA by inhibiting the active reabsorption of uric acid [38]. In recent years, the relationship between uric acid and 1, 5-AG has attracted much attention. In 2009, Koga et al. studied 158 male subjects with normal glucose tolerance and found that SUA was positively correlated with 1, 5-AG concentration. 1, 5-AG and SUA may share a common renal tubular transport system [39]. Another study in 2013 found that there was an independent positive correlation between 1, 5-AG and SUA in both T2DM and non-DM subjects [40]. In our study, we indeed found a positive relation between 1, 5-AG and SUA in the NGT, IGR, and T2DM groups (r = 0.168, r = 0.125, r = 0.14, respectively, $p < 0.05$). We suspect that 1, 5-AG and SUA share a common renal tubular transport system, causing 1, 5-AG to interfere with hyperuricemia in response to islet β-cell function in the HUA population of the T2DM group.

Interestingly, recent studies have shown that uric acid levels can also affect islet β-cell secretion, whether in diabetic or nondiabetic subjects [41]. A cross-sectional study was designed and performed on a total of 403 newly diagnosed T2DM patients and finally concluded that SUA may be considered as a predictor for β-cell function in clinical practice [42, 43]. Other studies have also supported this finding [44–46]. Our study also found that there was a positive correlation between SUA and HOMA-β in the T2DM group

(r = 0.148, p = 0.009). However, the mechanism that causes SUA to interfere with a positive correlation between 1, 5-AG and HOMA-β needs further exploration.

The mechanism of the positive correlation between 1,5-AG and islet β-cell secretion has also been deeply studied recently. One study confirmed that 1,5-AG could stimulate insulin release in a dose-dependent manner at a cytological level [18]. Another study suggested that 1,5-AG appeared to inhibit the activity of disaccharidase to inhibit postprandial blood glucose and insulin secretion [19], however, the exact mechanism still needs further exploration.

The current study has some limitations. First, it would be better to use more prospective or cohort studies to enhance the efficacy of the cross-sectional research. On this point, our team is currently involved and working hard on achieving this goal. Second, it would be more accurate to use the internationally recognized gold standard glucose clamp test. By taking into account of manoeuvrability, a more suitable method approved by domestic and foreign experts is applied in our study.

In general, this study is based on the findings from a large-scale and multicentric adult population in Jiangsu, China. As a result of this study, we make the following conclusions: 1. In the natural adult population of Jiangsu, China, 1,5-AG can reflect different glucose metabolism statuses. 2. 1,5-AG, HOMA-IR, and HOMA-β show significant differences among NGT, IGR, and T2DM groups. In the T2DM group, 1,5-AG was positively correlated with HOMA-β. For those in the NUA group within the T2DM group, 1,5-AG could be used as an auxiliary observation index reflecting the secretory function of β cells.

Conclusions

In this paper, we investigate the relationship between serum 1, 5-anhydroglucitol and β-cell function in Chinese adults with different glucose metabolism statuses. In a natural population from Jiangsu Province, China with no previous history of diabetes, there were significant differences in 1, 5-AG, HOMA-IR, and HOMA-β among the NGT, IGR, and T2DM groups. Therefore, 1, 5-AG can reflect different glucose metabolism statuses. In the T2DM group, 1, 5-AG was positively correlated with HOMA-β, and 1, 5-AG could also be used as an auxiliary observation index reflecting the secretory function of β cells for those in the NUA group within the T2DM group. From the results, we can obtain that 1, 5-AG can reflect different glucose metabolism statuses in the natural adult population of Jiangsu, China. We did not find a significant correlation between 1, 5-AG and HOMA-IR or HOMA-β in the NGT and IGR groups, so 1,5-AG may not be a predictor of β-cell function until a certain level of blood glucose elevation. In addition, 1, 5-AG, HOMA-IR, and HOMA-β show significant differences among NGT, IGR, and T2DM groups. In the T2DM group, 1, 5-AG was positively correlated with HOMA-β. For those in the NUA group within the T2DM group, 1, 5-AG could be applied as an auxiliary observation index reflecting the secretory function of β cells.

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Data availability All the data supporting the results are shown in the paper and can be applicable from the corresponding author.

Declarations

Ethical approval This study was approved in 2017 by the Clinical Ethics Committee of Zhongda Hospital, an affiliate of Southeast University (No.2017ZDSYLL006-P01), and all subjects signed informed consent forms before registration. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. This study was approved by the Clinical Ethics Committee of our hospital and all subjects signed informed consent forms before registration.

Conflicts of interest The authors declare that there are no conflicts of interest regarding the publication of this paper.

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ORIGINAL PAPER

Association between nesfatin-1 hormone levels, anthropometric measurements, and glucose regulation shortly after sleeve gastrectomy: A cross-sectional study

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Abstract

Objective This cross-sectional study was planned to evaluate the relationship between serum nesfatin-1 levels, glucose regulation and anthropometric measurements of individuals who had undergone sleeve gastrectomy.

Methods A total of 40 participants aged between 19 and 64 years with a body mass index (BMI) greater than 30 kg/m^2 who underwent sleeve gastrectomy participated in the study. Before and 1 month after the surgery, serum nesfatin-1, fasting insulin levels, anthropometric measurements and biochemical parameters were assessed.

Results Serum nesfatin-1 levels significantly decreased 4 weeks after the surgery (108.3 ± 58.35 pg/mL versus 74.6 ± 40.12 pg/mL: $p = 0.003$). Serum insulin levels (μIU/ml) showed a similar decrease ($p < 0.001$). Change in serum nesfatin-1 levels significantly correlated with change in BMI and body fat mass ($p = 0.009$, $p = 0.007$, respectively). Furthermore, serum nesfatin-1 levels significantly correlated with total cholesterol levels ($p = 0.037$). However, despite the significant decreases in both nesfatin-1 levels and glucose regulation markers, no correlation was found between them. The significant decrease observed in post-operative serum nesfatin-1 levels was found to be correlated with anthropometric measurements.

Conclusions This finding highlights the anorexigenic effects of nesfatin-1, and therefore, nesfatin-1 may be an effective factor in obesity treatment and diabetes.

Keywords Bariatric surgery · Type 2 diabetes · Nutrition · Nesfatin-1 · Obesity

Introduction

Obesity is a chronic disease associated with various comorbidities such as insulin resistance, type 2 diabetes, cardiovascular disease, hypertension, osteoarthritis and even some types of cancer [1]. Increasing frequency of obesity and concomitant diseases in the world necessitates effective strategies for treatment and prevention [2]. Treatment of obesity includes lifestyle changes such as dietary restrictions and increased physical activity, medication, and in some cases, surgery. Bariatric surgery is emerging as a valuable treatment option for patients with type 2 diabetes. The reason for this is the significant improvement of type 2 diabetes after bariatric surgery and increased evidence that gastrointestinal operations can directly affect glucose metabolism regardless of weight loss [3]. In our study, we preferred patients with sleeve gastrectomy because it is an easier procedure and provides weight loss in a short time.

Nesfatin-1 is peptide containing 82 amino acids that acts as a multifunctional metabolic regulator and plays an important role in metabolic control with its anorexigenic and antihyperglycemic effects [4]. It was first described by Oh S et al. in 2006. NUCB2 mRNA is a precursor of nesfatin-1. Although its receptor is still unclear, it is known to be widely expressed in various tissues such as the central nervous system, pancreatic islet cells, pituitary gland, adipose tissue and

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gastric and intestinal mucosa. Because of the wide distribution of NUCB2/nesfatin-1 in the hypothalamus and brainstem regions, nesfatin-1 is thought to have an integral role in regulating energy homeostasis [5]. Nesfatin-1 induces saturation and inhibits food and water intake by regulating the activities of target neurons in the brain as an anorectic factor [6]. Some studies have shown that central and peripheral injections of nesfatin-1 cause a significant reduction in food and water intake [7, 8]. Since nesfatin-1 has been shown to play a role in energy homeostasis and has an anorectic effect, it is thought to affect body weight and body composition [9]. For example, in one study, decreased levels of nesfatin-1 after bariatric surgery correlated with body mass index and fat mass [10].

Nesfatin-1, which is also expressed in pancreatic beta cells, synthesis in pancreatic beta cells is regulated by glucose levels. NUCB2/nesfatin-1 regulates glucose homeostasis by increasing insulin secretion from pancreatic beta cells in the presence of high glucose conditions [11]. For example, nesfatin-1 infusion increased insulin secretion in a mouse model and resulted in better glucose utilisation by activation of intracellular insulin signalling [12]. In vitro, NUCB2 mRNA is regulated in human pancreatic islets through glucolipotoxic conditions (high glucose and palmitate levels). Glycemic diseases may cause impaired regulation of NUCB2 mRNA and a decrease of nesfatin-1 release [13].

This cross-sectional study was planned to evaluate the relationship between serum nesfatin-1 levels and glucose regulation of individuals who had undergone sleeve gastrectomy.

Materials and methods

Study design

This cross-sectional study involved two visits. The participants were interviewed before the surgery and 1 month after the operation. Anthropometric measurements (height, body weight, waist and hip circumference, neck circumference, body mass index, body fat percentage), 24-h recall food consumption records and blood samples were collected at the baseline (before operation) and 1 month after the operation. Participants were selected among volunteers who visited the clinic during the research period and examined for eligibility by an interview. The sample size was calculated based on the difference in mean nesfatin-1 concentration between pre-surgery and post-surgery levels. In a study conducted by Dogan et al. [14], nesfatin-1 concentrations were 22.80 ± 14.16 (ng/ml) before surgery, 60.23 ± 52.92 (ng/ml) at 3 months post-surgery, and 96.99 ± 40.20 (ng/ml) at 6 months post-surgery. We hypothesized an average 50% increase in nesfatin-1 concentration at the 1-month post-surgery level and calculated the sample size as 40 subjects, using an effect size of 0.53, an alpha error of 0.05 and 95% statistical power (G*Power software, version 3.1.9.2, Franz Faul, Düsseldorf, Germany). There were 40 eligible participants in the study, and all volunteers were informed on the study and signed a written consent form.

Procedures

A total of 40 participants who underwent sleeve gastrectomy between October 2017 and October 2019 in the General Surgery Clinic of Ankara Atatürk Sanatorium Training and Research Hospital (formerly known as Keçiören Training and Research Hospital), aged between 19 and 64 years, with a body mass index greater than 30 kg/m^2 participated in this 4-week cross-sectional follow-up study. Patients with type 2 or type 1 diabetes were excluded from study. Furthermore, patients with positive hepatitis B antigen or antihepatitis C virus antibody, chronic liver disease due to other causes (HBV, HCV, Wilson's disease, hemochromatosis, alcoholic hepatitis), history of inflammatory disease and major abdominal surgery were excluded from the study.

Nesfatin-1 and insulin analyses

ELISA kits were used to analyse serum nesfatin-1 (Elabscience, USA) and insulin levels (DiaMetra, Italy) according to the manufacturer's protocol. In this assay, based on the standard sandwich ELISA method, diluted serum samples and nesfatin-1 standards were added to nesfatin-1 antibodies in 96-well plates and incubated to form the antigen–antibody complex. Proteins not bound were removed by washing, then the second antibody was added to the plate wells and incubated. Unbound proteins were removed by rewash. The appropriate substrate was added to the wells and incubated, and colour change was observed in the wells in proportion to nesfatin-1 concentration due to enzymatic reaction. Finally, the stop solution was added, and the plates were read in Biotek Synergy HTX microplate reader at 450-nm wavelength within 30 min. The same procedure was repeated for insulin. All samples were analysed in duplicates.

Statistical analyses

Statistical Package for Social Sciences (SPSS 22.0, Armonk, NY: IBM Corp) program was used in the statistical analyses. Sample size was assessed via power analysis in accordance with the previous research data with 95% confidence interval and 0.05 type-I error. Kolmogorov-Simirnov test was used to evaluate the normality of the data. Pearson correlation test was used to calculate the correlation. The mean differences were compared using either paired *t* test or Wilcoxon signed rank test. Multiple linear regression was used to further

analyse the associations. The data obtained are presented as number, percentage, mean, standard deviation, median and interquartile range. Results were considered significant when the p value was below 0.05 in the 95% confidence interval.

Results

A total of 40 patients who met the inclusion criteria agreed to participate in the study. Mean age of the individuals was 37.2 ± 8.97 years. At the 4-week follow-up, anthropometric measurements significantly decreased compared to preoperative values (Table 1) ($p < 0.001$). Mean BMI decreased from 46.9 ± 5.54 kg/m^2 to 42.7 ± 5.44 kg/m^2 ($p < 0.001$); mean body fat % decreased from 49.0 ± 1.89 to 46.4 ± 2.71 ($p < 0.001$). Serum nesfatin-1 levels (pg/ml) showed a significant decrease 4 weeks after the operation (Table 2) (108.3 ± 58.35 pg/ml versus 74.6 ± 40.12 pg/ml, $p = 0.003$). Postoperative HOMA-IR and HbA1c (%) levels were significantly decreased compared with the preoperative levels, whereas fasting blood glucose levels (mg/dl) did not change (Table 2) (3.5 ± 2.40 versus 1.4 ± 1.19: $p < 0.001$; 5.9 ± 1.06 (%) versus 5.1 ± 0.56 (%): $p < 0.001$; 106.9 ± 45.57 (mg/dl) versus 101.2 ± 26.71 (mg/dl): $p > 0.05$, respectively).

Correlations between changes in serum nesfatin-1 level and anthropometric and biochemical parameters are presented in Table 3. There were positive correlations between serum nesfatin-1 and BMI and body fat mass ($p = 0.009$, $p = 0.007$, respectively). Additionally, changes in serum nesfatin-1 levels were significantly correlated with changes in total cholesterol levels ($p = 0.037$).

Finally, multiple linear regression analysis showed changes in BMI, body fat mass and total cholesterol as predictors of changes in nesfatin-1 levels but not changes in glucose homeostasis markers (Table 4).

In addition, we observed that changes in carbohydrate intake were associated with changes in nesfatin-1 levels ($p < 0.05$). However, changes in energy intake per kg, protein intake and fat intake did not show any association with nesfatin-1 levels.

Table 1 Anthropometric characteristics of the subjects at baseline and 4th week follow-up

	Subjects ($n = 40$)				
	Baseline	4th week follow-up	Δ	% change	p
Weight (kg)	122.2 ± 14.62	111.1 ± 13.65	11.2 ± 2.60	9.16	**< 0.001**
BMI (kg/m^2)	46.9 ± 5.54	42.7 ± 5.44	4.2 ± 0.92	8.95	**< 0.001**
Body fat %	49.0 ± 1.89	46.4 ± 2.71	2.6 ± 1.58	5.30	**< 0.001**
Body fat mass (kg)	56.5 ± 9.98	49.5 ± 9.60	7.0 ± 1.56	12.38	**< 0.001**
Fat free mass (kg)	65.7 ± 10.68	61.6 ± 9.67	4.1 ± 1.32	6.24	**< 0.001**
Waist circumference (cm)	127.3 ± 10.57	120.3 ± 10.43	7.1 ± 2.07	5.57	**< 0.001**
Hip circumference (cm)	143.3 ± 13.57	137.4 ± 13.19	5.9 ± 1.99	4.11	**< 0.001**
Waist/hip ratio	0.89 ± 0.06	0.88 ± 0.06	0.01 ± 0.01	1.12	**< 0.001**
Neck circumference (cm)	39.3 ± 2.56	36.9 ± 2.12	2.3 ± 1.10	5.85	**< 0.001**

BMI body mass index

*Paired samples t test was used for continuous data. Data was presented as mean ± SD. $p < 0.05$ data were shown in bold

Table 2 Biochemical parameters of the subjects at baseline and 4th week follow-up

	Subjects ($n = 40$)				
	Baseline	4th week follow-up	Δ	% change	p
Nesfatin-1 (pg/ml)	108.3 ± 58.35	74.6 ± 40.12	33.7 ± 67.36	31.11	**0.003**
Fasting glucose (mg/dL)	106.9 ± 45.57	101.2 ± 26.71	5.8 ± 44.23	5.42	> 0.05
Insulin (μIU/ml)	14.6 ± 11.05	5.2 ± 4.74	9.4 ± 12.02	64.38	**< 0.001**
HOMA-IR	3.5 ± 2.40	1.4 ± 1.19	2.1 ± 2.77	60	**< 0.001**
HbA1C (%)	5.9 ± 1.06	5.1 ± 0.56	0.7 ± 0.92	11.86	**< 0.001**
Triglyceride (mg/dL)	167.7 ± 135.51	147.9 ± 69.47	19.8 ± 138.88	11.80	> 0.05
Total cholesterol (mg/dL)	177.2 ± 38.84	161.8 ± 30.38	15.5 ± 50.47	8.74	> 0.05

HbA1c hemoglobin A1c

*Paired samples t test was used for continuous data. Data was presented as mean ± SD. $p < 0.05$ data were shown in bold. 1508, 5456

Table 3 Correlations of changes in nesfatin-1 levels and anthropometric and biochemical parameters

	ΔNesfatin-1	
	r	*p*
Δ Weight (kg)	0.373	**0.018**
Δ BMI (kg/m^2)	0.410	**0.009**
Δ Body fat %	0.163	0.314
Δ Body fat mass (kg)	0.419	**0.007**
Δ Fat free mass (kg)	-0.244	0.130
Δ Waist/hip ratio	0.110	0.500
Δ HOMA-IR	0.083	0.611
Δ Insulin (μIU/ml)	0.155	0.339
Δ HbA1c (%)	0.204	0.206
Δ Glucose (mg/dL)	0.077	0.638
Δ Total cholesterol (mg/dL)	0.331	**0.037**
Δ Triglyceride (mg/dL)	0,130	0,423

BMI body mass index, *HbA1c* hemoglobin A1c

*Pearson correlation test was used. $p < 0.05$ data were shown in bold

Discussion

Metabolic and endocrine changes occur together in individuals who underwent bariatric surgery [15]. In our study, we aimed to show that the decrease in nesfatin-1 level may be responsible for some of the changes after bariatric surgery.

It is uncertain whether the decreased nesfatin-1 levels after sleeve gastrectomy surgery are directly related to weight loss, and further studies are needed. There are many mechanisms that affect weight loss. From a different perspective, an animal study has shown that decreased SGLT-3 levels after sleeve gastrectomy may contribute to lowering blood sugar and controlling body weight. This can be explained by the decreased expression of SGLT-3 causing downregulation of SGLT-1 expression, which prevents glucose uptake into the blood. It is thought that surgery can be used as a potential target for diabetes treatment and weight loss [16].

Nesfatin-1 can cross the blood–brain barrier [6]. Although the receptor of nesfatin-1 has not yet been identified, some animal studies showed that nesfatin-1 administration can cause a decrease in food intake [17, 18]. In our study, participants' food intakes were significantly decreased after the surgery, as expected. Although both nesfatin-1 levels and energy intake decreased after surgery, there was no correlation between these parameters. However, serum nesfatin-1 levels and carbohydrate intakes were correlated in our study. A study showed that serum nesfatin-1 levels of participants with metabolic syndrome were correlated with carbohydrate intake, similar to our results [19]. One study found that obese adolescents had higher levels of nesfatin-1 compared to the control group, and nesfatin-1 levels were correlated with carbohydrate and fat intake [20]. In another study, there was a negative correlation between the dietary intakes of energy, protein, fat and serum nesfatin-1 levels of obese patients [21]. These inconsistent results may be due to self-reported food intake records.

Studies on the relationship between nesfatin-1 and diabetes, gestational diabetes and insulin resistance are inconsistent. Li et al. [22] showed that nesfatin-1 levels were significantly lower in type 2 diabetes patients compared with the control group. In a study, nesfatin-1 levels were lower in pregnant women with gestational diabetes compared with the control group [23]. In contrast, one study reported that patients with type 2 diabetes had higher levels of nesfatin-1 than the healthy control group [24]. Furthermore, in a study with metabolic syndrome patients, no correlation was found between nesfatin-1 levels and HBA1c and insulin levels [5]. Similarly, in our study, after 4-week follow-up, serum nesfatin-1 and insulin levels decreased; however, changes in nesfatin-1 levels did not correlate with changes in glucose regulation markers such as insulin, HbA1c and fasting blood glucose.

Table 4 Association between changes in serum nesfatin-1 level, anthropometric measurements and biochemical parameters after surgery

	B	S.E	Beta coefficient	*p*	95% CI (lower/upper limit)
Δ BMI (kg/m^2)	29.7	10.73	0.410	**0.009**	8.0 / 51.4
Δ Body fat (kg)	18.1	6.38	0.419	**0.007**	5.2 / 31.1
Δ HbA1c (%)	14.6	11.5	0.204	0.206	−38.2 / 8.5
Δ Insulin (μIU/ml)	0.8	0.89	0.155	0.338	−0.9 / 2.6
Δ Glucose (mg/dL)	0.2	0.27	0.077	0.638	−0.4 / 0.6
Δ Triglyceride (mg/dL)	0.1	0.08	0.130	0.423	−0.4 / 0.6
Δ Total cholesterol (mg/dL)	0.5	0.20	-0.331	**0.037**	0.1 / 0.8

S.E standard error, *BMI* body mass index, *HbA1c* hemoglobin A1c

Multiple linear regression analysis was performed. $p < 0.05$ data were shown in bold

Anorexigenic effects of nesfatin-1 can cause a decrease in body fat and BMI. Several studies demonstrated that nesfatin-1 has anorexigenic effects, and also, nesfatin-1 levels are higher in individuals with obesity compared to individuals with normal BMI [20, 25]. Nesfatin-1 is expressed in many areas of the body including gastric cells. Obese individuals have larger stomach volume, suggesting that they may have higher levels of nesfatin-1 [26]. A study showed that plasma nesfatin-1 levels significantly decreased 12 months after sleeve gastrectomy ($p < 0.005$) and the change in nesfatin-1 was positively correlated ($p < 0.005$) with the change in BMI and negatively correlated ($p < 0.005$) with fasting blood glucose levels [27]. Similarly, in this study, changes in serum nesfatin-1 levels after surgery were correlated with the change of weight, BMI and body fat mass; however, there was no correlation with fasting blood glucose levels. In our study, we found no correlation between nesfatin-1 and fat-free mass or body fat percentage (Table 3). However, we did observe a positive correlation between nesfatin-1 and body fat mass. We also noticed that both parameters decreased, which we believe may be related to the direct release of nesfatin-1 from adipose tissue, as mentioned in the literature [28].

Furthermore, consistent with our results, a study showed that decreased nesfatin-1 and total cholesterol levels were correlated after bariatric surgery [10]. A study demonstrated that peripheral injunction of nesfatin-1 in high-fat diet induced obese mice decreased lipid accumulation in the liver and increased AMP-activated protein kinase (AMPK) activation. This finding may explain the significant correlation between cholesterol and nesfatin-1 levels in our study since AMPK activation inhibits cholesterol synthesis [28].

The literature contains conflicting results regarding the relationship between weight loss and nesfatin-1. We have mentioned the literature that is consistent with the results of our study above. However, contrary to our findings, a study on obese mice showed that NUCB2/nesfatin-1 levels increased in serum after sleeve gastrectomy and Roux-en-Y Gastric Bypass (RYGB) operations. The same study indicated that sleeve gastrectomy was more effective than RYGB in terms of nesfatin-1 levels, glucose and lipid metabolism [29]. Another study showed that nesfatin-1 levels increased 3 and 6 months after sleeve gastrectomy compared to the preoperative period, which is contrary to our results [14]. This difference may be due to the fact that nesfatin-1 mRNA expression levels decrease significantly during fasting but increase in the hypothalamus upon refeeding. The first month after surgery is typically characterised by a significant decrease in food intake [30]. We believe that the contradictory results in the literature may be due to slight increases in food intake in the postoperative 3rd and 6th months, as compared to the first month [14].

This study has limitations such as low number of participants and short follow-up period.

This study had several limitations. Firstly, the study duration may be too short to observe changes that are affected by nesfatin-1 levels. Secondly, the study was limited by small sample size; therefore, a larger and longer follow-up study is needed.

Conclusions

In conclusion, our results indicate that nesfatin-1 levels decrease in response to weight loss after bariatric surgery in obese subjects. In our study, serum nesfatin-1 levels and anthropometric measurements were correlated. There was no correlation between nesfatin-1 and glucose homeostasis markers. Bariatric surgery provides significant weight loss and treats many diseases, including insulin resistance and type 2 diabetes. Changes in nesfatin-1 level may be one of the mechanisms of this effect. Longer-term studies are needed for evaluating the effects of nesfatin-1 in the treatment of obesity and type 2 diabetes.

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Data Availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to ethical concerns.

Declarations

Ethics approval and consent to participate The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Hacettepe University Non-Interventional Clinical Research Ethical Board (GO 17/760–05).

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Association of microRNA-192, pentraxin-3, and transforming growth factor-beta1 with estimated glomerular filtration rate in adults with diabetic nephropathy

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Abstract

Objective Nephropathy is among the most pervasive complications of diabetes; it frequently results in end-stage renal disease and even death. However, current biomarkers for diabetic nephropathy (DN) have limited diagnostic utility. Thus, this present study aims to examine the associations of estimated glomerular filtration rate (eGFR) with plasma concentrations of microRNA-192 (miR-192), pentraxin-3 (PTX-3), and transforming growth factor-beta1 (TGF-β1) to identify biomarkers able to distinguish late-stage from early-stage DN.

Methods In total, 50 healthy volunteers and 271 diabetes patients were enrolled in this study. Participants were stratified into seven groups according to eGFR and glycated hemoglobin (HbA1c), healthy controls, diabetes without DN (G1), diabetes with mild renal impairment (G2), and 4 DN grades (G3a, G3b, G4, and G5).

Results DN groups exhibited increases in serum miR-192 ($p < 0.05$), PTX-3($p < 0.05$), TGF-β1($p < 0.05$), malondialdehyde ($p < 0.05$), and xanthine oxidase ($p < 0.05$) levels and reductions in glutathione-s-transferase ($p < 0.05$) and superoxide dismutase ($p < 0.05$) compared to healthy controls. Among patients, eGFR was negatively correlated with miR-192, PTX-3, and TGF-β1, and positively correlated with HbA1c. In receiver operating characteristic curve analysis, miR-192 and PTX-3 demonstrated good diagnostic performance in distinguishing early from advanced DN.

Conclusion Elevated serum miR-192 and PTX-3 are associated with lower eGFR in DN, suggesting their utility as diagnostic and prognostic biomarkers.

Keywords Diabetic nephropathy · Glomerular filtration rate · Noncoding RNAs · microRNA-192 · Pentraxin-3 · Transforming growth factor-beta1

Introduction

The most prevalent complication of type 1 and type 2 diabetes mellitus is diabetic nephropathy (DN), which will develop in approximately 40% of all diabetic patients. Moreover, DN progressed to end-stage renal disease (ESRD), a leading cause of diabetes-related mortality [1]. The chronic hyperglycemia characteristic of diabetes often results in excessive glucose metabolism and the overproduction of reactive oxygen species (ROS), which can destroy crucial cellular macromolecules, including structural proteins, enzymes, membrane lipids, and DNA. Ultimately, this oxidative damage results in organelle dysfunction, especially mitochondrial dysfunction and energy failure [2]. In addition, enhanced polyol pathway activity and activation of protein kinase C (PKC) and nicotinamide-adenine-dinucleotide-phosphate-oxidase (NADPO) may result in the formation of

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advanced glycosylation end products, which increase ROS generation and kidney damage [3].

Diabetic nephropathy (DN) characterized by excessive urinary albumin secretion, reduced glomerular filtration rate, and gradual kidney function decline, ultimately leading to kidney failure [4]. However, microalbuminuria is not a reliable diagnostic indicator of DKD. Hence, there is a need for more effective biomarkers to predict, diagnose, and monitor the disease.

Noncoding RNAs (ncRNAs), in particular, long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), provide significant contributions to the pathophysiology of renal disorders [5, 6]. MicroRNAs are highly conserved noncoding RNAs of 18–24 nucleotides that silence genes post-transcriptionally to control expression [7]. MicroRNA-192 (miR-192) is among the most highly expressed miRNAs in the renal cortex [8], suggesting potential contributions to DN pathogenesis. Transforming growth factor-beta-1 (TGF-b1) is an immune mediator that reduces inflammation by interfering with Toll-like receptor-dependent signaling, thereby preventing or reversing macrophage activation [9]. There are 33 cytokines in the TGF family, and they function by forming dimeric type I and type II serine/threonine kinase receptors [10]. These receptors control the expression of genes involved in the epithelial–mesenchymal transition, angiogenesis, immune system regulation, and growth arrest [11]. Given the contributions of oxidative stress and chronic inflammation to DN, we speculated that serum TGF-b1 concentration may also be associated with the DN stage. Finally, we have also explored the long noncoding RNA pentraxin-3 (PTX-3) as a potential biomarker. Long-noncoding RNAs are ncRNAs greater than 200 nucleotides that act as the primary regulators of gene expression by increasing or decreasing mRNA stability [12]. By blocking the angiogenic fibroblast growth factor reaction, PTX-3 prevents angiogenesis, induces restenosis, and advances atherosclerotic lesions [13]. In cardiovascular and renal disorders, PTX-3 has been identified as a sensitive biomarker of innate immunity and localized inflammatory responses [14, 15].

To evaluate the potential utility of serum miR-192, PTX-3, and TGF-β1 as biomarkers for diabetic nephropathy, we have examined the associations between serum concentrations and kidney function as measured by eGFR in a cohort of diabetic adults with DN of variable severity.

Methods and Materials

Study design

In total, 271 outpatients with type 2 diabetes mellitus (T2DM) were enrolled from the specialized diabetes and nephrology clinic of the Internal Medicine Department, Beni-Suef University Hospital, Beni-Suef, Egypt. Eligible patients were divided into six severity groups according to eGFR [16]. Fifty healthy adult males and females matched for age and sex were included as normal controls. This study was conducted following the Declaration of Helsinki and clinical practice recommendations, and study protocols were approved by the hospital Ethics Committee (BSU: 7–2021). Blood samples were collected between November 2020 and June 2021 after study approval. During laboratory visits, patient body parameters were also measured.

Inclusion criteria

Healthy controls ($n=50$) were selected based on the absence of significant health-related issues and no signs of diabetes/prediabetes [glycated hemoglobin (HbA1c) below 5.7%, fasting glucose level below 110 mg/dl, and postprandial glucose level below 140 mg/dl] or kidney dysfunction [eGFR ≥ 90 mL/min/ 1.73 m^2)]. Diabetic patients were divided into a non-nephropathy group (G1: HbA1c > 6.5%, eGFR ≥ 90 mL/min/ 1.73 m^2, $n=46$), a mild DN group (G2: HbA1c > 6.5%, eGFR 60–89 mL/min/1.73 m^2, $n=50$), and a DN group (HbA1c > 6.5%, eGFR < 60 mL/min/ 1.73 m^2). In turn, the DN group was stratified into severity groups as follows: G3a: HbA1c > 6.5%, eGFR 45–59 mL/min/1.73 m^2 ($n=43$); G3b: HbA1c > 6.5%, eGFR 30–44 mL/min/1.73 m^2 ($n=40$); G4: HbA1c > 6.5%, eGFR 15–29 mL/min/1.73 m^2 ($n=45$); G5 [ESRD]: HbA1c > 6.5%, eGFR < 15 mL/min/1.73 m^2 ($n=47$).

Exclusion criteria

Participants with a history of acute and chronic infections, malignancy, hepatic disease, diabetic retinopathy, and other endocrine dysfunctions were excluded from this study.

Biochemical assays

After an overnight fast, two 4-mL blood samples were obtained from all participants, one in ethylenediamine tetraacetic acid (EDTA)-treated tubes and the other in plain ones. Samples in plain ones were incubated at room temperature for 30 min and centrifuged at 4,000 g for serum isolation; meanwhile blood samples containing EDTA were frozen at − 80 °C until DNA extraction and HbA1c% determination. Glycated hemoglobin was measured using kits from Stanbio (Boerne, Texas, USA), blood glucose, creatinine, urea, uric acid, sodium, potassium, and calcium were measured in serum samples using dedicated kits from Spinreact (Girona, Spain). Fasting insulin was measured using radioimmunoassay kits from Diagnostic Products Corporation (Los Angeles, CA, USA). Insulin resistance was measured according to the homeostatic model (HOMA-IR),

where HOMA-IR = [(Fasting Insulin, μU/ml) × (Fasting Glucose, mmol/L)]/22.5 according to Matthews et al. [17]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate eGFR in adults [18]. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid method [19], xanthine oxidase (XO) activity using the methodology of Ozer et al. [20], and superoxide dismutase (SOD) and glutathione-s-transferase (GST) activities according to a previously described method [21] using kits from Biodiagnostic (Giza, Egypt).

MicroR-192, PTX-3, and TGF-β1 assays

The total RNA was isolated from the serum samples using the Direct-zol RNA Miniprep Plus kit (Cat # R2072, Zymo Research, Irvine, CA, USA), and RNA quality was assessed using a Beckman dual spectrophotometer (Brea, CA, USA). Isolated RNA samples were reverse-transcribed; then, expression levels were measured by quantitative real-time PCR using a One-Step RT-PCR kit (Cat # 12594100, Thermo Fisher, USA) and the following primer sequences: F: 5′TGACCTATG AATTGACAGCCGT-3′ and R: 5′ ATCCAGTGCAGGGTC CGA-3′ for miR-192; F: 5′ AATGCTGTGTCTCTGTCA-3′ and R: 5′ ACATACCAATAACAATGAACAATG-3′ for PTX-3; F: 5′AACACATCAGAGCTCCGAGAA-3′ and R: 5′GTC AATGTACAGCTGCCGCAC-3′ (NM-000660.2) for TGF-β1; F: 5′GGCGGCACCACCATGTACCCT-3′ and R: 5′ AGG GGCCGGACTCGTCATACT-3′ (NM-001101.3) for β-actin (the internal control for TGF1-β); F: and R: for GAPDH (the internal control for PTX-3). Expression of miRNA-192 was normalized to U6 expression. The RQ of each target gene was quantified and normalized to the specified internal control according to the calculation $2-\Delta\Delta Ct$ [22].

Statistical analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) 22.0 (SPSS Inc., Chicago, IL, USA). Demographic and clinical parameters are presented as mean ± standard error of the mean (SEM). Group means were compared via one-way analysis of variance with post hoc Duncan's multiple range tests for pairwise comparisons. A $p < 0.05$ was considered statistically significant for all tests. Associations between eGFR and target gene expression levels were evaluated via Pearson's correlation coefficient. Receiver operating characteristic (ROC) curves were constructed by plotting sensitivity on the Y-axis versus 1-specificity on the X-axis at various cut-off values. The diagnostic accuracy for each cut-off was evaluated by measuring the area under the ROC curve (AUC). At least 50% of performance was considered adequate.

Results

The average age of the study population was 49.40 ± 13.50 years, and the majority were males (51.71%). There were marked variations in body mass index (BMI), systolic blood pressure, and diastolic blood pressure among the healthy control, diabetic without DN (G1), diabetic with mild impairment (G2), and diabetic nephropathy (G3a, G3b, G4, and G5) groups ($p < 0.05$) (Table 1). Compared to the healthy controls, all diabetic groups exhibited significantly higher fasting blood sugar (FBS), HbA1c%, Homa-IR, and serum urea, creatinine, uric acid, and potassium levels. Additionally, there were significantly lower levels of serum insulin, serum calcium, and eGFR ($p < 0.05$). Sodium levels were found to be significantly higher in G2, G3a, G3b, and G4 groups as compared to healthy controls (Table. 1). On the other hand, a significant decline ($p < 0.05$) was noted in sodium concentrations at G5 (ESRD) compared to healthy controls and patients in earlier stages of DN (G2, G3a) (Table 1).

The findings demonstrate that individuals with diabetes have significantly higher levels of MDA and XO, indicating the presence of chronic oxidative stress. Moreover, all diabetic patient groups showed considerably lower levels of SOD and GST compared to healthy controls, as depicted in Fig. 1. Notably, as DN progressed to its later stages (G3b, G4, G5), MDA and XO levels continued to rise significantly ($p < 0.05$). SOD and GST activities showed significant declined, as shown in Fig. 1A, B, C, D for the early stages (G2 and G3a).

Serum expression levels of miR-192, PTX-3, and TGF-β1 were significantly elevated in DN stages (G3a, G3b, G4, G5) as compared to healthy controls (Fig. 2) and in later stages (G3b, G4, G5) compared to early stages (G2, and G3a) (Fig. 2A, B, C). Moreover, miR-192, PTX-3, and TGF-β1 levels were negatively correlated with eGFR in stages G2, G3a, G3b, G4, and G5 (for G2; miR-192: $r = -0.643$, $p < 0.001$; for PTX-3: $r = -0.523$, $p < 0.001$; for TGF-β1: $r = -0.570$, $p < 0.001$) (Table 2), whereas these correlations did not reach significance in G1. The glycemic biomarker (HbA1c) was also determined to be positively correlated with serum expression levels of miR-192, PTX-3, and TGF-β1 (Table 2) in all diabetic stages (G1, G2, G3a, G3b, G4, and G5), and these correlations were higher through disease progression from diabetic stage (G1) (miR-192: $r = 0.794$, $p < 0.001$; PTX-3: $r = 0.722$, $p < 0.001$; TGF-β1: $r = 0.706$, $p < 0.001$) to ESRD (G5) (miR-192: $r = 0.913$, $P < 0.001$; PTX-3: $r = 0.878$, $p < 0.001$; TGF-β1: $r = 0.889$, $p < 0.001$).

Serum PTX-3 discriminated participants with DN from healthy controls with 96.68% sensitivity and 100% specificity (AUC = 0.993; 95% confidence interval [CI] = 0.985–1.001;

Table 1 Demographic, diabetic and kidney profiles of healthy controls and diabetic-nephropathy groups

Group Parameter	Healthy controls	G1	G2	G3a	G3b	G4	G5
Age (Year)	43.71 ± 1.15^{a}	51.08 ± 0.66^{b}	56.12 ± 0.89^{c}	60.02 ± 0.91^{d}	63.02 ± 0.98^{e}	63.88 ± 0.81^{e}	59.06 ± 0.85^{d}
Gender, no. (%)							
Male	27 (54)	20 (43)	26 (52)	20 (46)	23 (57)	24 (53)	26 (56)
Female	23 (46)	26 (47)	24 (48)	23 (54)	17 (43)	21 (47)	21 (44)
BMI (Kg/m^2)	28.06 ± 0.33^{a}	33.08 ± 0.52^{cd}	32.16 ± 0.63^{bcd}	31.41 ± 0.41^{bc}	33.62 ± 0.65^{d}	30.36 ± 0.52^{b}	31.28 ± 0.59^{bc}
SBP (mmHg)	124.22 ± 1.08^{a}	125.69 ± 0.87^{ab}	130.94 ± 1.31^{b}	140.76 ± 1.65^{c}	142.13 ± 1.88^{c}	143.12 ± 2.37^{c}	146.26 ± 3.04^{c}
DBP (mmHg)	82.98 ± 0.55^{a}	82.91 ± 0.58^{a}	85.88 ± 0.75^{ab}	88.18 ± 0.98^{b}	88.85 ± 1.08^{b}	88.24 ± 1.57^{b}	87.16 ± 1.71^{b}
FBS (mg/dl)	82.06 ± 0.92^{a}	165.61 ± 3.73^{bc}	194.12 ± 7.62^{d}	181.21 ± 7.28^{cd}	190.30 ± 6.42^{d}	163.84 ± 3.24^{b}	173.23 ± 5.88^{bc}
HbA1c (%)	4.73 ± 0.05^{a}	8.81 ± 0.09^{b}	9.67 ± 0.16^{d}	9.12 ± 0.16^{bc}	9.82 ± 0.13^{d}	9.44 ± 0.10^{cd}	9.21 ± 0.17^{c}
Fasting insulin (mLU/L)	11.25 ± 0.09^{e}	10.52 ± 0.08^{d}	8.43 ± 0.05^{c}	8.07 ± 0.04^{b}	7.96 ± 0.04^{ab}	7.82 ± 0.04^{a}	7.88 ± 0.05^{a}
HOMA-IR	2.27 ± 0.03^{a}	4.29 ± 0.09^{f}	4.04 ± 0.16^{ef}	3.61 ± 0.15^{cd}	3.74 ± 0.12^{de}	3.16 ± 0.06^{b}	3.35 ± 0.10^{bc}
Creatinine (mg/dl)	0.92 ± 0.02^{a}	0.94 ± 0.01^{a}	1.01 ± 0.02^{ab}	1.26 ± 0.02^{b}	1.58 ± 0.04^{c}	3.09 ± 0.07^{d}	7.22 ± 0.26^{e}
Urea (mg/dl)	22.26 ± 0.46^{a}	23.89 ± 0.52^{a}	27.84 ± 0.65^{a}	36.37 ± 1.04^{b}	51.22 ± 1.98^{c}	88.55 ± 2.74^{d}	114.70 ± 3.73^{e}
Uric acid (mg/dl)	4.33 ± 0.06^{a}	4.98 ± 0.10^{b}	5.36 ± 0.11^{b}	5.79 ± 0.12^{c}	6.11 ± 0.26^{cd}	6.42 ± 0.15^{d}	6.51 ± 0.19^{d}
Sodium (mEq/l)	140.66 ± 0.66^{b}	144.36 ± 0.72^{c}	144.90 ± 0.75^{c}	147.09 ± 0.97^{cd}	149.35 ± 1.42^{d}	146.80 ± 1.78^{cd}	134.43 ± 0.80^{a}
Potassium (mEq/l)	4.39 ± 0.07^{a}	4.42 ± 0.06^{a}	4.51 ± 0.07^{a}	4.54 ± 0.07^{a}	4.76 ± 0.08^{b}	6.13 ± 0.06^{d}	5.72 ± 0.09^{c}
Calcium (mg/dl)	9.47 ± 0.07^{d}	9.09 ± 0.07^{c}	9.04 ± 0.09^{c}	8.59 ± 0.10^{b}	8.98 ± 0.11^{c}	9.03 ± 0.11^{c}	7.90 ± 0.10^{a}
eGFR (mL/min/1.73 m^2)	92.93 ± 0.31^{f}	90.92 ± 1.85^{f}	70.11 ± 0.81^{e}	52.31 ± 0.64^{d}	39.66 ± 0.72^{c}	20.07 ± 0.49^{b}	7.87 ± 0.36^{a}

Data were expressed as mean ± SE. Values that share the same superscript symbol are not significantly different

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBS* fasting blood sugar, *HbA1c%* glycated hemoglobin, *HOMA-IR* homeostatic model assessment for insulin resistance, *eGFR* estimated glomuler filtration rate, *G1* stage 1 (kidney damage with normal or increased GFR ≥ 90), *G2* stage 2 (kidney damage with mildly decreased GFR 60–89), *G3a* stage 3a (moderately decreased G FR 30–59), *G3b* stage 3b (moderately to severely decreased GFR 30–44), *G4* stage 4 (severely decreased GFR 15–29), *G5* stage 5 (kidney failure, GFR < 15), all GFR inmL/min/1.73m^2

$p < 0.001$; Fig. 3A), thus suggesting its utility as a diagnostic marker. Indeed, serum PTX-3 level discriminated late stages of DN (G3b, G4, G5) from early stages (G2, G3a) with 97.83% sensitivity and 94.41% specificity (AUC = 0.994, $p < 0.001$; Fig. 3B). Similarly, serum miR-192 differentiated participants with DN from healthy controls and late stages of DN (G3b, G4, G5) from early stages (G2, G3a), as shown in Fig. 3C, D.

Discussion

Chronic hyperglycemia can induce DN, which, in turn, can progress to chronic kidney failure and even death [23]. Microalbuminuria is the standard biomarker for early DN detection and diagnosis, but its efficiency in estimating disease stage remains limited [24]. To effectively diagnose and treat DN in its early stages, it is imperative to identify more sensitive indicators of DN progression. Here, we show that serum levels of miR-192 and PTX-3 can distinguish early- from late-stage DN with high sensitivity and specificity among Egyptian diabetes patients with widely varying eGFR and other clinicodemographic parameters such as BMI, SBP, and DBP. In addition, there were linear increases in FBS, HbA1c%, creatinine, urea, uric acid, and potassium with DN severity, consistent with other Egyptian studies [25, 26], while fasting insulin, calcium, and eGFR declined with DN severity. Serum sodium was also significantly higher in G2, G3a, G3b, and G4 compared to healthy controls but lower in stage 5 (ESRD) compared to earlier stages. Therefore, these factors may yield even more accurate staging and prognosis.

Elevated blood sugar levels have the potential to trigger a surge in ROS production, leading to harmful effects on the renal kidney tubes and podocytes. With time, this damage can transform into renal fibrosis [27]. Malondialdehyde and XO levels were significantly higher, while SOD and GST levels were noticeably lower in all diabetic patient groups as compared to healthy controls, in accordance with Lodhi and colleagues [28], who found that diabetic rats had considerably higher MDA and lower SOD levels than the controls. Additionally, there were substantial differences in MDA, XO, SOD, and GST levels between the early and late stages of DN. Patients with ESRD were found to have higher serum XO activity than healthy individuals and those with chronic renal failure [29]. Our findings are also consistent with Bessa and coworkers, who reported that GST, SOD, glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH) were negatively correlated with albumin creatinine

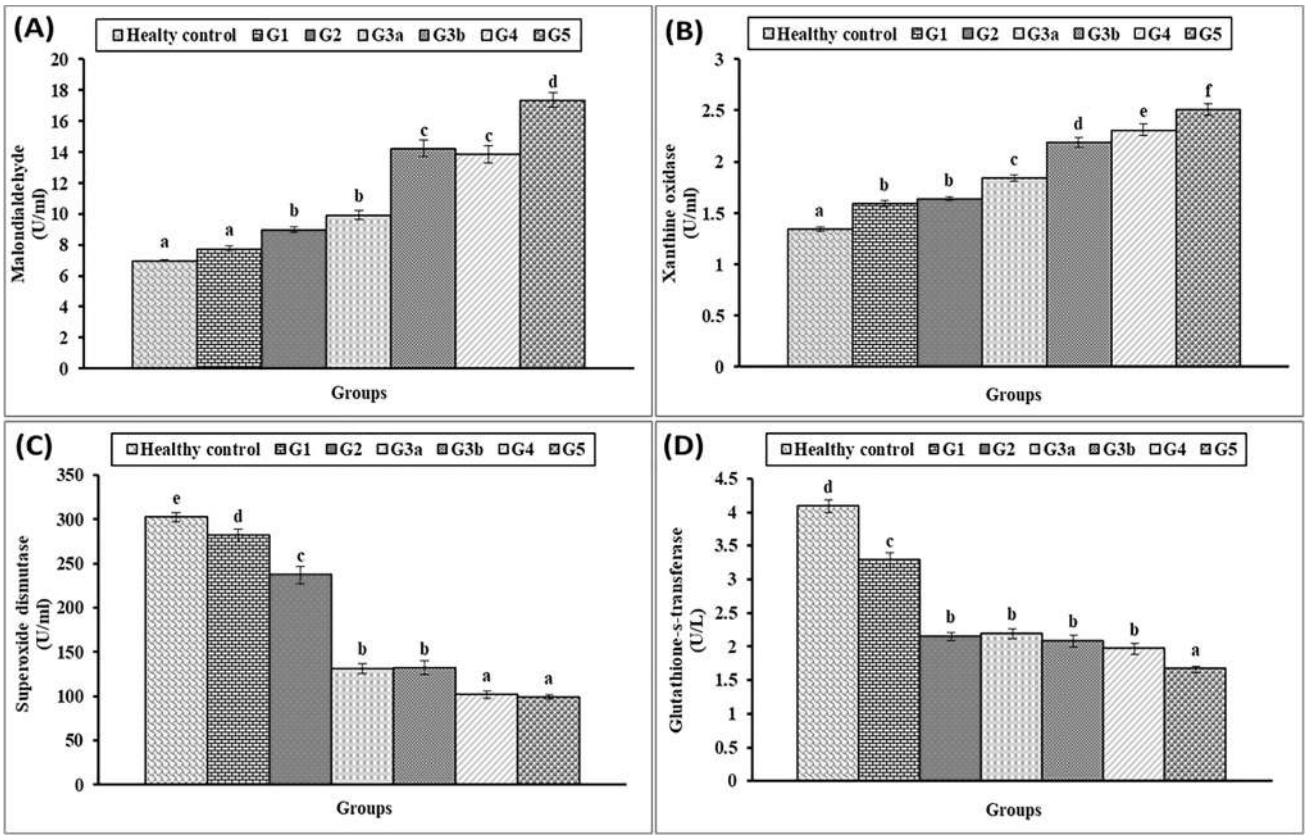

Fig. 1 Oxidative stress profiles of healthy controls, diabetic and diabetic nephropathy groups. Data are expressed as mean ± SEM. Insignificant differences between two groups according to Duncan's post hoc multiple comparison tests are indicated by the same superscript symbol

ratio and positively with eGFR, two crucial indices of renal function [30]. These findings indicate that oxidative stress is a chronic process that contributes substantially to the progression of DN.

Noncoding RNAs are essential for many cellular functions, as more than 30% of the human genome is regulated by lncRNAs and miRNAs [31]. MicroRNA-192 has been widely expressed in the human kidney and is critical for maintaining normal kidney function [32]. Expression of miR-192 was elevated significantly in all DN stages relative to healthy controls, and there was a significant difference between late and early stages, consistent with Saadi and colleagues [33], who found higher blood levels of miR-192 in participants with more advanced DN than T2DM patients with normal albuminuria. They also found a strong inverse relationship between eGFR and miR-192 and a positive correlation between miR-192 levels and the degree of glycemia as indicated by HbA1c. In the current study, miR-192 levels were positively correlated with HbA1c in all investigated groups and negatively correlated with eGFR except for stage 1. Chien and colleagues reported no observable change in serum miR-192 levels between type 2 diabetes patients with and without kidney disease. However, compared to patients with moderately elevated urinary albumin excretion (UAE), those with considerably elevated UAE had significantly greater serum miR-192 [34]. One of the potential mechanisms by which miR-192 could impact DKD and kidney fibrosis is through E-box repressor Smad-1 interacting protein (Zeb2), which attaches to E-box enhancer elements in the Col1a2 gene and accelerates collagen production in response to TGF-β1 [35]. As DN progresses, elevated TGF-β1 expression in renal cells would induce both fibrosis and hypertrophy [36]. Other miRNAs (miR-216a/217 and miR-200 family) may also altered by this process (Zeb1/2 targeted by miR-192). Moreover, Akt kinase may stimulate fibrosis via miR-192 as Akt stimulation in mouse mesangial cells (MCs), which could result in the elevation of DKD markers like extracellular matrix (ECM) genes as well as hypertrophy and apoptosis inhibition [37]. Consequently, miR-216a can target RNA binding protein (Ybx1) and a P-bodies component, leading to TGF-β1-induced collagen expression in mouse MCs [38].

The long pentraxin PTX-3 is a member of the same family as serum amyloid P and hs-CRP [39]. In response to vascular inflammation, PTX-3 expressed in vascular endothelial cells, primary proximal renal tubular epithelial cells,

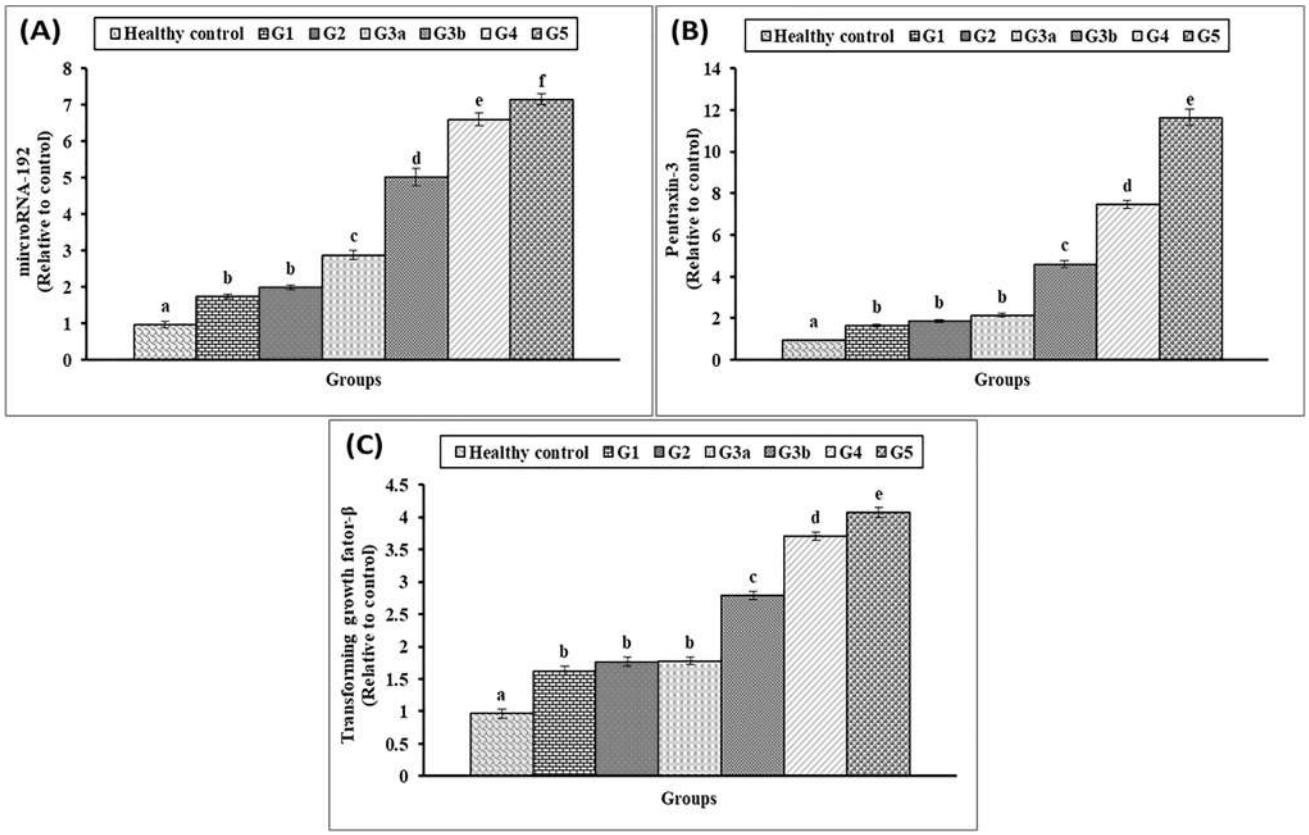

Fig. 2 Serum levels of (**A**) microRNA-192, (**B**) pentraixn-3, (**C**) transforming growth factor-beta in healthy controls, diabetic and diabetic nephropathy groups. Data are expressed as mean ± SEM. Insignificant differences between two groups according to Duncan's post hoc multiple comparison tests are indicated by the same superscript symbol

Table 2 Correlations between miR-192, PTX-3 and TGF-β with eGFR and HbA1c% among diabetic and diabetic-nephropathy groups

Parameters	eGFR						HbA1c%					
Groups	miR-192		PTX-3		TGF-β		miR-192		PTX-3		TGF-β	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
G1	0.090	> 0.05	0.112	> 0.05	0.061	> 0.05	0.794	< 0.001***	0.722	< 0.001***	0.706	< 0.001***
G2	−0.643	< 0.001***	−0.523	< 0.001***	−0.570	< 0.001***	0.837	< 0.001***	0.732	< 0.001***	0.732	< 0.001***
G3a	−0.776	< 0.001***	−0.724	< 0.001***	−0.710	< 0.001***	0.838	< 0.001***	0.833	< 0.001***	0.757	< 0.001***
G3b	−0.842	< 0.001***	−0.860	< 0.001***	−0.876	< 0.001***	0.892	< 0.001***	0.926	< 0.001***	0.933	< 0.001***
G4	−0.929	< 0.001***	−0.932	< 0.001***	−0.943	< 0.001***	0.934	< 0.001***	0.952	< 0.001***	0.949	< 0.001***
G5	−0.949	< 0.001***	−0.921	< 0.001***	−0.944	< 0.001***	0.913	< 0.001***	0.878	< 0.001***	0.889	< 0.001***

Correlation was significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

G1 stage 1 (kidney damage with normal or increased GFR ≥ 90), *G2* stage 2 (kidney damage with mildly decreased GFR 60–89), *G3a* stage 3a (moderately decreased GFR 30–59), *G3b* stage 3b (moderately to severely decreased GFR 30–44), *G4* stage 4 (severely decreased GFR 15–29), *G5* stage 5 (kidney failure, GFR < 15), all GFR in mL/min/1.73 m^2

primary MCs, and renal fibroblasts [40]. The expression of PTX-3 was markedly elevated in all DN stages but to a greater extent in the late stages than in the early stages. These findings are in line with earlier studies by Dawood and colleagues and Wang and colleagues, who both reported a substantial elevation in PTX-3 levels with DN progression and suggested that PTX-3 can act as a biomarker for both prognostic and diagnostic purposes before the onset of overt chronic kidney disease (CKD) [15, 41]. By preventing fibroblast growth factor signaling, PTX3 prevents angiogenesis, increases restenosis, and increases advanced atherosclerotic lesions [13]. In this present study, PTX-3 and HbA1c were

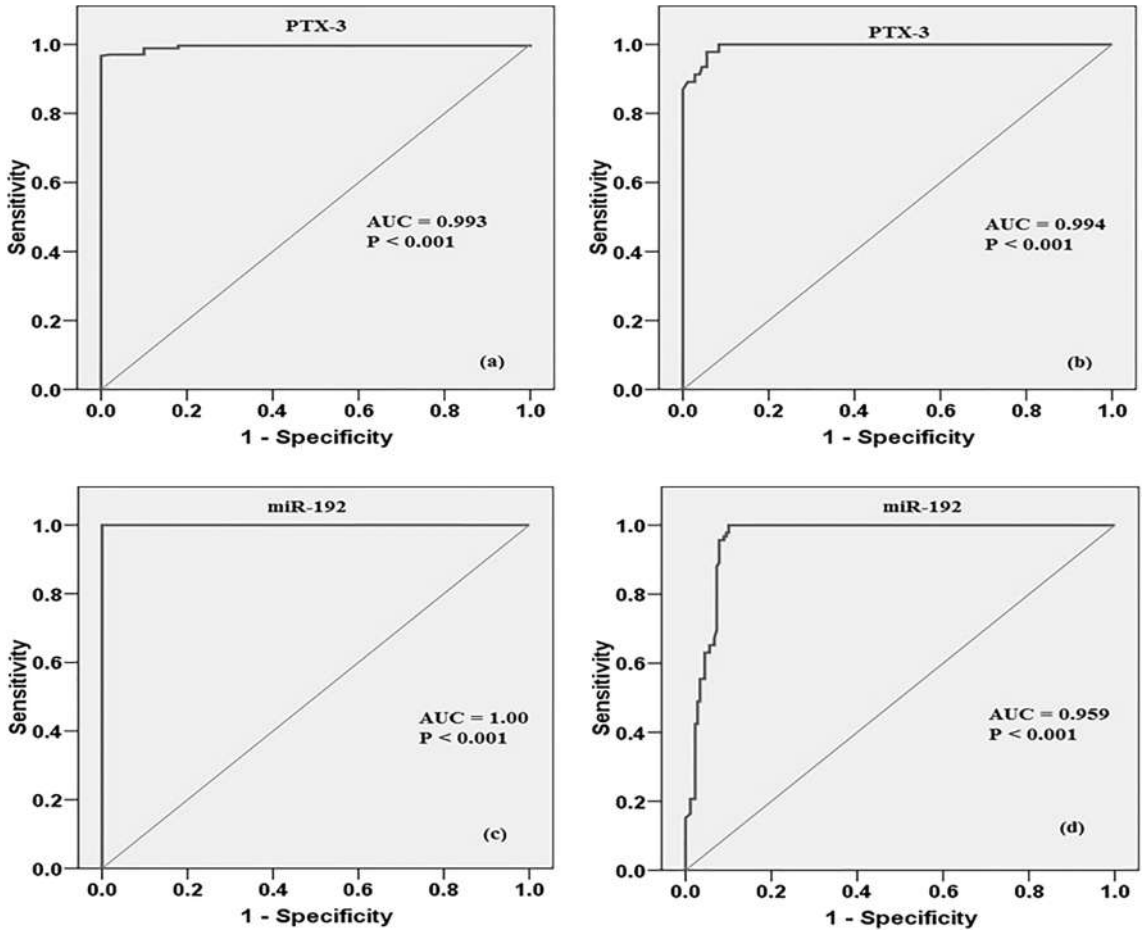

Fig. 3 Receiver operating characteristic (ROC) curves for pentraixn-3 and microRNA-192 in (**a, c**) early diabetic groups and (**b, d**) late-stage diabetic nephropathy groups

determined to be strongly and positively correlated during all DN stages, consistent with Takashi et al. [42], who found that PTX-3 was significantly higher in patients with diabetes and has a notable correlation with HbA1c and UAE. There was a negative correlation between eGFR and PTX-3. Tong et al. [43] also observed higher PTX-3 levels in CKD patients than in healthy controls. Furthermore, PTX-3 was positively associated with protein-energy wasting, cardiovascular disease, and mortality and negatively correlated with eGFR in these patients.

Transforming growth factor-beta may also contribute to the pathogenesis of DN. In the proximal tubules, TGF-β1 stimulates renal cell hypertrophy, controls the formation of ECM molecules such as type I and type IV collagen, and induces the production of chemokines [44]. Further, TGF-β1 also slows down matrix disintegration by blocking proteases, thereby accelerating the development of glomerulosclerosis and tubulointerstitial fibrosis [45]. In addition to TGF-β1, ROS generation activates NF-κB, angiotensin II/ TGF-β1/ smad, and PKC signaling pathways, which can induce ECM protein accumulations and fibrosis. Further, signaling elements, including PKC, TGF-β1, and angiotensin II, also promote ROS generation, causing oxidative stress damage and DN [46]. ROS accumulation induces the expression of numerous pro-fibrotic growth factors, including TGF-β1, VEGF, and connective tissue growth factors, which accelerate ECM protein formation and kidney dysfunction [47].

Expression of TGF-β1 was elevated in all stages of DN and higher in late-stage than early-stage patients. These results align with Qiao et al. [48] Shukla et al. [49], who reported that serum TGF-β1 expression was elevated in type 2 diabetes patients and considerably higher in T2DM patients with nephropathy. There were a strong positive correlation between TGF-β1 and HbA1c in all DN stages and a negative correlation with eGFR. Shaker et al. found positive correlations between TGF-β1 and glucose and HbA1c levels [50]. Additionally, John and Yadla reported that TGF-β elevation in T2DM patients was associated with the severity of kidney damage [51].

Finally, the results of the ROC analysis demonstrate that serum miR-192 and PTX-3 can accurately distinguish DN patients from healthy controls, with serum miR-192 exhibiting diagnostic potential as it can also discriminate late from early stages. These results align with Saadi et al. [33], who reported that miR-192 effectively distinguished T2DM patients with and without DKD and that plasma expression correlated positively with albuminuria. Hence, miR-192 and serum PTX-3 may be promising circulating biomarkers that can effectively detect and monitor disease progression at an early stage.

This current study has several limitations, including the sample size of each DN grade. In addition, other variables known to be associated with DN, such as smoking, various medications, degree of physical activity, and inflammatory mediators, were not examined or controlled.

Conclusion

Serum miR-192, PTX-3, TGF-β1, MDA, and XO were elevated, while SOD and GST levels lowered in DN patients relative to healthy controls. Moreover, It has been noted that the alterations in the later stages of the disease are notably more substantial compared to the initial stages. Additionally, plasma expression levels of miR-192, PTX-3, and TGF-β1 were negatively correlated with eGFR and positively correlated with HbA1c. Our findings suggest that miR-192 and PTX-3 can act as diagnostic biomarkers for discriminating patients in the late stages of DN from those in the early stages.

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Authors' contributions AAM and NAH contributed to the study's conception and design. Material preparation, data collection was performed by ZRN. Statistical analyses were performed by ZRN, AAM, BM, NAH and AEA. The first draft of the manuscript was written by ZRN. AAM, BM, NAH and AEA critically revised the manuscript. All authors reviewed and edited the final draft of the manuscript.

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Data availability This article has all the data that were created or analysed during this study.

Declarations

Ethics approval and consent to participate This study was conducted in compliance with the Declaration of Helsinki, and approved by the (BSU: 7-2021). Written informed consent was obtained from each participant in the study.

Patient consent for publication Patient consent for publication was covered by the informed consent document.

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

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ORIGINAL ARTICLE

Association of the rs7903146 variant (IVS3C>T) of TCF7L2 with the prevalence of the metabolic syndrome and its components in population from Ahvaz cohort study: a case-control study in Iran

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Abstract

Background The metabolic syndrome consists of a combination of metabolic abnormalities and genetic predisposition that both contribute significantly to its development. Numerous studies have established a strong association between single nucleotide polymorphisms (SNPs) of the rs7903146 variant in the TCF7L2 gene and the metabolic syndrome (MetS) as well as type 2 diabetes.

Objective The aim of this study was to assess the impact of rs7903146 on MetS and its components.

Methods For this cross-sectional study, 325 individuals aged 25 to 86 who were selected from the baseline data of the Ahvaz cohort study were examined. Body mass index, blood pressure, fasting blood glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured following standard protocols. MetS subjects were identified based on the National Cholesterol Education Program guidelines. Genotyping was conducted using the PCR-RFLP method.

Results Our findings revealed that individuals with the CT genotype of rs7903146 had an increased risk of MetS (OR 2.24; 95% CI, 1.26–3.98; $p < 0.006$). This genotype was also found to be associated with a higher risk of hypertension and low HDL cholesterol ($p < 0.05$). Moreover, plasma triglyceride levels were slightly higher in individuals with TT and CT genotypes, although not significantly so ($p = 0.06$).

Conclusion In conclusion, the CT genotype of the TCF7L2 rs7903146 polymorphism exhibited higher odds for MetS. While lifestyle factors and other genes are also implicated in MetS, our findings suggest that studying TCF7L2 polymorphisms in high-risk groups could contribute to the development of genotype-specific prevention or treatment strategies. However, further research is required to validate these results.

Keywords Metabolic syndrome · TCF7L2 · rs7903146 · RFLP

Introduction

Metabolic syndrome (MetS) is characterized by a combination of metabolic risk factors, including hypertriglyceridemia, low HDL cholesterol, abdominal obesity or high BMI, glucose intolerance or insulin resistance, hypertension, and microalbuminuria that increases susceptibility to diabetes and cardiovascular diseases [1].

Global statistics indicate that the prevalence of MetS is on the rise in European and Asian countries. In Iran, studies have estimated the prevalence of MetS to be between 21.9 and 31.1% [2].

While the exact pathogenesis of MetS remains unknown, it is important to acknowledge that it predominantly affects

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populations with high calorie intake and limited physical activity. Additionally, genetic predisposition plays a significant role in its development [3]. However, many of the genes involved in MetS are not yet well understood.

The TCF7L2 gene, located on chromosome 10q25.3, encodes a protein that plays an essential role in the Wnt signaling pathway. This pathway regulates adipocyte differentiation, adipokine secretion profiles, adipogenesis, and β-cell function [4, 5]. Disruption of this pathway can contribute to adipocytokine dysfunction and insulin resistance, both of which are risk factors for MetS [6, 7]. Abnormalities in the Wnt pathway, therefore, can lead to metabolic disorders [8]. Single nucleotide polymorphisms (SNPs) in the TCF7L2 gene can disrupt the Wnt signaling pathway [9].

Numerous studies have found an association between the rs7903146 SNP in TCF7L2 and MetS, as well as an increased risk of type 2 diabetes due to reduced insulin secretion [9–11]. However, some studies have reported no association between the rs7903146 variant and insulin resistance or MetS [12]. It is important to note that conflicting results across different populations may be attributed to variations in sample sizes or specific ethnicities.

Early identification, educational programs, and appropriate treatment can be effective in managing the complications of MetS [13], Therefore, it is crucial to conduct indigenous research to determine whether the TCF7L2 rs7903146 polymorphism predisposes individuals to MetS. In this study, we investigated the associations between the TCF7L2 SNP (rs7903146) and the prevalence of MetS and its components in individuals participating in the Ahvaz cohort study. Findings from this study can inform effective health programs in the future.

Materials and methods

Study design and participants

The data and sub-samples for this research were obtained from a 5-year follow-up cohort of the adult population (aged 25 to 86 years) in Ahvaz. The study population consisted of 142 men and 183 women. The diagnosis of metabolic syndrome was based on the Adult Treatment Panel III (ATP III) criteria, requiring at least three of the following five components: abdominal obesity (waist circumference ≥ 102 cm in men and ≥ 88 cm in women), TG ≥ 150 mg/dl or use of medications to manage triglycerides, HDL ≤ 40 mg/dl in men and ≤ 50 mg/dl in women, systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg or use of antihypertensive medication, and FBS ≥ 100 mg/dl or use of blood sugar-lowering drugs [7, 14].

Clinical analysis

Anthropometric and biochemical data were obtained from the 5-year follow-up cohort of the adult population in Ahvaz. The measurement of anthropometric and biochemical parameters was previously presented by Shahbazian et al. [15] in 2013.

DNA isolation and genotyping

The rs7903146 variant was genotyped using the PCR-RFLP method. Genomic DNA was purified from leukocytes in EDTA blood samples using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The integrity and purity of the extracted DNA were evaluated using 1% agarose gel electrophoresis and NanoDrop (Thermo Scientific, USA) at wavelengths of 260 and 280 nm, respectively. The PCR reaction was performed as follows: step one, 5 min at 95 °C for enzyme activation; step two, 35 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. The sequences of the primers used for PCR are provided in Table 1.

The rs7903146 variant was genotyped using the PCR-RFLP method. The PCR-amplified product resulted in a 188-bp fragment. Subsequent digestion of the PCR product with the RsaI enzyme generated specific fragments based on the genotypes. Specifically, the presence of the TT genotype produced a 188-bp fragment, the CC genotype produced 159-bp and 29-bp fragments, and the CT heterozygous genotype produced all three fragments (188-bp, 159-bp, and 29-bp). The digestion products were separated by electrophoresis on a 2.5% agarose gel and visualized using a safe stain (Yektatajhiz Inc., Iran) under a UV transilluminator (Quantum ST4, France).

Statistical analysis

Statistical analysis was conducted using SPSS version 23.0 (IBM Corporation). The Hardy-Weinberg equilibrium was assessed using a simple chi-square test. Anthropometric and biochemical characteristics among genotypic groups were compared using chi-square tests for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Multivariable binary logistic regression, adjusted for age and gender, was performed to determine the independent association of different genotypes and alleles of rs7903146 with the prevalence of MetS. The associations were presented as odds ratios (ORs) with corresponding 95% confidence intervals

Table 1 The sequences of the primers used in the study

Forward sequences: 5′-ACAATTAGAGAGCTAAGCACTTTTTAG GTA-3′
Reverse sequences: 5′-GTGAAGTGCCCA AGCTTCTC-3′

(CIs). A significance level of $p < 0.05$ was considered statistically significant. The comparison was made between the CC genotype (as the dominant model) and carriers of the minor alleles (CT and TT) in the analysis.

Results

Characteristics of the study participants based on TCF7L2 rs7903146 genotype

The anthropometric and clinical characteristics of the study participants were analyzed based on their TCF7L2 rs7903146 genotype at the beginning of the study. The distribution of genotypes was found to be in accordance with Hardy-Weinberg equilibrium ($p > 0.05$). Although there was a tendency for carriers of the TT genotype to experience a decrease in plasma TG levels, the results did not reach statistical significance ($p = 0.06$). Furthermore, there were no significant differences observed in other variables based on genotype (refer to Table 2).

Association between TCF7L2 rs7903146 and MetS

Logistic regression analysis revealed that individuals with the CT genotype had a significantly higher prevalence of metabolic syndrome (MetS) compared to those with the CC genotype ($p = 0.004$; OR, 2.34; CI, 0.31–1.80). The dominant genetic model test indicated that carriers of the T allele (CT+TT) had a significantly higher prevalence of MetS compared to non-carriers ($p = 0.01$; OR, 2.06; CI, 1.18–3.60) (refer to Table 3).

Association between TCF7L2 rs7903146 and MetS components

Individuals with the CT genotype exhibited a significantly higher prevalence of hypertension compared to those with the reference genotype (CC) ($p = 0.02$; OR, 2.06; CI, 1.11–3.82). Under a recessive model, T allele carriers (CT+TT) demonstrated a significant difference in the prevalence of hypertension compared to other genotypes ($p = 0.02$; OR, 2.07; CI, 1.12–3.83), while the TT genotype exhibited a protective effect against hypertension compared to the (CT+CC) genotype ($p = 0.04$; OR, 0.34; CI, 0.12–0.95). Furthermore, the prevalence of low HDL cholesterol was significantly higher in CT carriers compared to the reference genotype ($p = 0.02$; OR, 1.76; CI, 0.49–2.20). However, in the dominant model, individuals with the T allele (TT+CT) showed lower HDL cholesterol levels than those with the CC genotype, although this difference did not reach statistical significance ($p = 0.07$) (refer to Table 4).

Table 2 Clinical characteristics of the study participants according to TCF7L2 rs7903146 genotype

Clinical data	Mean[b]/percent data level by genotype			*p* value[a]
	TT	CT	CC	
Number of participants (%)	43.69%	42.77%	13.54%	
Age (years)	47.0 ± 13.4	46.0± 13.4	47.7 ± 13.3	0.71
Sex (Male/female)	57/85	65/74	20/24	0.51
BMI (kg/m^2)	27.5± 5.0	28.0 ± 7.3	27.7±4.3	0.87
Waist circumference(cm)	91.0 ± 12.0	91.0± 11.0	93.0±11.0	0.55
Systolic blood pressure (mmHg)	115.2±15.5	116.0±14.2	112.1±13.2	0.34
Diastolic blood pressure (mmHg)	69.7±16.16	71.4± 14.4	71.3±11.0	0.62
Fasting plasma glucose (mg/dL)	104.2 ± 39.3	108.0±41.5	118.±60.0	0.18
Triacylglycerol (mg/dL)	143.0± 79.0	152.7±90.0	119.0±66.1	0.06
HDL cholesterol (mg/dL)	47.4 ± 9.3	45.3± 10.0	48.0 ± 8.2	0.11
Smoking (%)	10.6%	8.7%	11.6%	0.80
Abdominal Obesity (%)	58.5%	54.0%	63.6%	0.49
Hypertriglyceridemia (%)	36.6%	36.7%	22.7%	0.19
Low HDL cholesterol (%)	44.4%	53.2%	38.6%	0.15
Hypertension (%)	25.4%	28.8%	20.5%	0.52
Hyperglycemia (%)	40.1%	36.0%	43.2%	0.62
Metabolic syndrome, NCEP-ATPIII	39.3%	48.6%	12.1%	0.33

[a]All *p* values are for univariate logistic regression model, and $p < 0.05$ indicates significant differences

[b]The comparison between groups was based on the means ± standard deviation

Table 3 Association between TCF7L2 rs7903146 polymorphism and MetS based on logistic regression analysis

Rs7903146	Crude model		Adjusted model[b]	
	OR (95%CI)	*p* value	OR (95% CI)	*p* value
CC	Reference	-----	Reference	-----
CT	2.24 (1.26–3.98)	0.006*	2.34 (0.31–1.80)	0.004*
TT	1.33 (0.56–3.13)	0.51	1.78 (0.78–4.10)	0.17
C	1.17 (0.53–2.57)	0.69	---	----
T	1.99 (1.15–3.46)	0.01*	2.061 (1.18–3.60)	0.01*
TT+CT vs. CC(D)	1.99 (1.15–3.46)	0.01*	2.061 (1.18–3.60)	0.01*
TT vs. CC+CT(R)	0.85 (0.39–1.87)	0.69	---	-----

D dominant, *R* recessive, *OR* odds ratios, *CI* confidence interval

[b]Adjusted model, adjusted for age and gender

*Significant *p* values

Discussion

The metabolic syndrome is a complex condition influenced by various factors, including disrupted adipocytokine secretion and insulin resistance, leading to a pro-inflammatory and pro-thrombotic state [6, 7]. While metabolic syndrome is characterized by a combination of metabolic abnormalities, the prevalence of these risk factors can vary among different ethnic groups [7]. Several genomic studies have focused on the genetic susceptibility to metabolic syndrome, revealing significant associations with specific single nucleotide polymorphisms (SNPs) in genes such as FTO, TCF7L2, APOA5, APOC3, and IL6 [16].

TCF7L2 is a transcription factor involved in the Wnt signaling pathway [9] and expressed in various human tissues [17]. The rs7903146 polymorphism in the TCF7L2 gene has been extensively studied and strongly associated with metabolic syndrome and type 2 diabetes [16].

In this Ahvaz cohort study, we also found that the T allele and particularly the CT genotype of rs7903146 were linked to an increased risk of developing metabolic syndrome. This finding is consistent with previous research showing that T allele carriers who consume high levels of polyunsaturated fatty acids (PUFA) are more susceptible to metabolic syndrome, diabetes, and cardiovascular diseases [18]. Meta-analysis studies have further supported the strong association between rs7903146 and type 2 diabetes mellitus (T2DM) in diverse populations of Caucasian, East Asian, South Asian, etc. [19]. Furthermore, a study conducted by Mustafa et al. on the Iraqi Kurdish population indicated that individuals carrying the T allele are more susceptible to type 2 diabetes mellitus (T2DM) [20]. This association has been further confirmed in other

Table 4 The association between *TCF7L2 rs7903146* polymorphism and MetS components

TCF7L2 rs7903146	Abdominal obesity (W.C ≥ 102 cm in men, ≥ 88 cm in women)		High triglycerides (TG ≥ 150 mg/dl)		Low HDL cholesterol (HDL ≤ 40 mg/dl in men, ≤ 50 mg/dl in women)		High blood pressure (systolic BP ≥ 130 mmHg and or diastolic BP ≥85mmHg)		Hyperglycemia (FBS ≥ 100 mg/dl)	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
CC	Reference	----	Reference	----	Reference	---	Reference	-----	Reference	-----
CT	0.84 (0.51–1.39)	0.51*	1.96 (0.54–7.05)	0.86*	1.76 (0.49–2.20)	0.02*	2.06 (1.11–3.82)	0.02*	1.24 (0.64–2.39)	0.51*
TT	0.76 (0.39–1.59)	0.47*	2.07 (0.58–7.42)	0.30*	0.93 (0.90–3.92)	0.85*	0.70 (0.24–2.01)	0.51*	0.79 (0.28–2.27)	0.67*
TT+CT vs. CC	0.80 (0.50–1.28)	0.36	0.94 (0.48–1.83)	0.85	1.53 (0.96–2.42)	0.07*	2.07 (1.12–3.83)	0.02*	1.10 (0.59–2.05)	0.75
TT vs. CC+CT	0.82 (0.41–1.04)	0.57	0.48 (0.14–1.63)	0.24	0.69 (0.35–1.39)	0.31	0.34 (0.12–0.95)	0.04*	0.71 (0.26–1.90)	0.49

*Data are adjusted according to age and sex. Statistical significance was determined at $p < 0.05$; values that was statistically significant was underlined

studies conducted on the Iranian population [21] and the Southern Brazilian population [22].

In contrast, studies conducted by Zheng et al. did not find any association between variants of rs11196218 or rs7903146 in the TCF7L2 gene and type 2 diabetes (T2DM) or fasting levels of proinsulin/insulin ratios in the Chinese population. Interestingly, the rs11196218 variant was identified as the most high-risk locus in the Chinese population [23]. Similarly, Marzi et al. [24] and Saadi et al. [25] did not observe a significant association between TCF7L2 variants and insulin resistance or metabolic syndrome in the MONICA/KORA study and Emirati subjects, respectively. The contradictory results observed in complex diseases like metabolic syndrome can pose a significant challenge [26], and it is likely that these inconsistencies arise from differences in sample size, ethnic heterogeneity, variations in defining metabolic syndrome, and the inherent heterogeneity of the syndrome itself [27].

Our study also revealed a significant association between the CT genotype and high blood pressure as well as lower levels of plasma HDL cholesterol. This is in line with findings from other cohorts, which demonstrated that the TCF7L2 rs7903146 polymorphism and reduced insulin secretion after glucose consumption were associated with increased incidence of hypertension [28]. However, our study found that the TT genotype was more protective against hypertension, contradicting this finding.

We did not observe an association between the TT genotype and metabolic syndrome components such as high triglycerides, low HDL cholesterol, hyperglycemia, hypertension, and abdominal obesity [29]. In contrast, Perez-Martinez et al., studying the effects of rs7903146 on postprandial lipid metabolism in elderly subjects ($\geq$ 65 years), have shown that minor allele carriers have higher fasting plasma TG levels. Factors such as age and adiponectin levels may contribute to the variation in lipid profiles among TCF7L2 T allele carriers [30].

On the other hand, it was suggested that the decrease in triglyceride levels may be due to the inhibition of adipose tissue lipolysis and adipogenesis by the TCF7L2 minor variant [28]. A large body of evidence suggests that the Wnt/TCF7L2 signaling pathway is critical for adipocyte differentiation and the regulation of adipogenesis [9, 28]. The mentioned study also showed that among healthy young males, subjects with homozygous alleles have shown a worse postprandial lipid profile (a trend towards higher plasma triglycerides).

Based on the available evidence, it seems that the effect of the TCF7L2 rs7903146 polymorphism on MetS components is different and related to many conditions so that the T allele has a protective role in some of them and an enhancing role in others. Therefore, it complicates the interpretation of the results and makes other factors more prominent in the development of the metabolic syndrome.

It is important to consider that metabolic syndrome is a multifactorial condition influenced by various risk factors, and our study solely focused on the role of the TCF7L2 rs7903146 polymorphism. Further research should examine the involvement of other related genes and consider lifestyle factors, diet, and nutrition in the study population.

Conclusions

In conclusion, our study supports the association between the CT genotype of the TCF7L2 rs7903146 polymorphism and increased risk of hypertension and low HDL cholesterol, as well as higher odds of developing metabolic syndrome. Although TCF7L2 polymorphisms in high-risk populations could potentially inform genotype-specific prevention and treatment strategies, further research is warranted to validate these findings.

Author contribution NM conceived and supervised the study; MR and HS performed experiments; MTB and BC analyzed data; ND and MR wrote the paper. All authors read and approved the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical clearance, consent of participant The study protocol was approved by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (HLRC-9505), and informed consent was obtained from all participants.

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ORIGINAL ARTICLE

Expression of NLRP3 and superoxide dismutase-2 (SOD2) in the gingival tissues of periodontitis patients with and without type 2 diabetes mellitus: a case–control study

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Abstract

Objective To evaluate the interaction between the NLRP3 inflammasome and superoxide dismutase-2 (SOD2) to provide an insight into the complex host response in periodontitis patients with type 2 diabetes mellitus (T2DM), who have an inherent upregulation of inflammatory cytokines and reactive oxygen species (ROS).

Methods Gingival tissues were collected from 51 patients divided into three groups: group 1, systemically and periodontally healthy; group 2, systemically healthy with periodontitis; and group 3, periodontitis with T2DM. Immunohistochemistry was performed to evaluate the expression of the NLRP3 inflammasome and SOD2 in terms of mean percentage, intensity and staining intensity distribution (SID).

Results The mean percentage levels of NLRP3 and SOD2 expression were statistically significantly higher in group 3 ($49.16 \pm 6.45\%$ and $48.22 \pm 11.66\%$, respectively) when compared to groups 2 and 1 ($p < 0.001$). While the groups with periodontitis with or without T2DM had statistically significant differences in these variables, the highest expression was found in the periodontitis patient group with T2DM ($p < 0.001$). NLRP3 and SOD2 mean percentages were positively correlated in groups 1 and 2 but were negatively correlated in group 3.

Conclusion NLRP3 inflammasome is significantly upregulated in periodontitis patients with T2DM. The negative correlation between SOD2 and NLRP3 in periodontitis patients with T2DM probably indicates insufficient endogenous SOD2 to effectively counteract the excessive oxidative burden and inflammatory state, thereby compounding periodontal destruction in cohorts with T2DM. Further, NLRP3 and SOD2 can be used as biomarkers of periodontal disease progression in periodontitis cohorts modified by DM.

Keywords Periodontitis · Type 2 diabetes mellitus · Inflammasomes · Immunohistochemistry

Introduction

The pathogenesis of periodontitis, which though microbial biofilm initiated, is predominantly a host-driven immuno-inflammatory disease of the periodontium. While insufficient inflammation can lead to persistence of these toxic stimuli, excessive inflammation in certain conditions can cause an exaggerated release of pro-inflammatory cytokines in response to the microbial challenge, thereby resulting in the destruction of the surrounding tissues referred to as 'bystander damage' [1]. Primary amongst the various local, systemic and environmental conditioning factors of the periodontal tissues that amplify the host immuno-inflammatory reactions in periodontitis is diabetes mellitus (DM), which is a significant systemic risk modifier with a wide range of adverse effects on the periodontium [2].

Periodontal disease and DM have been proven to have a bidirectional link which further potentiates periodontal destruction, with periodontitis being recognised as a co-morbidity associated with DM [3, 4]. In diabetics with periodontitis, hyperglycaemia causes extensive production of free radicals, with the resultant increased oxidative stress being a critical driver of periodontal tissue destruction in these patients [5, 6]. The elevated reactive oxygen species serve as

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immuno-stimulatory by-products, which can activate certain intracellular macromolecular switches called the inflammasomes. These inflammasomes promote the maturation and release of the pre-eminent proinflammatory cytokines—interleukin 1ß and interleukin 18—further underscoring their significance in the innate immune response [7]. This priming of the tissues causes the periodontium to react to the microbial assault with a more exaggerated inflammatory reaction than required, even as it fails to eliminate the microbial factors from the gingival sulcus, thereby perpetuating gingival inflammation.

The NLRP3 inflammasomes in particular play a pivotal role in diabetes mellitus as well as in other inflammatory and autoimmune diseases like rheumatoid arthritis and atherosclerosis [8, 9]. The NLRP3 inflammasome contributes to the pathogenesis of diabetes mellitus by its upregulation of systemic inflammation and subsequent elevation of circulatory IL-1ß and IL-18, thereby contributing to worsening of insulin sensitivity [10]. The hyperglycaemic state in diabetes is characterised by accumulation of advanced glycation end products and oxidative stress. This hyper-inflammatory state, along with the infectious challenge from bacteria in periodontitis, is responsible for the over-activation of the NLRP3 inflammasome in these patients [11]. Amongst the various reactive oxygen species (ROS), the mitochondrial ROS are known to be the key molecules that trigger the activation of the NLRP3 inflammasome, resulting in a pro-inflammatory state [12, 13]. These macromolecular components and the various cytokines associated with the NLRP3 inflammasome have been found to be elevated in the saliva of diabetic patients with periodontitis, when compared to the clinically healthy state [14]. The gingival concentrations of the NLRP3 inflammasome has been reported to be present in higher quantities in the diabetic patients with periodontitis [10].

While acknowledging the above-mentioned relationship of ROS, DM, and periodontitis, it is pertinent to highlight that the hallmark of periodontal disease is the elicitation of protective responses, in the form of antioxidants to counter the oxidative stress in the periodontal tissues. Superoxide dismutase-2 (SOD2) is a principal antioxidant enzyme which is a mitochondrial protein that converts superoxide radicals to H_2O_2 and diatomic oxygen [15].

Inflamed gingival tissues in systemic health as well as in type 2 DM (T2DM) have been reported to express higher levels of SOD2 as compared to healthy tissues [6, 16]. SOD2 is seemingly of significant importance in periodontal disease pathogenesis since this isotype of SOD targets mitochondrial ROS which is known to trigger the NLRP3 inflammasome. The levels of SOD2 seems to increase with the severity of periodontitis, and its insufficiency has been implicated in the upregulation of the NLRP3 inflammasome while the consistent overexpression of SOD2 suppresses the expression of NLRP3 inflammasome [17]. However, to our knowledge, the literature lacks studies that assess the possible interactions between the above mentioned key biological products NLRP3 and SOD 2 in periodontitis patients with type 2 DM.

This study therefore sought to assess these markers of unique but interconnected biological processes to provide us an insight into the multidirectional pathways that lead to the progression of periodontal disease complicated with a systemic condition such as diabetes mellitus. An understanding of this relationship would be the basis for further extrapolation of their suitability as biomarkers for periodontal disease activity.

Materials and methods

Study design and ethical clearance

This observational, cross-sectional study was conducted at the outpatient clinics of the Department of Periodontics and Oral Implantology, between September 2019 and August 2020. The study was approved by the Institutional Scientific and Ethical Review Board prior to the commencement of the study (SRMDC/IRB/2018/MDS/No.508). Written informed consent was obtained from each of the participants before enrolment.

Sample size calculation

Sample size was calculated based on the study by Garcia Hernandez AL et al. (2019) based on the percentage of NLRP3 staining in periodontitis and in periodontitis associated with type 2 diabetes mellitus, i.e., 90% and 40%, respectively. nMaster Version 2.0 (Department of Biostatistics, CMC Vellore, India) was utilised to determine sample size. With a power of 90% and alpha error of 5%, the required sample to reach statistical significance was 17 in each group, i.e., a total of 51 samples.

Subject recruitment

Complete periodontal examination was carried out by a single calibrated examiner followed by radiographic evaluation (orthopantomogram) and assessment of glycated haemoglobin levels (HbA1c) for those subjects giving a history of T2DM. The following clinical parameters were evaluated—site-specific gingival index (Loe and Silness, 1963), -specific plaque index (Silness and Loe,1964), site-specific probing pocket depth (PPD), site-specific clinical attachment level (CAL), full mouth bleeding scores (FMBS), and full mouth plaque scores (FMPS). All the subjects were non-smokers between ages 18 and 65 years with at least 20 remaining teeth.

Following recruitment, the participants were categorised into three groups based on specific inclusion and exclusion criteria: group 1, systemically and periodontally healthy; group 2, systemically healthy with generalised stage III/IV grade A periodontitis; and group 3, generalised stage III/IV, grade B periodontitis with T2DM and HbA1c < 7%. This segregation was based on the 2017 World Workshop on 'The Classification system for Periodontal and Peri-Implant Diseases and Conditions, given by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP)' [18].

Inclusion and exclusion criteria

Subjects were included in group 1 if they had no or negligible bleeding on probing, PPD ≤ 3 mm, no clinical attachment loss, and no bone loss. Normoglycaemic, non-smoker subjects diagnosed with stage III or IV periodontitis based on PPD ≥ 6 mm, CAL ≥ 5 mm, bone loss until the middle or apical third of the root visualised on the radiograph, missing teeth due to periodontitis either ≤ 4 (stage III) or ≥ 5 (stage IV), with periodontal disease in > 30% of the teeth were included in group 2. Subjects diagnosed with stage III/IV periodontitis (inclusion criteria same as group 2) and type 2 DM, HbA1c < 7% under oral hypoglycaemics for a duration of more than 6 months were included in group 3. The subjects were diagnosed with type 2 diabetes mellitus, based on the American Diabetic Association, standard of medical care in Diabetes 2011 [19].

Subjects were excluded if they were pregnant or lactating, undergoing orthodontic treatment, diagnosed with autoimmune diseases or systemic conditions or disorders that could influence the periodontium, taking or having taken antibiotics or anti-inflammatory drugs in the previous 3 months, underwent periodontal therapy in the previous 6 months, diagnosed with T2DM with HbA1c ≥ 7% or diabetic complications, or on insulin therapy.

Gingival tissue samples were collected after administration of local anaesthesia with 2% lignocaine and 1:80,000 adrenaline using a no. 15 BP blade. Internal bevel and sulcular incisions were placed on the buccal/palatal/interproximal aspects of the teeth and the gingival tissues were removed with a non-toothed tissue holding forceps and placed in a sterile Eppendorf tube with neutral buffered formalin (pH 7.0) for fixation. The gingival tissue samples were taken from the sites with the deepest probing pocket depth from periodontally involved hopeless prognosis teeth in groups 2 and 3 and samples were collected during crown lengthening procedure or from periodontally healthy teeth extracted for orthodontic treatment in group 1.

Immunohistochemistry procedure

Gingival tissues were processed into paraffin embedded tissue blocks, antigen retrieval was performed, and following manufacturer instructions slides were incubated with the following primary antibodies: NLRP3/NALP3 Antibody, NBP2-12,446 (Novus Biologicals, Littleton, USA), and SOD2/Mn-SOD Antibody, NB100-1992 (Novus Biologicals, Littleton, USA). The tissues were then incubated with secondary antibodies, stained using DAB chromogen and finally counterstained with haematoxylin. The secondary antibody alone was used as the negative control [8].

Expression and quantification of NLRP3 and SOD

The slides were viewed for expression of NLRP3 (Fig. 1a, b, c) and SOD2 (Fig. 2a, b, c) under a light microscope at 40 × magnification. The total number of stained positive cells in every field was counted, and marker expression was seen by calculating the mean of eight fields expressed as percentage for the respective sample (mean%). The proportion of positively stained cells and their intensity of staining were also assessed for each visualized field. The staining intensity distribution (SID) scores for respective fields were calculated by multiplying the proportion score and the staining intensity. The SID score for each sample was then obtained by calculating the mean of eight fields [20, 21].

Statistical analysis

SPSS Version 17 compatible with Microsoft Windows was used for all the statistical analyses. The data was expressed as mean and standard deviation. Following evaluation for normality by Kolmogorov–Smirnov and Shapiro–Wilk tests, non-normal data were further assessed using Kruskal–Wallis followed by Mann–Whitney *U* test for continuous data. For parametric data, one-way analysis of variance with a post hoc Tukey HSD was employed. Pearson's correlation coefficient analysis/Kendall's tau-b correlation was used to examine the relationship between two related variables. Chi-squared test was used to analyse the association between the two attributes; $p < 0.05$ was considered to be statistically significant.

Results

Descriptive statistics of the study

The gender distribution and clinical parameters of the subjects are given in Table 1. The evaluated clinical parameters showed significant difference between group 1 with

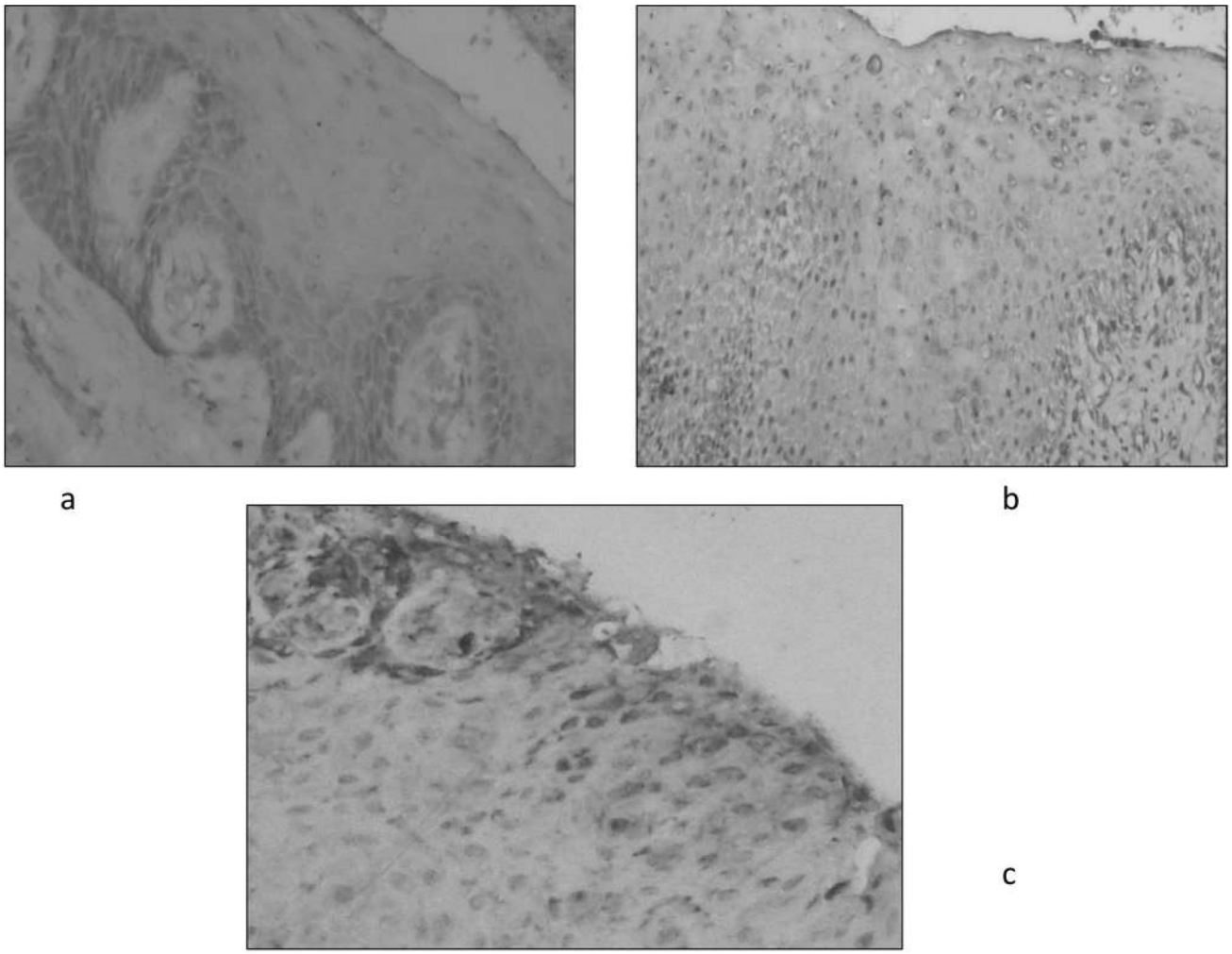

Fig. 1 **a**, **b**, **c** NLRP3 staining in groups 1, 2, and 3

2 ($p < 0.001$) and group 1 with 3 ($p < 0.001$). However, on comparing group 2 with 3, no statistically significant differences were observed.

NLRP3 mean% and SID

The samples from group 1 reflected gingival health with stratified squamous epithelium and a relatively inflammation free underlying connective tissue. However, the sections from groups 2 and 3 presented with minor epithelial hyperkeratosis, nuclear pyknosis, and vacuole formation. Inflammatory cell infiltration was evident in the connective tissue. The immunohistochemical reactivity was positive for NLRP3 in both the epithelium and connective tissue in all samples analysed (Fig. 1a, b, c). A statistically significant difference was seen on comparing the NLRP3 mean% between groups. The NLRP3 mean% of group 3 was greater when compared to groups 1 and 2 with group 1 having the lowest expression of positive cells ($p < 0.001$) (Table 2). NLRP3 mean% negatively correlated with mean PPD in group 1 and positively correlated with PPD and CAL in groups 2 and 3 but without any statistical significance. On intergroup comparison, NLRP3 SID score indicated statistically significant difference with $p < 0.001$. Group 3 had the highest score followed by group 2 and least expression in group 1 (Table 1).

SOD2 mean% and staining intensity distribution

The SOD2 mean% scores of the three groups revealed that group 1 had the least score while group 3 had the highest score. Similarly, the staining intensity distribution progressively increased from group 1, being the lowest, to group 3 ($p < 0.001$) (Table 2; Fig. 2a, b, c). Mean PPD in group 1 (healthy) individuals showed a statistically significant negative correlation with SOD2 mean% ($r = -0.698$, $p = 0.002$) and SOD2 SID ($r = -0.059$, $p = 0.01$).

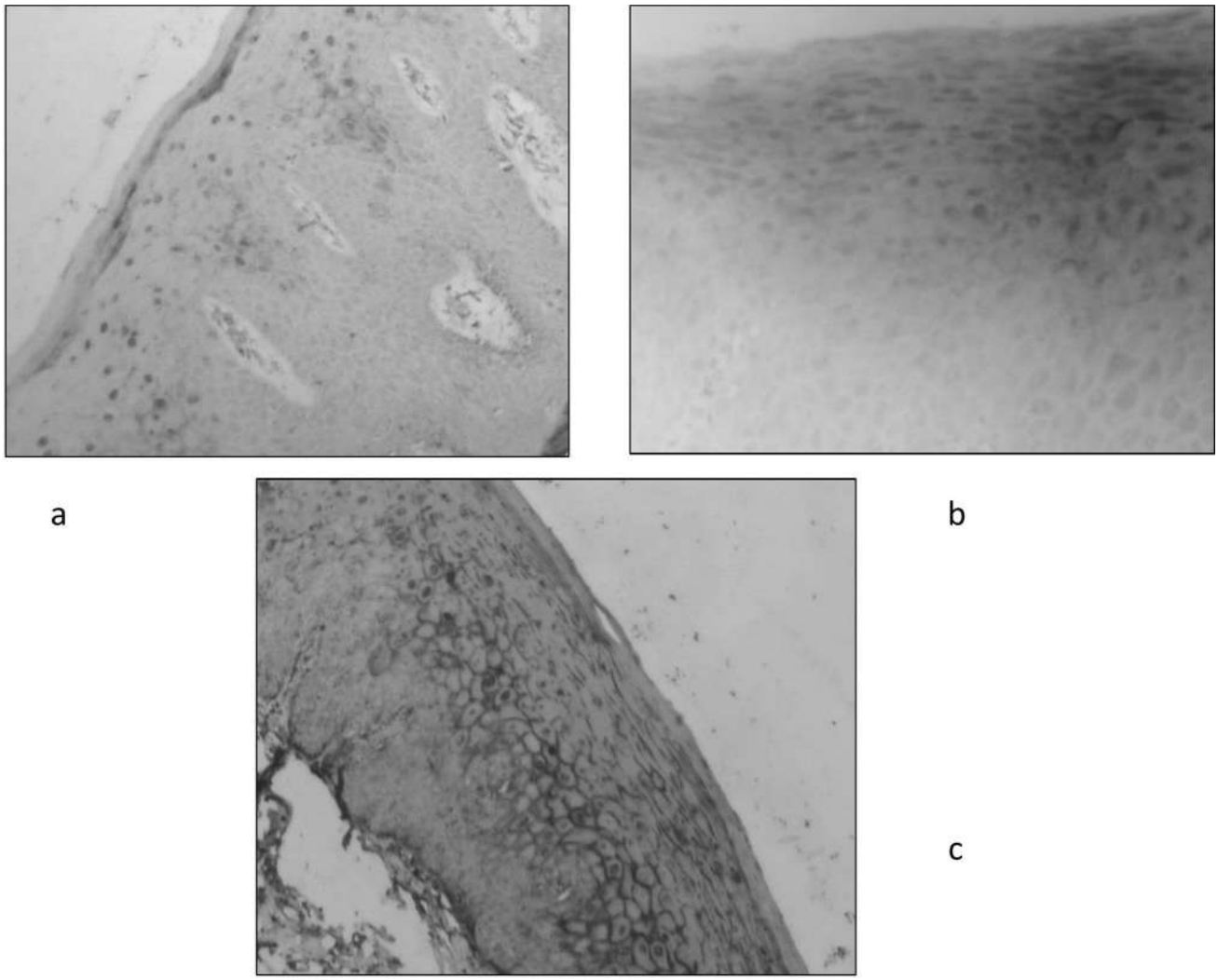

Fig. 2 **a**, **b**, **c** SOD2 staining in groups 1, 2, and 3

Correlation analysis between NLRP3 and SOD2 IHC parameters

The mean% and SID scores of NLRP3 and SOD2 showed no significant correlation.

Discussion

The current study sought to evaluate the levels of the NLRP3 inflammasome and SOD2 in the gingiva of periodontitis patients with diabetes mellitus since these biologic products are known to play pivotal roles as proinflammatory and anti-inflammatory agents in these diseases. The NLRP3 inflammasome is linked to the pathogenesis of diabetes mellitus as well as periodontitis [22, 23]. Early evidence by Bostanci et al. in 2009 presented elevated mRNA expression of NLRP3 in the gingiva of gingivitis, periodontitis, and aggressive form of periodontitis patients in contrast to healthy controls [24]. They found a positive correlation between NLRP3 mRNA expression and IL-1β and IL-18 in the tissues studied. Belibasakis et al. reported that the putative periodontal pathogen *Aggregatibacter actinomycetemcomitans* enhances NLRP3 upregulation and causes inflammation irrespective of its virulence factors [25]. Xue et al. in 2015 immunohistochemically analysed NLRP3, NLRP2, and AIM2 expression in gingival tissue samples and reported a greater intensity of expression of NLRP3 inflammasome amongst periodontitis patients when compared to healthy controls [26]. Guzman et al. in 2017 highlighted the possibility of evaluating NLRP3 inflammasome as a possible biomarker to identify stages of active periodontal breakdown in the disease pathogenesis of periodontitis [27]. Huang et al. in 2015 concluded that, in patients with T2DM and chronic periodontitis, hyperglycaemic condition may aggravate

Table 1 Descriptive statistics of the clinical and immunohistochemical parameters evaluated in the study population

Parameters		Mean ± SD			
		Group 1	Group 2	Group 3	*p* value
Gender (%)†	Male, *n* (%)	8 (47.1%)	7 (41.2%)	9 (52.9%)	0.790
	Female, *n* (%)	9 (52.9%)	10 (58.8%)	8 (47.1%)	
Site specific Probing pocket depth (mm)‡		2.53 ± 0.51	8.41 ± 1.69	7.59 ± 1.37	< 0.001**
Site specific Clinical attachment level (mm)‡		0.00 ± 0.00	7.76 ± 2.19	6.94 ± 2.07	< 0.001**
Full mouth gingival index‡		0.00 ± 0.00	1.06 ± 0.46	1.02 ± 0.31	< 0.001**
Full mouth plaque index‡		0.46 ± 0.20	1.11 ± 0.35	1.16 ± 0.41	< 0.001**
NLRP3 mean%‡		11.63 ± 8.14	31.22 ± 9.24	49.16 ± 6.45	< 0.001**
NLRP3 SID‡		1.53 ± 0.80	3.12 ± 1.45	6.12 ± 1.87	< 0.001**
NLRP3 intensity ‡		1.41 ± 0.51	1.76 ± 0.56	2.53 ± 0.51	< 0.001**
SOD2 mean%‡		25.04 ± 7.17	36.88 ± 6.38	48.22 ± 11.66	< 0.001**
SOD2 SID‡		2.71 ± 1.45	4.41 ± 1.18	7.00 ± 2.40	< 0.001**
SOD2 intensity ‡		1.65 ± 0.49	2.29 ± 0.59	2.76 ± 0.44	< 0.001**

†Pearson chi-square test

‡Mann–Whitney *U* test

**$p < 0.05$, statistically significant

$p > 0.05$, not significant

Table 2 Intergroup comparison of the evaluated IHC parameters

Parameter	Pairwise comparison	*p* value
NLRP3 Mean%	Group 1/group 2	< 0.001**
	Group 1/group 3	< 0.001**
	Group 2/group 3	< 0.001**
NLRP3 SID	Group 1/group 2	0.007 **
	Group 1/group 3	< 0.001**
	Group 2/group 3	< 0.001**
SOD2 Mean%	Group 1/group 2	< 0.001**
	Group 1/group 3	< 0.001 **
	Group 2/group 3	< 0.001 **
SOD2 SID	Group 1/group 2	0.018 **
	Group 1/group 3	< 0.001 **
	Group 2/group 3	< 0.001 **

**$p < 0.05$, statistically significant. $p > 0.05$, not significant

inflammation in their gingival tissue by activating the NLRP3 pathway and upregulating IL-1ß [12].

Consistent with the above-mentioned studies, this study also revealed statistically significant increase in the expression of NLRP3 inflammasome as it progressed from a state of systemic and periodontal health, to periodontitis, that further increased in patients with periodontitis and type 2 diabetes mellitus. In this study, the expression of NLRP3 is presented in terms of mean percentage and staining intensity distribution. These two variables were significantly higher in periodontitis patients with diabetes mellitus, when compared to the periodontitis group and the healthy controls. The values in the diabetes mellitus cohort revealed 49.16 ± 6.45 mean% of NLRP3 expression and a greater staining intensity distribution of 6.12 ± 1.87, which were both statistically highly significant. This indicates greater NLRP3 expression and activation in the diabetic cohort with periodontitis when compared to the other two groups evaluated in this study. Here, it is to be noted that, the NLRP3 inflammasome was also expressed by the healthy group which establishes its role in innate immunity as the host is subjected to constant bacterial challenge in health too.

In the next part of this study, we evaluated the levels of the antioxidant enzyme SOD2 in the gingival tissues of the three study groups and also correlated its levels to the NLRP3 levels. The rationale behind this comparison is the fact that, this isotype of superoxide dismutase is targeted against the mitochondrial reactive oxygen species—one of the key inducers of the NLRP3 inflammasome–mediated periodontal destruction [12, 13]. Literature suggests that the level of SOD2 increases with the severity of periodontitis and that SOD2-deficient cells show a significant upregulation of the NLRP3 inflammasome components [17]. However, there is no data regarding the association between the NLRP3 inflammasome and SOD2 in periodontitis modified by diabetes mellitus.

In the current study, the group with periodontitis modified by DM had the highest mean percentage of SOD2 of 48.22 ± 11.66, when compared to the other two groups and this difference was statistically significant. The SOD2 staining intensity distribution also progressively increased from group 1 (which being the lowest) to group 3—being

the highest with 7.00 ± 2.40. The SOD2 levels negatively correlated with mean PPD in group 1 (correlation coefficient 0.698, p value ≤ 0.002), indicating that with minimal levels of inflammation, there is not much requirement for upregulation of this antioxidant. In group 2 and group 3 on the other hand, PPD were positively correlated with mean SOD2 levels without statistical significance. This observation highlights the requirement for the antioxidant enzyme SOD2 to be present in higher levels to offset the oxidative stress caused by inflammatory periodontal destruction in group 2 and 3 patients as compared to health.

In contrast to our observation of elevated SOD2 in disease states, many other conditions have implicated lowered levels of SOD2 in diseased state like cardiomyopathy with neuronal degeneration, various cancers, and diabetic inflammatory kidney disease. A possible explanation for this observation could be attributed to the fact that the aetiology of periodontitis is multifactorial and the upregulation or downregulation of a single component does not influence its severity greatly. Also, periodontitis has stages of both quiescence and active destruction and the elevated levels in diseased state in controlled diabetics in this study might correspond to relatively quiescent phase of the disease and therefore, uncontrolled diabetic patients with periodontitis might exhibit lower levels of SOD2 corresponding to active phase of periodontal destruction.

There was a negative correlation seen between the two variables in periodontitis patients with DM. Higher mean percentage levels of NLRP3 as compared to SOD2 in this group might suggest that with increase in severity of the disease, endogenous SOD2 production could be insufficient to counteract the overt activation of the NLRP3 inflammasome due to excessive oxidative burden in these patient groups [28, 29].

Insulin resistance and ensuing pre-diabetes have been associated with NLRP3 activation and the production of IL-1ß mediated via decreased gene expression and decreased tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) [30, 31]. Additionally, pre-diabetic individuals have higher serum NLRP3 levels, which positively correlated with insulin resistance markers [32, 33]. Therefore, the low-grade inflammation effected by NLRP3 could be modulated by other systemic diseases like periodontitis. This has been demonstrated in several longitudinal prospective cohort studies that suggest that the risk for incident pre-diabetes and diabetes was increased in those with moderate or severe periodontitis [34–36]. The significance of elevated levels of these systemic inflammatory markers such as NLRP3 in the pathophysiology of both of these chronic inflammatory disorders is thus emphasised. Furthermore, decreased superoxide dismutase levels have been documented in pre-diabetic individuals highlighting the importance of oxidative stress in the aetiology [37, 38]. Similar trend has been observed in periodontitis mediated by increased ROS generation and reduced anti-oxidant levels [16, 17]. Therefore, examining the significance of NLRP3 and SOD2 in pre-diabetic individuals with periodontitis could further enhance the existing knowledge on the involvement of these biomarkers in the pathogenesis of both of the aforementioned diseases, and is a potential limitation of this study.

The role of DM as a significant modifier of periodontal disease by its effects on NLRP3 inflammasome and SOD2 antioxidant is addressed for the first time in this study. However, this study did not include gingivitis group which could have provided a valuable insight into transitional levels of these molecules from a reversible periodontal disease, i.e., gingivitis to irreversible periodontal breakdown—periodontitis. Further, the diabetics included in this study were controlled diabetic patients, and the inclusion of uncontrolled diabetics could have given us a better understanding of the contribution of diabetes mellitus as a modifier of periodontal disease.

Conclusion

To conclude, we have presented data regarding the levels of the pro inflammatory inflammasome NLRP3 and the anti-inflammatory antioxidant SOD2 in periodontitis modified by type 2 diabetes mellitus for the first time in periodontal literature. The knowledge of the roles of these important biologic modulators of inflammation gained from this study could be the basis for future research, to make use of NLRP3 and SOD2 as chairside salivary biomarkers to accurately diagnose the current status of periodontal disease as well as in the monitoring of the treatment of periodontitis.

Author contributions Dr. GV was involved in the conceptualisation, study design, acquisition of data, drafting the article, and final approval of the submitted version.

Dr. DJV was involved in the conceptualisation, study design, acquisition of data, analysis, drafting the article, and final approval of the submitted version.

Dr. SV was involved in the conceptualisation, study design, acquisition of data, analysis, drafting the article, revising the article, and final approval of the submitted version.

Dr. DA was involved in the conceptualisation, study design, acquisition of data, analysis, drafting the article, revising the article, and final approval of the submitted version.

Dr. PSGP was involved in the conceptualisation, drafting the article, revising the article, and final approval of the submitted version.

Dr. SS was involved in the conceptualisation, drafting the article, revising the article, and final approval of the submitted version.

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Data Availability The authors agree to make data and materials presented in their paper available upon request.

Declarations

Ethical clearance Ethical clearance was obtained from the institutional review board of SRM University. The clearance number is SRMDC/IRB/2015/MDS/No.505.The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Conflict of interest The authors declare no competing interests.

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ORIGINAL PAPER

High expression of the glutathione S-transferase A2 and neuropilin-2 genes affects pancreatic islet β-cell function

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Abstract

Objective Type 2 diabetes mellitus (T2DM) seriously affects human life and health. The aim of this study is to investigate the molecular mechanisms underlying the pathogenesis of T2DM through functional studies of pancreatic β-cell line in vitro.

Methods In this study, a high-glucose- and high-fat-induced model of Min6 cells was constructed, and their cellular functions and insulin secretion levels were detected. Transcriptome sequencing and differentially expressed genes (DEGs) screening and identification were eventually performed.

Results We successfully constructed a T2DM model of high-fat- and high-glucose-treated Min6 cells and found that their migration rate, survival rate, and insulin secretion capacity were reduced. Through transcriptome sequencing and bioinformatics analysis, we finally selected the glutathione S-transferase A2 (Gsta2) and neuropilin-2 (Nrp2) genes. After overexpressing Nrp2, we found that PARP1 protein levels were elevated and apoptotic pathways were activated. Cell viability and survival were significantly reduced, apoptosis was increased, and insulin secretion capacity was reduced. Overexpression of Gsta2 significantly increased the apoptosis of Min6 cells, but no increase in Nrp2 expression was observed.

Conclusions The results suggest that Nrp2 regulates apoptosis in Min6 cells and that there may be a link between this molecule and pathological apoptosis of pancreatic β-cells in T2DM patients. However, Gsta2 was not found to be an upstream regulator of Nrp2 in our cell line. Therefore, Gsta2 regulation of apoptosis in Min6 cells may be achieved through other apoptotic pathways.

Keywords Type 2 diabetes mellitus · Islet β-cells · Gsta2 · Nrp2 · Mechanistic studies

Introduction

Type 2 diabetes mellitus (T2DM) is an expanding global health problem that affects 300 million people and will affect 578.4 million by 2030 and 702 million by 2045 [1, 2]. T2DM is a complex chronic disease caused by multiple aetiologies and characterised by insulin resistance (IR) and chronic hyperglycemia [3, 4]. Patients with T2DM often have pathological changes in pancreatic structure and function. The pathogenesis is still unclear and may involve genetic factors, diet and environment, life stress, and behavioural disorders [5]. T2DM can also cause a variety of complications, including ketoacidosis, diabetic nephropathy (DN), diabetic cardiomyopathy (DCM), and diabetic peripheral neuropathy (DPN) [6]. Therefore, T2DM requires continuous medical care and patient self-management to regulate abnormal blood glucose levels and to bring blood glucose levels, lipid levels, and blood pressure close to normal [7]. Strategies are needed to effectively prevent and reduce the occurrence and development of diabetic complications.

Insulin secreted by pancreatic β-cells plays an important role in glucose homeostasis and metabolic control [8]. Insulin resistance alone will not cause T2DM when accompanied by pathological damage and failure of pancreatic β-cells. In patients with T2DM, the structure and function of islet β-cells

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are altered. This change is manifested by low secretory activity and an inability to proliferate, increased oxidative stress, endoplasmic reticulum stress, hypoxic stress, and cytokine exposure, which ultimately leads to abnormal apoptosis, an inability to proliferate pathological differentiation and autophagy of islet β-cells [9, 10]. The decrease in the number and quality of pancreatic β-cells directly leads to a decrease in insulin secretion and an increase in blood glucose levels. Increasing blood glucose further impairs β-cell function and increases apoptosis. Pancreatic β-cells cannot secrete excess insulin to compensate for insulin resistance, which eventually forms a vicious cycle [11]. Therefore, promoting pancreatic β-cell secretion and proliferation whilst inhibiting progressive islet β-cell apoptosis is an important direction for T2DM treatment.

Glutathione S-transferase A2 (Gsta2) is a functional antioxidant response element (ARE) that plays a role in the defence against oxidative stress [12]. Upregulation of Gsta2 may be a compensatory mechanism for the elevated oxidative stress associated with obesity, and downregulation of Gsta2 weakens the defence against oxidative stress [13]. However, overexpression of Gsta2 in turn damages cells and leads to apoptosis [14]. Neuropilin (NRP) is a multifunctional non-tyrosine kinase receptor. The family members include Nrp1 and Nrp2 [15]. Nrp2 is a single-chain transmembrane glycoprotein expressed in islet cells and pancreatic tumours [16]. In different cell types, Nrp2 has different effects on cell proliferation and survival. Nrp2 acts as a coreceptor for vascular endothelial growth factors (VEGFs), and the combined action of Nrp2 and VEGF-2 may enhance VEGF-A- and VEGF-C-induced signalling pathways in different ways to promote cell survival and migration [17]. However, Gsta2 has been proposed to be an upstream regulator of Nrp2 in human umbilical vein endothelial cells (HUVECs) under the stimulation of blood low-shear stress (LSS). LSS promotes apoptosis and accelerates atherosclerosis in HUVECs by upregulating Nrp2 to activate the poly ADP-ribose polymerase 1 (PARP1) signalling pathway [18, 19]. Therefore, differences in Nrp2 function may be closely related to stimulation conditions and specific microenvironments.

Apoptosis and decreased insulin secretion of pancreatic β-cells aggravate T2DM. The molecular mechanisms of pancreatic β-cell injury need more exploration. This study will construct a high-glucose- and high-fat-induced model of Min6 cells to investigate the molecular mechanisms underlying the pathogenesis of T2DM.

Materials and methods

Cell culture

Min6 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (China). Cells were cultured in RPM1-1640 (Gibco) containing 10% foetal bovine serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2. Cells were passaged when they reached 80–90% growth. Cells were passaged every 2 days, and all experiments were performed with cells at generations 2–7 after cell recovery and 90% cell fusion. High-fat cell additive palmitic acid purchased from Kunchuang Biotechnology (China). The D-glucose solution was purchased from Pricella (China).

CCK8 assay

The CCK8 kit was purchased from MedChemExpress (USA). Different cell density gradients were set, and the cells were inoculated in 96-well plates. Incubation at 37 °C and 5% CO_2 was performed for 4–6 h until the cells fully adhered to the wall. The CCK8 assay was performed under light-proof conditions. Ten percent CCK8 working solution in complete medium was added to each well and incubated for 2–4 h at 37 °C with 5% CO_2 under light-proof conditions.

Cell migration assay

First, cells were inoculated in 12-well plates at 1×10^5 cells/well. The cells were incubated at 37 °C with 5% CO_2 for 4–6 h until the cells completely adhered to the wall. The scratch insert was removed, and the 12-well plate was washed 2–3 times with PBS. Subsequently, 1 ml of RPM1-1640 containing 1% foetal bovine serum and 1% penicillin/streptomycin was added to each well and incubated at 37 °C and 5% CO_2. Different times were set for microscopic observation and photographing. Finally, the pairs of scratches were analysed using NIH ImageJ software 1.43.

Flow cytometry

An Annexin V- FITC/PI apoptosis assay kit was purchased from Solarbio (China). Cells of different groupings were collected at 1×10^6 cells/ml each and washed 3 times. Flow cytometry (FCM) samples were then prepared using the following steps: first, cells were resuspended in a binding buffer with Annexin V FITC and PI added. Next, the mixture was incubated in the dark at room temperature for 15 min. Finally, the binding buffer was added to the mixture, and the mixture was shaken slightly. Cells were subjected to flow cytometry within 1 h, and data were analysed using FlowJo 10.0 software.

ELISA

A mouse insulin ELISA kit was purchased from Elabscience (China). First, the cells were inoculated in 6-well plates at 3×10^5 cells/well and incubated at 37 °C and 5% CO_2 for 24 h. Subsequently, the complete medium, palmitic acid,

and glucose were mixed for a total of 2 mL, replacing the previous medium followed by incubation at 37 °C and 5% CO_2 for 48 h. Finally, the cell membranes were disrupted, and the supernatant was taken after centrifugation to detect the insulin concentration.

Lentivirus transfection

The lentiviruses overexpressing Gsta2 and Nrp2 and the HitransG P transfection enhancement solution used in this experiment were obtained from Genechem (China). First, 6×10^5 cells were added to each well of a 6-well plate. The cells were incubated at 37 °C and 5% CO_2 for 24 h. Subsequently, the complete medium, HitransG P transfection enhancement solution, and lentivirus were mixed for a total of 500 μL, replacing the previous medium. The cells were incubated at 37 °C and 5% CO_2 for 16 h. Finally, the complete medium was replaced, and the culture was continued.

RNA isolation, RNA sequencing, and sequencing analysis

Total RNA was extracted from the experimental group (T) cocultured with high glucose and high fat for 48 h and the control group (C) was cultured normally. Each group included 3 samples (T1, T2, T3, C1, C2, C3). And mRNA was extracted with the RNA Easy Fast Total Cellular RNA Extraction Kit (TIANGEN, China) according to the manufacturer's instructions. The extracted RNA samples were quantified using a NanoDrop 2000 (Thermo Scientific, USA) and cDNA libraries were constructed (Agilent Technologies, USA). Equal samples from each library were pooled and sequenced with a HiSeq PE150 (Illumina, USA). The GENOME's Sequencing Data Platform performed RNA quantity and quality measurements, library preparations, RNA sequencing, and analyses. The expression levels and differential expression analyses were estimated using DESeq2 v16.3.1 (selection criteria: adjusted p-value < 0.05 and $|\log_2$ fold change$| \geq 1$). A volcano plot displayed the logarithm of the p-value on the y-axis and the logarithm of the fold-change on the x-axis. A heat map was used to visualise gene expression patterns across different samples. A Venn diagram between four groups was performed. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) were performed with p-value < 0.05. All RNA sequencing-related analyses were performed using the R environment for statistical computing.

Real-time qPCR (RT-qPCR) analysis

TB Green® Premix Ex TaqTM was purchased from TaKaRa (Japan). Relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. The primers used for qPCR (Table 1) were all from Tsingke (China). Total RNA was extracted from the cells to synthesise complementary DNA strands. Subsequently, qPCR was performed using the premixed system.

Western blotting

The primary antibodies Bak Antibody, Bcl-2 Antibody, and Caspase-3 Antibody and the secondary antibody β-Actin (13E5) Rabbit mAb were purchased from Cell Signalling Technology (USA). Cells were lysed in RIPA buffer with protease inhibitors and phosphatase inhibitors to obtain protein extracts. After incubation on ice for 30 min, the cells were centrifuged at 12,000 rpm for 15 min at 4 °C. The protein supernatant was collected, and the protein content was determined by the bicinchoninic acid protein assay. Samples were mixed in a 5 × loading buffer and denatured at 100 °C for 10 min. Twenty micrograms of protein were added to sodium dodecyl sulphate-PAGE gels along with a prestained

Table 1 Primer sequences of the candidate genes

Gene name	Forwards primer (5′–3′)	Reverse primer (5′–3′)
Slc7a11	TTACCACCATCAGTGCGGAG	GCAACAAAGATCGGGACTGC
Hif1a	CATCAGTTGCCACTTCCCCA	TCGCCGTCATCTGTTAGCAC
Srxn1	GCACAACGTACCAATCGCC	TCACGAGCTTGGCAGGAATG
Areg	CCATGCACTGCCAAGTTTCAG	TCCACACCGTTCACCAAAGT
Kdm7a	CCTTCACCCCACCAAGAGAC	TCACACGAAGAAGCGAGCAT
Atg4a	AAACCCCTGCTGCTCATTGT	GCCCCTAAAGACTGTGGCAT
Nrp2	GGCGTTTGTACGCAAGTTCA	CTTCGGATGTCAGGGGTGTC
Gsta2	ACCGTTACTTGCCTGCCTTT	GCTGGCATCAAGCTCTTCAAC
Nduf3	ATGGCTTCGAGGGACATCCT	CACTGGTTCAGCCACTACCC
Osr1	TGATGAGCGACCTTACACCTG	TGTGAGTGTAGCGTCTTGTGG
Ears2	AGCTGCCTATCCCTGTTTCTG	GATAGCAGGCTTGGGGTCTA
Mat2a	GCTCCTTCGTAAGGCCACTT	GGGCATCAAGGACAGCATCA
GAPDH	CAGTGGCAAAGTGGAGATTGTTG	TCGCTCCTGGAAGATGGTGAT

protein marker (Thermo, #26,616). The separated proteins were transferred to PVDF membranes (Immobilon, China). The membranes were blocked with skim milk powder dissolved in TBST buffer at room temperature for 2 h and then incubated with primary antibody overnight at 4 °C. The membranes were washed 3 times in TBST for 5 min each time and then incubated with the corresponding secondary antibodies at room temperature for 1 h. Finally, the membranes were washed 3 times in TBST. Protein bands were observed by chemiluminescence. The intensity of the bands was quantified using NIH ImageJ software 1.43.

Statistical analysis

All quantitative results are presented as the mean ± standard deviation. GraphPad Prism software (version 8.0) was used for statistical analysis. For normally distributed data, differences between two groups were compared using independent samples *t*-test or nonparametric test analysis, and $p < 0.05$ was considered to be statistically significant.

Results

Min6 cell function is impaired by high-glucose and high-fat culture

First, we examined the growth curve of Min6 cells and determined the optimal seeding plate density of 5000 cells/well (Fig. 1A). To model the cell damage of high glucose and high fat, we determined the optimal concentration of glucose treatment, which was 30 mM [20, 21]. The optimal treatment concentration of palmitic acid was 0.3 mM (Fig. 1B). The treatment time was 48 h. The half maximal inhibitory concentration for palmitic acid treatment for 48 h was 0.28 mM (Fig. 1C). Second, we examined Min6 cells treated with glucose and palmitic acid at different time points, and their migratory capacity was significantly reduced (Fig. 1D). The apoptosis rate was 25.03% at 24 h and 56.50% at 48 h, both of which were significantly higher than that of the control (Fig. 1E). Moreover, we examined Min6 cells treated with high glucose and high fat, all of which had a significantly reduced insulin secretion capacity (Fig. 1F). Finally, examining apoptosis-related proteins in the Min6 cell injury model, we found that the expression levels of Bcl-2 were significantly reduced and those of Bak and Caspase-3 were significantly increased (Fig. 1G). In conclusion, our results indicate that after coculture with 30 mM glucose and 0.3 mM palmitic acid for 48 h, the function of Min6 cells is disrupted, insulin secretion levels are reduced, and apoptosis-related signalling pathways are activated.

Transcriptome sequencing analysis and DEG screening

To explore the effect of high glucose and high fat on Min6 cells, we performed transcriptome sequencing and analysis. A total of 222 DEGs were identified, including 127 upregulated genes and 95 downregulated genes (Fig. 2A and B). With the Venn diagram, we found 102 DEGs in the intersections of all four groups (Fig. 2C). The KEGG pathway analysis showed that the DEGs were enriched in the cell adhesion molecules, cell cycle, MAPK signalling pathway, NF-κB signalling pathway, IL-17 signalling pathway, etc. (Fig. 2D). GO analysis revealed that the DEGs were crucial to cell membranes and macromolecular complexes in cellular components, metabolic process, and cell cycle in biological processes, and binding and catalytic activities of biomolecules in molecular functions (Fig. 2E).

According to the sequencing results and related research background, we screened 12 genes as candidate genes (Table 2). The expression levels of the 12 genes were examined using RT-qPCR and Gsta2 and Nrp2 were significantly upregulated (Fig. 2F). The protein expression levels of Gsta2 and Nrp2 were also significantly increased (Fig. 2G). Based on the above results, we ultimately selected Gsta2 and Nrp2 as target genes for further research.

Overexpression of the Gsta2 and Nrp2 genes impaired the function of Min6 cells

To explore the role of the Gsta2 and Nrp2 genes in Min6 cells, we constructed lentiviral vectors overexpressing Gsta2 and Nrp2. Normal Min6 cells were transfected separately using lentiviruses, and the fluorescence status of the transfected cells was observed under a fluorescence microscope. The highest fluorescence abundance was found when the transfection time was 72 h (Fig. 3A). Moreover, we used 2.55 μg/mL puromycin to screen the stable positive cells. Subsequently, RT-qPCR showed a significant increase in the RNA levels of the Gsta2 and Nrp2 genes compared to those of the empty vector group (Fig. 3B). And the protein expression levels were significantly elevated (Fig. 3C). Next, we examined the viability and survival of Min6 cells after transfection with lentivirus and found that the viability and survival of the Min6 cells overexpressing Nrp2 were significantly reduced after 48 h (Fig. 3D and E). However, in migration assays, we found that Gsta2 and Nrp2 had no significant effect on the migration of Min6 cells (Fig. 3F). Using flow cytometry, we found that overexpression of Gsta2 and Nrp2 significantly increased the apoptosis of Min6 cells (Fig. 3G).

In addition, we examined the effect of overexpression of Gsta2 and Nrp2 on the level of insulin secretion in Min6 cells under high-glucose and high-fat conditions. The assay

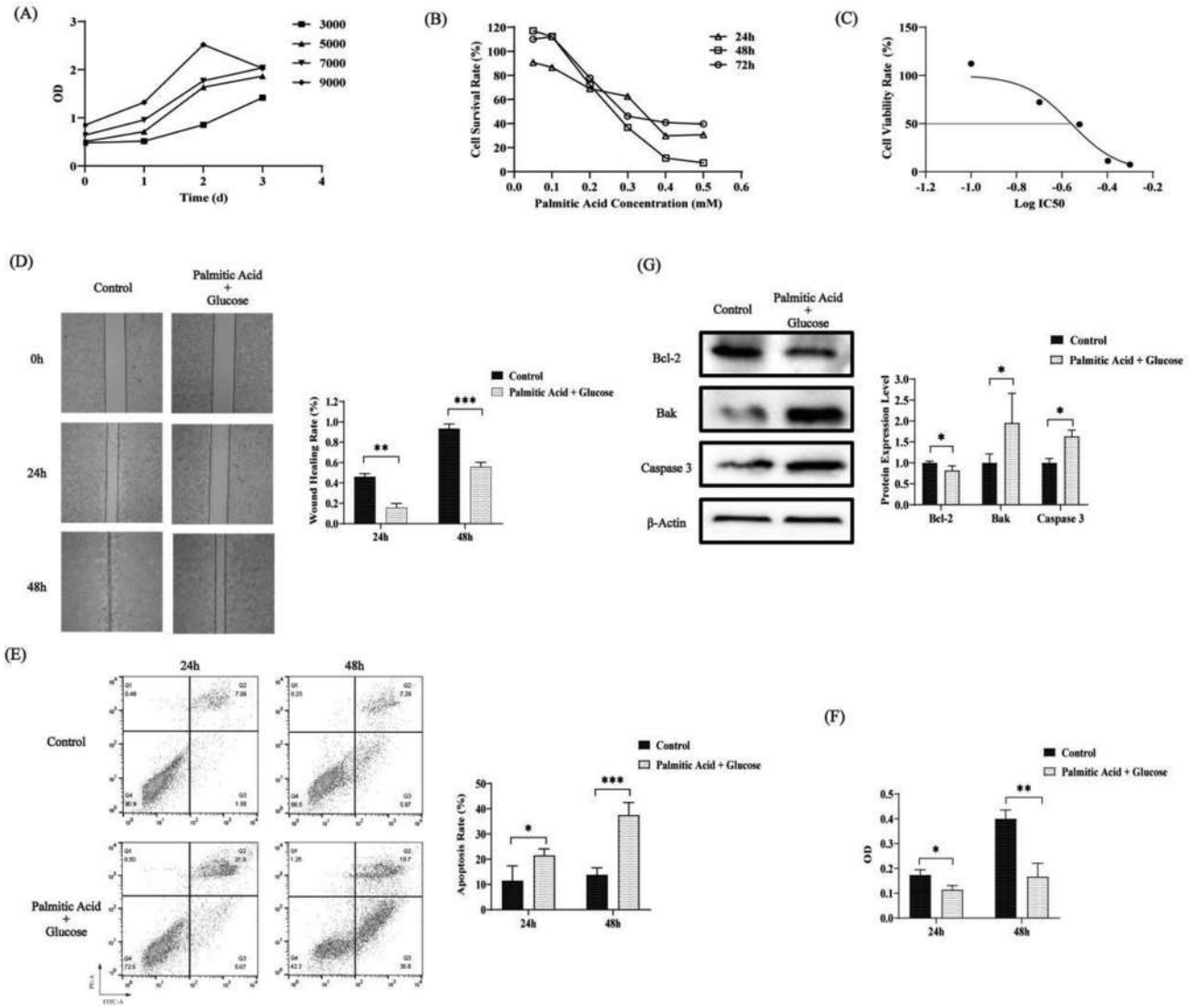
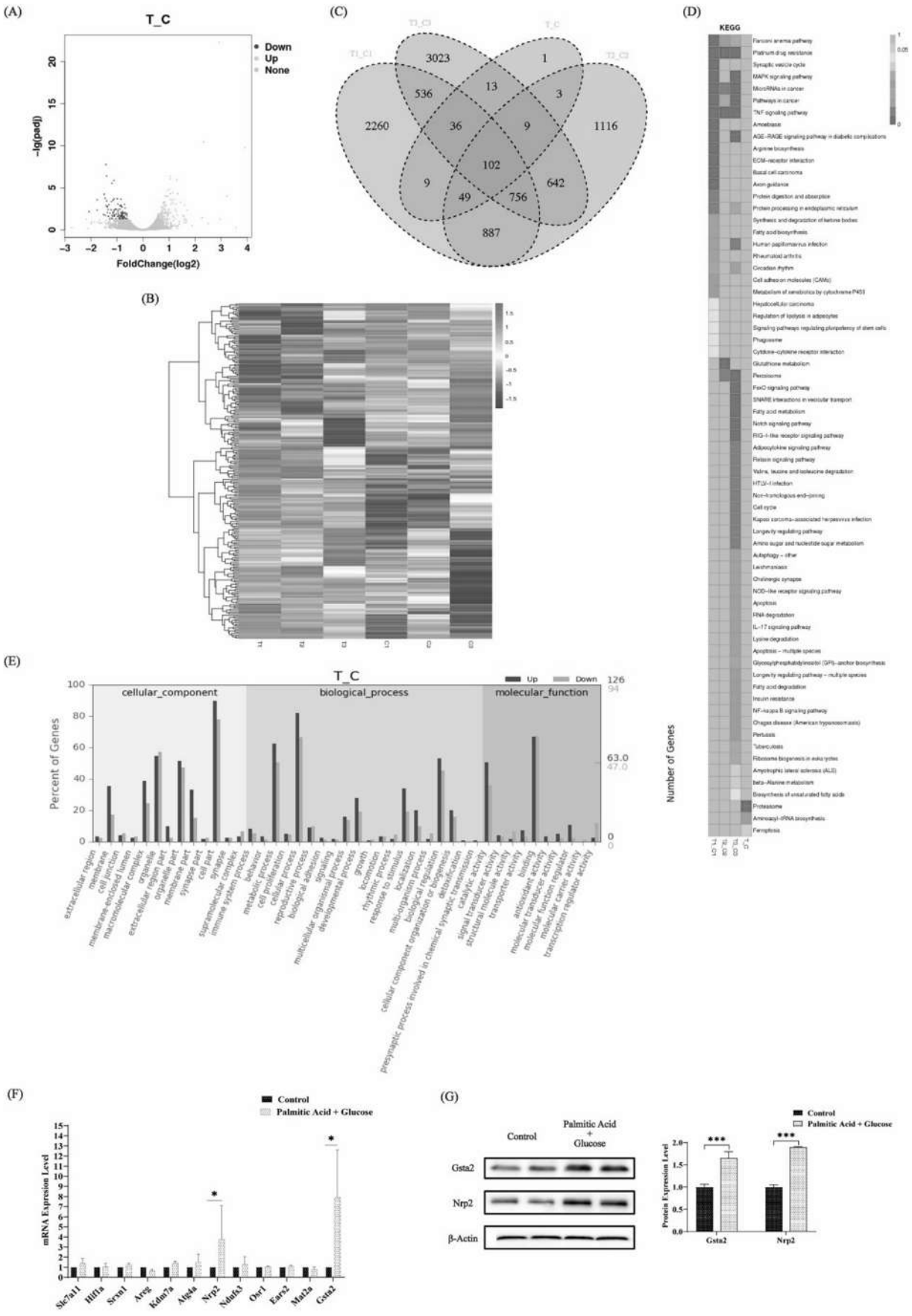


Fig. 1 High-glucose and high-fat culture-impaired Min6 cell function. **A** Growth curves of Min6 cells at different cell densities and times detected by CCK8 assays. **B** The survival rate of Min6 cells under different palmitic acid concentrations and treatment durations. **C** The half maximal inhibitory concentration of Min6 cells at 48 h of palmitic acid treatment. **D** Migration assay to detect the migration of Min6 cells under different times of high-glucose and high-fat treatment. **E** Flow cytometry assay to detect the apoptosis rate of Min6 cells under different times of high-glucose and high-fat treatment. **F** ELISA was performed to detect the insulin secretion level of Min6 cells under high-glucose and high-fat treatment at different times. **G** Western blot to detect the expression level of apoptosis-related proteins in Min6 cells after 48 h of high-glucose and high-fat treatment. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

showed that the insulin secretion level of the Min6 cells overexpressing Nrp2 was significantly reduced, whilst the insulin secretion level of the other groups only tended to decrease (Fig. 3H). In conclusion, we found that overexpression of Nrp2 significantly decreased Min6 cell viability and survival, increased apoptosis, and decreased insulin secretory capacity. Overexpression of Gsta2 significantly increased the apoptosis of Min6 cells. However, the migratory ability of both groups was not significantly different from that of the control group, indicating that Gsta2 and Nrp2 could not affect the migratory ability of Min6 cells.

Overexpression of the Nrp2 gene affects the expression of apoptosis-related proteins

Finally, we focused on the expression of proteins related to apoptosis. We examined the protein expression level of PARP1 in Min6 cells. Overexpression of Nrp2 significantly increased the protein levels of PARP1, Bak, and Caspase 3, whilst the protein level of Bcl-2 was significantly decreased (Fig. 4A). Subsequently, under high-glucose and high-fat treatment conditions for 48 h, we similarly found that overexpression of Nrp2 significantly increased the protein


(A)
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642
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KEGG
Number of Genes
(E)
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126
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Percent of Genes
cellular_component
biological_process
molecular_function
(F)
Control
Palmitic Acid + Glucose
mRNA Expression Level
Slc7a11
Hif1a
Srxn1
Areg
Kdm7a
Atg4a
Nrp2
Ndufa3
Osr1
Eafr2
Mat2a
Gsta2
(G)
Control
Palmitic Acid + Glucose
Gsta2
Nrp2
β-Actin
Protein Expression Level
Control
Palmitic Acid + Glucose
Gsta2
Nrp2

◂**Fig. 2** Transcriptome sequencing analysis and DEGs screening. The palmitic acid and glucose treatment groups were numbered T1, T2, and T3, and the control groups were numbered C1, C2, and C3. **A** Volcano plot of DEGs. Genes with a significant change of more than 1.0-fold were selected. The yellow dot represented upregulated significant genes and the blue dot represented downregulated significant genes. **B** Heat map of DEGs. The horizontal coordinates represent sample clustering, and the vertical coordinates represent gene clustering. Red represents high-expression genes; blue represents low-expression genes. **C** Venn diagram. DEGs in the T_C, T1_C1, T2_C2, and T3_C3 groups. **D** KEGG enrichment pathway plot. The horizontal coordinate represents the above clustering situation, and the vertical coordinate represents the KEGG term. The redder the colour is, the more significant the enrichment is. **E** GO statistical histogram of DEGs. The results of GO statistics of DEGs in the T_C group. **F** RT-qPCR to detect the expression levels of 12 initially screened differentially expressed mRNAs in Min6 cells under high-glucose and high-fat treatment. **G** Western blot detection of the protein expression levels of two genes, Gsta2 and Nrp2, in Min6 cells under high-glucose and high-fat treatment. $*p < 0.05$, $***p < 0.001$

expression levels of PARP1, Bak, and Caspase 3 and significantly decreased the protein levels of Bcl-2 in Min6 cells (Fig. 4B).

Next, to verify whether Gsta2 is an upstream regulator of Nrp2, we examined the intracellular levels of Nrp2 after overexpression of Gsta2. The protein expression of Nrp2 was not significantly upregulated (Fig. 4C). In conclusion, we confirmed that Nrp2, as a key gene in Min6 cells, regulates apoptosis through the PARP1 signalling pathway.

Discussion

T2DM is a chronic disease that poses a threat to patients, and its incidence is increasing year by year [22]. T2DM is characterised by damaged islet β-cells, inadequate insulin secretion, and peripheral insulin resistance [23]. Compared to normal human islet β-cells, islet β-cells in T2DM patients become less responsive to glucose early in the disease [24]. Subsequent glucose toxicity, lipotoxicity, inflammatory factors, and oxidative stress impede pancreatic β-cell function, further affecting islet β-cell numbers and insulin secretion. In animal experiments [25, 26], continuous high-glucose and high-fat feeding was shown to lead to a progressive decline in β-cell function [27].

To explore the effect of high glucose and high fat on islet β-cell function, we constructed a Min6 cell T2DM model using palmitic acid and glucose. The optimal concentration of palmitic acid was 0.3 mM, and the optimal concentration of glucose was 30 mM [20, 21]. When palmitic acid and glucose were used, simulating high-glucose and high-fat treatment for 48 h, we found a significant decrease in the survival rate of Min6 cells, a significant decrease in migration, a significant increase in apoptosis, and a significant decrease in the ability to secrete insulin. Studies showed that palmitic acid–induced apoptosis of Min6 cells could directly inhibit Bcl-2 gene expression by upregulating miR-34a expression [28]. Hyperglycaemia-induced apoptosis in pancreatic β-cells is associated with the balance between proapoptotic Bax proteins and antiapoptotic Bcl-2 proteins [29]. In addition, Bak, another member of the Bcl-2 family, shares a similar function to Bax [30]. We treated Min6 cells with palmitic acid and glucose for 48 h and found that the expression level of Bcl-2 was decreased, Bak expression was increased, Caspase 3 expression was increased, and the apoptotic pathway was activated.

Gene abnormalities of β-cells will further damage cell function, hinder insulin secretion, and aggravate the progression of diabetes [31]. β-cell-specific deletion of the trans-Golgi residing small GTPase ARFRP1 led to elevated blood glucose levels in mice [32]. And downregulation of ARFRP1 in Min6 cells interfered with the function of the plasma membrane, thereby impairing glucose-stimulated

Table 2 The 12 candidate genes. Up indicates upregulated genes after high-glucose and high-fat treatment, and down indicates downregulated genes after high-glucose and high-fat treatment

Gene name	Log2FC	*P* val	*P* adj	Expression
Slc7a11	2.328357046	6.05E–15	3.55E–11	up
Hif1a	1.013438578	1.50E–08	1.61E–05	up
Srxn1	1.328305761	6.70E–08	5.25E–05	up
Areg	1.118438633	7.26E–07	0.000297338	up
Kdm7a	1.132611701	1.92E–06	0.000625986	up
Atg4a	1.073482017	3.04E–06	0.000891522	up
Nrp2	1.036153882	7.83E–05	0.009315409	up
Gsta2	3.222481118	1.48812E–07	1.48812E–07	up
Ndufs3	− 1.344935923	2.30E–05	0.003867648	down
Osr1	− 1.369360485	6.41E–05	0.008677281	down
Ears2	− 1.055567876	1.67E–05	0.003168464	down
Mat2a	− 1.453869322	3.23E–07	0.000180458	down

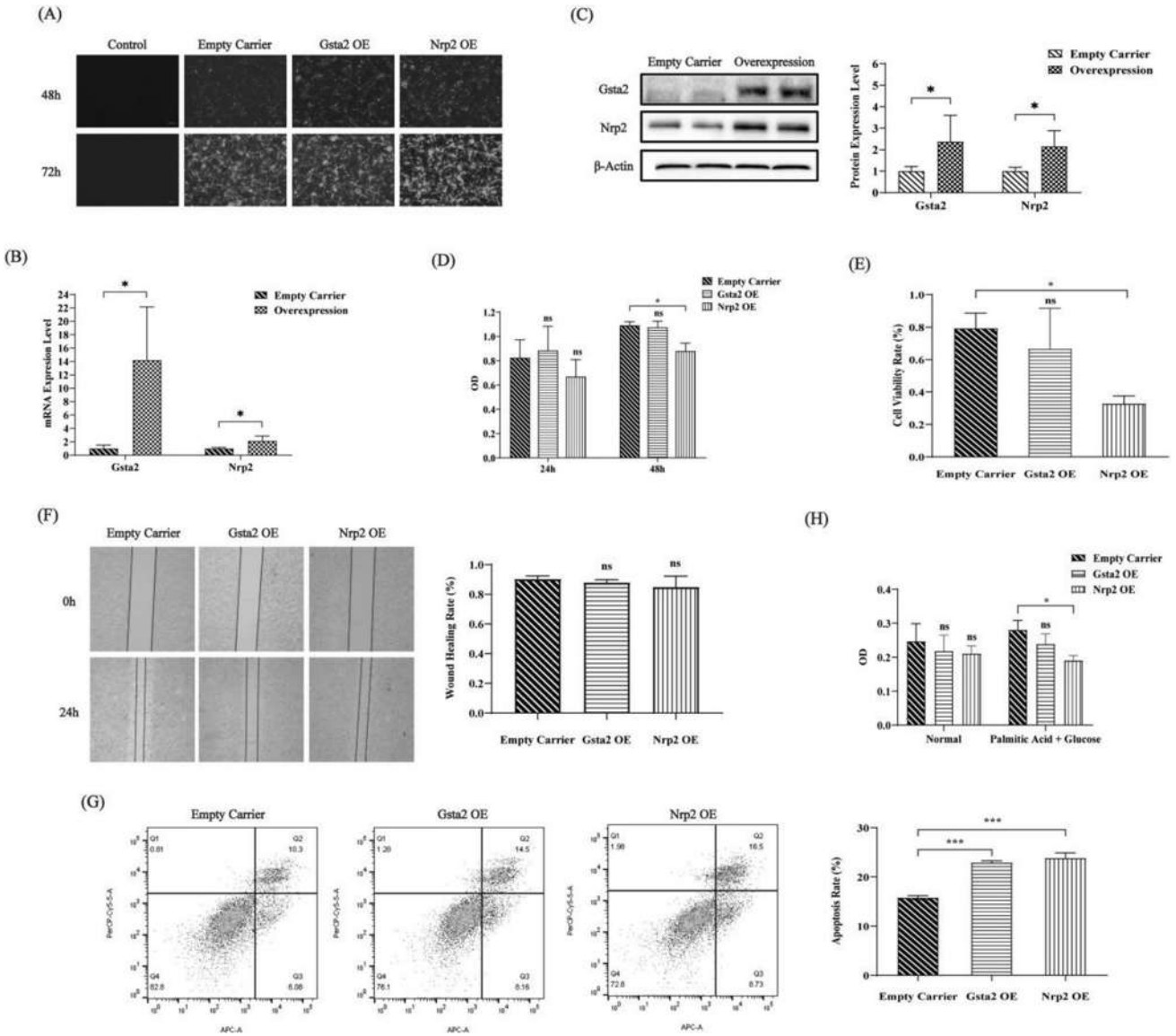

Fig. 3 Overexpression of the Gsta2 and Nrp2 genes impaired the function of Min6 cells. **A** Fluorograms of Min6 cells after lentiviral transfection for 48 and 72 h. **B** RT-qPCR to detect the mRNA expression levels of Gsta2 and Nrp2 after lentivirus transfection. **C** Western blot detection of the protein expression levels of Gsta2 and Nrp2 after lentivirus transfection. **D** Viability of Min6 cells after lentiviral transfection for 24 and 48 h. **E** The survival rate of Min6 cells overexpressing Gsta2 and Nrp2 after high-glucose and high-fat culture for 48 h. **F** Migration assay to detect the migration ability of Min6 cells overexpressing Gsta2 and Nrp2 after 48 h of high-glucose and high-fat culture. **G** ELISAs to detect the insulin secretion level of Min6 cells overexpressing Gsta2 and Nrp2 after 48 h of high-glucose and high-fat culture. **H** Flow cytometry detection of the apoptosis rate of Min6 cells overexpressing Gsta2 and Nrp2 after 48 h of high-glucose and high-fat culture. $*p < 0.05$, $***p < 0.001$. NS no significance, OE overexpression

insulin release from β-cells [32]. Sheng et al. discovered the pathogenic role of Smad3 in type 2 diabetes β-cell loss and dysfunction via the Pax6-dependent mechanism [33]. They found that genetic deletion of Smad3 largely increased the serum insulin levels, and prevented metabolic abnormalities of db/db mice, which suggested that islet-specific silencing of Smad3 might represent a novel therapeutic strategy for T2DM prevention and treatment [33]. In this study, a total of 102 DEGs were identified, which might lead to abnormal β-cell function and impede the synthesis and secretion of insulin. These genes are an abundant resource for studying the function of pancreatic β-cells in the future.

Gsta2 and Nrp2 are associated with cell proliferation and apoptosis; Gsta2 overexpression increases apoptosis in Parkinson's disease models, Nrp2 has different effects on cell proliferation and survival in different cell types, and Nrp2 upregulation promotes apoptosis in human umbilical vein endothelial cells [14]. Nrp2 integrin (α9ERK 1) binding

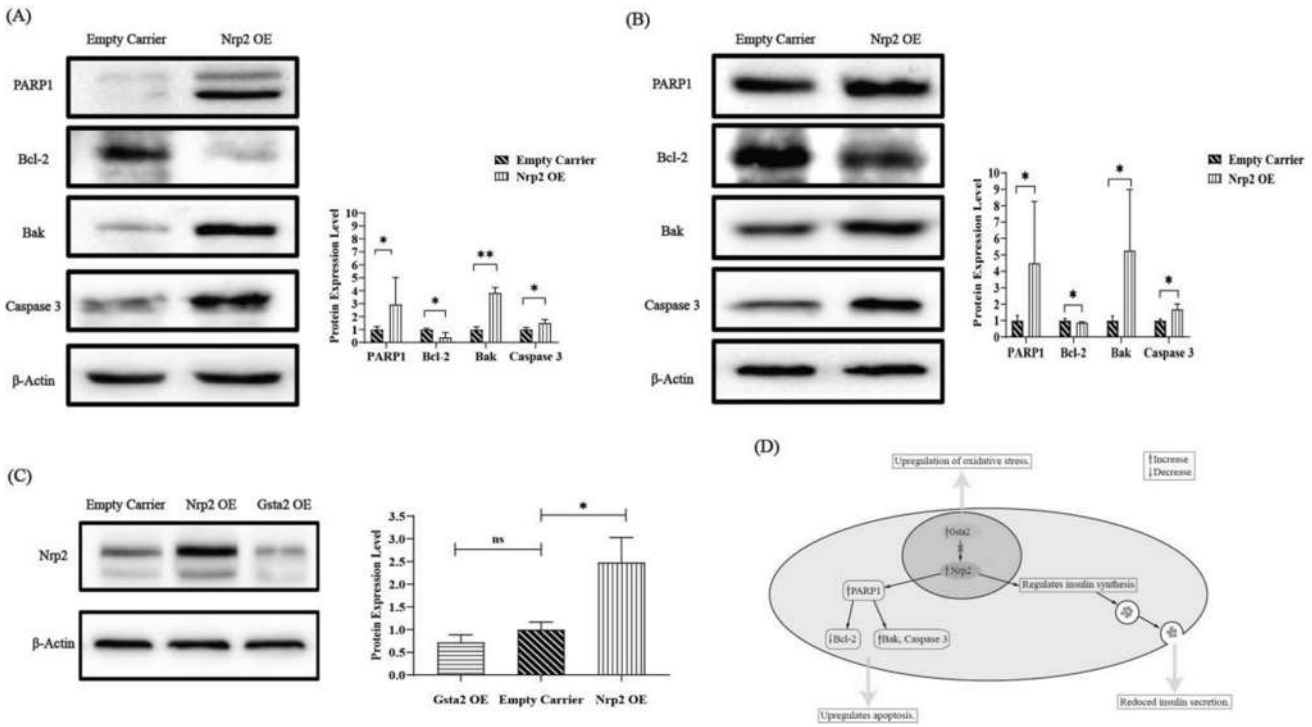

Fig. 4 Overexpression of the Nrp2 gene affects the expression of apoptosis-related proteins. **A** Western blot detection of apoptosis-related protein expression levels in Min6 cells overexpressing the Nrp2 gene. **B** Western blot detection of apoptosis-related protein expression levels in Min6 cells after 48 h of high-glucose and high-fat culture. **C** Western blot detection of Nrp2-associated protein expression levels in Min6 cells overexpressing Nrp2 and Gsta2. **D** Flow diagram of the regulation of related apoptotic pathways. $*p < 0.05$, $**p < 0.01$, NS no significance, OE overexpression

in lymphatic vessel endothelial cells activates the β/ERK signalling pathway and promotes their migration, germination, and tubule formation [34]. To explore the functions of Gsta2 and Nrp2 in Min6 cells, we constructed and transfected overexpression lentiviral vectors of Gsta2 and Nrp2. Under normal culture conditions, overexpression of Nrp2 reduced Min6 cell viability. When Min6 cells were cultured with palmitic acid and glucose, we found that overexpression of Nrp2 significantly reduced cell survival, increased apoptosis, and significantly decreased insulin secretion levels. Overexpression of Gsta2 significantly increased the apoptosis rate and decreased cell survival, but the decrease was not significant. In addition, Min6 cells overexpressing Nrp2 showed the lowest tolerance to palmitic acid and glucose, so we further investigated the molecular mechanism of Nrp2 gene regulation in Min6 cells.

One study used the JASPAR and animal TFDB databases to predict transcription factors that may bind to Nrp2 and regulate its transcriptional levels, including Gsta2, and demonstrated that Gsta2 regulates angiogenesis and lymphangiogenesis through Nrp2 [17]. Furthermore, Gsta2 deletion could downregulate Nrp2 expression in endothelial cells [18]. However, in our study, we found that Gsta2 may not be an upstream regulator of Nrp2, probably due to the difference in cell types.

PARP1 protein plays an important role in the induction of cell death and the regulation of its gene expression. Depending on whether Caspases are involved, PARP1 protein-mediated cell death can be classified as Caspase-independent (type I) and Caspase-dependent (type II) [35]. The PARP1 protein was shown to be activated in atherosclerotic plaques. Inhibition or downregulation of PARP1 protein expression could interfere with atherosclerosis formation and promote the regression of formed plates [36]. Nrp2 accumulation in human umbilical vein endothelial cells can lead to PARP1 overexpression, which depletes cellular energy, which in turn decreases the level of Bcl-2 expression, increases the level of Bax expression, and ultimately leads to apoptosis [18]. We found that Nrp2 increased PARP1 expression levels, decreased Bcl-2 expression levels, increased Bak expression levels, and increased Caspase 3 expression, suggesting that overexpression of Nrp2 can activate the apoptotic signalling pathway. Under palmitic acid and glucose coculture conditions, Nrp2 similarly regulated PARP1 and activated the apoptotic pathway.

However, this research work is based on a cell model, and all results need to be validated in animal and clinical trials. To prevent β-cell destruction in long-term T2DM patients, we will continue to focus on the role of these genes related to pancreatic β-cells.

Conclusions

The present study shows that Gsta2 and Nrp2, as key genes in pancreatic β-cells, function to regulate cell viability, apoptosis, and insulin secretion. This study indicates that Nrp2 affects the biological functions of pancreatic β-cells by regulating the PARP1 apoptotic pathway, as well as the apoptosis-related proteins Bcl-2, Bak, and Caspase 3. However, Gsta2 did not act as an upstream regulator of Nrp2 in Min6 cells, controlling the function of Nrp2 (Fig. 4D).

Author contribution Jiarui Zhang, Wenzhe Wu, and Jundong He designed the study and wrote and revised the manuscript. Lichenlu Huang and Yongqin Zheng participated in the analysis of experiments and sequencing. Jundong He and Yikun Zhou gave the final approval for the version to be submitted. All authors read and approved the final manuscript.

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Data Availability All data in this article are original to all authors. The data underlying this article will be shared on reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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All awardees are encouraged to submit their work to the RSDDI Journal IJDDC

CALL for RESEARCH PROPOSALS for GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI

The proposals may of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization, of funding sought from RSSDI

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
Upload your Research proposals on the RSSDI Online Research Grant Platform.

When to apply
Proposals will be accepted every quarter of a year. The first month will be for the proposal submission, the second month for the scrutiny of the submitted proposals and the third month for the grant disbursement. This cycle will repeat for each quarter.

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health-care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmachari Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh