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EDITORIAL

Advancing the understanding and management of diabetic peripheral neuropathy

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Published online: 6 February 2024

India faces a significant burden of diabetes-related complications, including both microvascular and macrovascular issues. As the prevalence of diabetes mellitus (DM) rises in the country, so does the number of people affected by these complications. Diabetic peripheral neuropathy (DPN) is particularly prevalent, affecting anywhere from 18.8 to 61.9% of individuals with DM in India [1]. Early detection of DPN is crucial for reducing associated complications. Implementing screening services at primary healthcare facilities can play a vital role in achieving early diagnosis and improving health outcomes for individuals with DM.

"Role of sensory feedback in postural control of the patients with diabetic neuropathy" by Reisi et al. [2] published in this current issue aimed to evaluate the role of sensory information in patients with type 2 diabetes. The study utilized stabilogram-diffusion analysis to evaluate balance in patients with T2DM, revealing distinct local and central modes of postural control. Notably, the parameter under assessment should exhibit random walk motion. Linear metrics indicated overall instability, particularly in the anterior–posterior (AP) direction, with greater instability observed in central control, which relies on sensory feedback. This finding underscores the impact of T2DM on sensory information crucial for posture. The study's insights suggest that exercise programs aimed at improving posture in T2DM patients should prioritize proprioception, considering the compromised sensory function in this population.

"The relationship between neuron-specific enolase, high sensitivity C reactive protein, and diabetic peripheral neuropathy in Chinese patients with type 2 diabetes: A prospective nested case–control analysis" by Xie et al. [3] in this current issue explored the potential value of serum NSE and hs-CRP in predicting DPN in patients with type 2 diabetes. The findings suggest that higher serum NSE levels are associated with increased neuroinflammatory responses and can predict the development of diabetic peripheral neuropathy (DPN) in individuals with type 2 diabetes over an average of 5.1 years. NSE could potentially serve as a specific marker for neurological damage in DPN, offering valuable insights for early diagnosis and treatment strategies.

"Prospective study of clinical, biochemical, and radiological characteristics of diabetic Charcot neuroarthropathy at a tertiary care centre" by Aruna et al. [4] published in this current issue aimed to assess demographic, clinical, biochemical, radiological profile and treatment response in diabetic patients with Charcot neuroarthropathy (CN). The diabetic CN was found to be present in 0.49% of diabetic patients in the fifth to sixth decades of life with bilateral foot involvement in only 4% of patients. Diabetic patients developing CN had higher BMI, poor glycemic control, a longer duration of DM, and a higher prevalence of neuropathy and retinopathy complications. Early diagnosis and appropriate offloading lead to clinical remission in the majority of acute Charcot neuroarthropathy (ACN) and healing as well as prevention of foot ulceration in chronic Charcot neuroarthropathy (CCN).

"Changes of sweat gland function in type 2 diabetes mellitus patients with peripheral neuropathy" by Liu et al. [5] aimed to assess sweat gland function, analyze relevant damage indicators, and investigate autonomic nerve function impairment in patients with T2DM-DPN, potentially offering new diagnostic and treatment approaches. In the early stages of T2DM-DPN, many patients may not experience noticeable symptoms, making early screening and diagnosis crucial for effective treatment. Hence, tracking sweat gland function can aid in monitoring the progression of T2DM neuropathy. Accurate assessment of sweat gland

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function damage can enhance clinicians' ability to monitor autonomic neuropathy and accurately gauge the onset, progression, and severity of T2DM-DPN.

"The importance of active B12 measurement in the diagnosis of vitamin B12 deficiency in type 2 DM patients using metformin" by Yildiz et al. [6] in this issue aimed to investigate the benefit of active B12 measurement in diagnosing B12 deficiency, as it might be a better alternative in the follow-up of B12 levels in type 2 DM patients using metformin. Higher doses of metformin, exceeding 1000 mg/day, were associated with a significant reduction in both total B12 and active B12 levels. There was also a negative correlation between the dose of metformin and these B12 levels. The study highlighted the importance of closely monitoring patients with type 2 diabetes mellitus who are taking high doses of metformin for potential B12 deficiency.

All patients with diabetes should undergo screening for diabetic neuropathy using a recommended evidence-based screening algorithm. Patients with diabetes should undergo assessment for diabetic peripheral neuropathy (DPN) at the time of type 2 diabetes diagnosis and 5 years after the onset of type 1 diabetes, followed by annual evaluations thereafter [7].

A deeper understanding of additional risks, especially those that can be modified like components of MetS, is crucial in identifying diabetes patients who are most vulnerable to developing DPN. Recognizing these modifiable risks also paves the way for potential interventions aimed at slowing down the progression of DPN [8].

The current management of diabetic neuropathy emphasizes improving glycemic control in type 1 diabetes patients and lifestyle changes in type 2 diabetes patients, along with addressing neuropathic pain. For type 2 diabetes, the optimal therapeutic approach involves lifestyle modifications like diet and exercise, as well as managing lipid and blood pressure levels [9].

Overall, these studies contribute to our understanding of DPN and offer potential avenues for improving its diagnosis and management. Further research in this field is essential to develop more effective strategies for preventing and treating this debilitating complication of diabetes mellitus.

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REVIEW ARTICLE

Decoding the mystery of non-nutritive sweeteners

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Abstract

Background This commentary explores the landscape of non-nutritive sweeteners, further delves into the historical trajectory and the contemporary surge in the consumption of non-nutritive sweeteners (NNS), along with the accompanying controversies concerning their safety.

Objective The aim is to provide a comprehensive understanding of the influences of NNS, drawn from global studies and diverse perspectives.

Methods The commentary synthesizes findings from global studies, notably the NutriNet-Santé cohort, exploring associations between specific NNS and health consequences such as cerebrovascular events and malignancies. Additionally, it examines the research on consequences of NNS on gut microbiota and explores concerns linked to gestational diabetes, fetal exposure, and health of the offspring.

Results While caution is advised during pregnancy and fetal development due to potential risks, NNS show promise in weight management and short-term dietary goals when used cautiously in lower amounts. The commentary underscores the necessity for inclusive, long-term studies to guide evidence-driven policies and guidelines.

Conclusion While the article underscores the complexities and debates surrounding non-nutritive sweeteners (NNS), it also sheds light on the positive aspects. In the Indian context, where the intake of sweeteners is relatively low and mainly limited to beverages (tea or coffee), NNS appear to be safe, but prudent use is advocated. The article emphasizes the value of public education on NNS usage and concludes that, overall, NNS are reasonably safe when consumed in moderation. Continued research is needed to elucidate their intricate effects on health and impact on global health outcomes.

Implications The article concludes with clear guidelines for using NNS in India, highlighting the need for informed decision-making and ongoing research to elucidate their broader health consequences.

Keywords Non-nutritive sweeteners · Low-calorie sweeteners · Artificial sweeteners · Non-caloric sweeteners

Navigating the sweet spectrum: the perplexing mystery of non-nutritive sweeteners

Throughout the ages, the sweet taste has remained one of humanity's most cherished flavors. However, for many years, it has been common knowledge that consuming excessive sugar can lead to detrimental health consequences. Non-nutritive sweeteners (NNS) have garnered a significant position among the most sought-after alternatives to sugar. They were primarily used before the 1950s for cost reasons because they were less expensive than sugar [1]. These sweeteners have the potential to satisfy sweet cravings without contributing to the excessive caloric load and risks of dental caries associated with sugar consumption [2, 3]. The growing prevalence of obesity, diabetes, and metabolic

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syndrome, along with increased consumer awareness, has triggered a progressive trend towards the use of low/zero caloric artificial sweeteners. NNS when consumed within acceptable daily limits (ADI) can help in limiting carbohydrate and energy consumption as a means to optimize weight [4]. Thus, their use has skyrocketed since the 2000s due to their presence in "lower calorie" food products and pharmaceuticals [5]. However, their usage has sparked controversies and concerns that merit thorough exploration in a balanced commentary.

Low-calorie sweeteners, artificial sweeteners, and non-caloric sweeteners have been designated as NNS by the American Heart Association since they do not provide any nutritional advantages such as of vitamins and minerals [6]. Aspartame, acesulfame-k, neotame, saccharin, and sucralose are USFDA (United States Food and Drug Administration)–approved NNS for use within the acceptable daily intake (ADI) levels in foods and beverages [7]. While these sweeteners have received approval from the USFDA, recently some concerns regarding the safety and long-term effects of certain sweeteners have arisen thereby leading the World Health Organization to advise restricting the consumption of non-sugar sweeteners in adults without diabetes, whenever possible [8, 9].

Guidelines and research on NNS: showing their safety

Numerous research studies and reviews have showcased the safety of NNS. The RSSDI-ESI (Research Society for the Study of Diabetes in India (RSSDI) and the Endocrine Society of India (ESI) recommendations 2020) advocates that artificial sweeteners when consumed in prescribed doses within the ADI are deemed to be safe [10]. Recently, a WHO (World Health Organization) meta-analysis indicated that NNS may have no effect on glucose metabolism and result in decreased body weight when combined with short-term dietary restriction [11]. The American Diabetes Association (ADA) advised, in the recent past, replacing up to one-third of white granulated sugar with artificial sweetener in diabetes-aware recipes [12]. NNS-containing beverages, when used as a replacement for sugar-sweetened beverages (SSBs), may provide cardiometabolic benefits among overweight/obese/type 2 DM (T2D) individuals [13]. A recent study from India investigating the effects of replacing added sugar with NNS in overweight and obese participants demonstrated significant reductions in adiposity indices and improvements in cardiometabolic parameters [14]. Furthermore, the study findings indicate that this substitution also led to reduced cardiometabolic risk factors among individuals with type 2 diabetes T2D [15].

Unsweetening the debate: evaluating NNS and the diseases risk data

Nonetheless, recent discoveries in several research studies have examined the potential adverse health consequences stemming from the excessive and extended consumption of NNS especially when used maybe solely for calorie restriction or weight reduction in people without diabetes, as detailed below:

A European Prospective Investigation into Cancer and Nutrition cohort of 477,206 participants from 10 European countries with a follow-up for 11.5 years found that daily consumption of approximately 6 servings per week of combined soft drinks (sugar sweetened and artificially sweetened) was positively associated with hepatocellular carcinoma [16]. After analyzing data from 24 population-based epidemiological studies, which included a total of 93,095 participants and 20,749 individuals with metabolic syndrome (MetS), a meta-analysis revealed significant positive associations between NNS and MetS risk. Their findings indicated that the risk of MetS increased by 31% with each 250 ml/day increase in NNS-sweetened beverage consumption [17]. The WHO meta-analysis on NNS published in 2022 suggests that NNS may temporarily substitute sugar in overweight/obese patients, yet it is unclear about the risks of cardiometabolic diseases and other long-term effects [8, 9]. Following this, the CARDIA study concluded that habitual, long-term aspartame and saccharin intake independent of total caloric intake and diet quality are related to greater volumes of visceral, intermuscular, and subcutaneous adipose tissue [18]. Recently, ADA 2023 proposed the link between artificial sweeteners consumption and an increasing incidence of T2D suggesting that these may not be safe sugar substitutes [19].

Bitter truths and sweet surprises: the gut-NNS connection

The impact of sweeteners on gut microbiota composition is still under debate. Even though there are some lacunae in the evidence associated with the health effects of NNS in both healthy and non-healthy populations, the USFDA, EFSA (European Food Safety Authority), and Codex Alimentarius consider them safe and well-tolerated, as long as the consumption is limited to NNS-specific ADI [20]. A number of the bacterial groups that exhibited alterations after consuming NNS had previously been linked to type 2 diabetes in humans [21]. However, a recent 12-week study investigated the effects of sucralose in coffee, tea, and fruit juices on the gut microbiome of 38 Asian Indian adults with type 2 diabetes. While no significant changes were observed in the gut microbiome, sucralose consumption resulted in significant reductions in body weight and body fat percentage [22]. The

findings from both short- and long-term human cohorts consuming NNS indicate that individuals have unique responses to NNS, which may be linked to the variations in their gut micro biota composition and function [23]. RSSDI 2022 recommends that artificial sweeteners should be restricted as they alter the diversity of the gut micro biome and can increase insulin resistance [24].

Table to cradle: are NNS safe in pregnancy?

According to a study, sucralose and stevia are considered to be the safest sugar substitutes for pregnant women. However, there is limited research on the safety of acesulfame-K and polyols during pregnancy. Saccharin is not recommended for pregnant women as it crosses the placenta. Pregnant women with hyper phenylalaninemia should avoid aspartame [25].

An observational study linked consumption of NNS-containing beverages to an increased risk of gestational diabetes mellitus (GDM) [26]. According to a systematic review and meta-analysis study, NNS consumption was associated with an increased risk of preterm birth, higher birth weight, and a shorter gestational age [27]. NNS was also speculated to have the ability to influence fetal metabolic programming. These observations raise the concern about transplacental fetal exposure to NNS [28]. Traces of limited NNSs can be found in amniotic fluid and breast milk, attracting attention to the probable effect of long-term exposure on the health of the fetus/infant. This effect of chronic exposure of the fetus/infant to low levels of several NNSs is not clear [29]. Based on human studies, maternal NNS consumption during pregnancy has been associated with an increase in offspring's weight. Additionally, it is intertwined with higher infant BMI at 1 year, as well as an increased risk of the infant being overweight [30, 31]. One study found a significant positive correlation between intrauterine NNS exposure and birth size, as well as an elevated risk of overweight or obesity in children aged 7 years [32]. The safety and potential consequences of NNS consumption during pregnancy warrant further investigation, as limited research suggests associations with adverse outcomes for both mother and infant. It would be prudent to advise all pregnant women to avoid NNS during the pregnancy period.

Deciphering the enigma: aspartame and dietary methanol

A large French population–based prospective cohort study (NutriNet-Santé 2009–21) of 103,388 adults with a follow-up duration of 9 years revealed that aspartame consumption was linked to an increased risk of cerebrovascular events, and excess acesulfame potassium and sucralose consumption was linked to an increased risk of coronary heart disease [33]. Moreover, when compared with 102,865 non-consumers, the intake of aspartame and acesulfame-K was associated with an elevated overall risk of obesity-related malignancies. Notably, aspartame exhibited a more significant risk, particularly in relation to breast cancer with a follow-up duration of 7.8 years [34].

Aspartame was classified by IARC (International Agency for Research on Cancer) as possibly carcinogenic to humans (IARC Group 2B) based on a few evidence of carcinogenicity in humans. However, despite this classification, JECFA (Joint FAO/WHO Expert Committee on Food Additives) reaffirmed the acceptable daily intake of 40 mg/kg body weight for aspartame. In response to this, WHO has reported continuing to monitor with vigilance along with IARC and support further research on the connection between aspartame exposure and long-term health effects [35].

Breaking sweet barriers: exploring non-nutritive sweeteners in type 1 diabetes research

While most children with type 1 diabetes can consume NNS within safe ranges (NNS-specific ADI), caution is necessary for those consuming higher levels of the NNS [36]. Furthermore, study findings suggest that children with type 1 diabetes in Canada generally consume NNS below acceptable daily intake (ADI) levels, indicating a low likelihood of significant impacts on blood glucose control or body weight. However, the study's small sample size and limitations emphasize the need for further research to validate or refute these results [37]. In general, NNS are not advised for children below 12 years of age.

Tying the threads: bitter or better?

Despite the growing body of research and heightened interest in NNS, our understanding of their effects remains significantly limited. Notably, there is a paucity of human studies on nutritive sweeteners, underscoring the need for more comprehensive investigations. To address this knowledge gap, the establishment of robust randomized control trials (RCTs) incorporating substantial population samples over extended periods is of paramount importance. By subjecting NNS to rigorous scrutiny through such well-designed RCTs, we can thoroughly assess potential adverse effects and safety considerations associated with these non-caloric sweeteners.

While certain long-term cohort studies have identified correlations between non-nutritive sweetener consumption and disease risks, it is essential to acknowledge the inherent limitations of these investigations, often confined to specific

populations or geographic regions. Consequently, their generalizability on a global scale is restricted. To overcome this limitation, it becomes imperative to conduct further long-term cohort studies that encompass diverse populations from various regions worldwide. Specifically, more studies are needed in Indian populations' where the consumption of NNS is not huge or is it in large quantities as in the West and in the Middle East. Such an inclusive approach aims to yield a comprehensive evaluation of the safety and potential health implications of NNS.

By assimilating data from wide-ranging and diverse populations, the insights gained from these longitudinal studies hold promising prospects for informing the development of health protocols, facilitating evidence-based decision-making, and ultimately safeguarding public health. As researchers, our commitment lies in striving for a nuanced and thorough understanding of the effects of NNS, laying the groundwork for evidence-driven policies and guidelines that can positively impact global health outcomes.

Diverse research data highlights the potential adverse health effects of artificial sweeteners, sparking public debate. Further study is needed to understand their impact on inflammatory pathways and non-communicable disease development. Though NNS may help limit the total energy intake (TEI) of sugar to lesser than 10%TEI, the long-term safety of NNS still remains uncertain. A higher risk of hypertension, insulin resistance, raised blood glucose, abdominal obesity, and dyslipidemia has been associated with long term and consumption of NNS in large quantities whether this is related to excess calorie consumption or other confounders is not clear. The impact of NNS on the cardiovascular system is still unclear, and further research is required [38]. Until comprehensive, long-term studies offer conclusive evidence, it is advisable to avoid intake of large doses of NSS [8]. This calls for informed choices and awareness, aiming for safer alternatives and promoting healthier lifestyles. Moreover, it is not clear whether the adverse effects of NNS apply to all or only to specific ones like Aspartame.

What did the WHO meta-analysis conclude?

The 2022 meta-analysis conducted by the World Health Organization (WHO) examined and advised that NNS consumption below the acceptable daily intake is generally considered safe. While the analysis did not establish a direct link to cardiometabolic disorders, it highlighted the need for caution regarding potential long-term effects. Short-term use may benefit those dealing with weight issues. An additive does not pose safety concerns provided the anticipated daily intake is less than or equal to the ADI [7]. There is an urgent need for appropriately planned randomized controlled studies to review their effectiveness in different populations. The WHO made it very clear that their cautions regarding NNS did not apply to those with diabetes. It was mainly means for those using NNS in large quantities with weight reduction as their goal for which the WHO felt that the NNS was better avoided.

What about use of NNS in India?

In India, most of the NNS intake is related to their coffee and tea where only a small amount of the NNS is added. However, it is important to note that aspartame is not heat stable hence better avoided in hot beverages [39]. The consumption of sweetened beverages using artificial sweeteners is, as of now, not very high in India. Given the low intake of sweeteners which are well within the acceptable daily intake (ADI) allowed for sweeteners, there is not much of concern, as of now, regarding the use of sweeteners in India. However, learning from the experience in the West where there is indiscriminate and large-scale use of sweeteners has resulted in some adverse effects, it would be prudent to advise use of artificial/alternate sweeteners only in limited quantities and not to go overboard with their use. Moreover, the use of artificial sweeteners to prepare deserts or sweets, where sweeteners would be used, should be discouraged. We should also educate people that just because sugar is replaced with a sweetener, a sweet dish like a dessert does not become healthy, since it may still be calorie dense, which also depends on the type and quantity of fat and carbohydrate used for the preparation. ADA recommends health care providers should continue prescribing reduction in consumption of sugar and calories with or without the usage of NNS. At the same time, moderate quantities of sweeteners can be consumed by people living with diabetes with a sweet tooth, provided it reduces the total calorie and carbohydrate consumption. Since NNS have been subjected to thorough safety assessments by regulatory bodies, one could be reassured that as of now, population-specific USFD-approved sweeteners can be considered reasonably safe within their acceptable daily intake [40].

'TEN ' Guidelines for use of Non-nutritive sweeteners (NNS) in India

1. Avoid all NNS in pregnancy.
2. Preferably avoid all NNS in children.
3. Aspartame should not be added to hot beverages.
4. Aspartame must be avoided by those with Phenylketonuria.
5. Do not use NNS to prepare desserts and sweet dishes where large quantities may be required for optimizing taste.
6. Remember just because sugar is removed and replaced with NNS, a dessert or sweet can still be unhealthy because of high calorie and fat content.
7. Do not use NNS as part of weight reduction program as their efficacy is not established.
8. Use of NNS particularly stevia or sucralose in small quantities along with tea and coffee appears to be safe.
9. WARNING: Do not exceed Acceptable Daily Intake (ADI) of sweeteners.
10. More Indian data (particularly long term) is required.

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Declarations

Competing interests Zydus Wellness has supported studies on Sucralose done by the Madras Diabetes Research Foundation.

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REVIEW ARTICLE

Diabetes self-care activities among patients with type 2 diabetes: A systematic review and meta-analysis

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Abstract

Objectives The important role of prevention in controlling chronic diseases such as diabetes, the major impact of self-care behaviors as preventive behaviors, and the lower cost of self-care measures should be considered. Therefore, this study was conducted to systematically review and meta-analyze the status of diabetes self-care activities status in type 2 diabetic patients worldwide.

Methods As far as we know, our study is the first systematic based meta-analysis estimating the pooled score of diabetes self-care activities (SDSCA) and its dimensions among type 2 diabetic patients. Keywords were searched in Scopus, Web of Science, Google Scholar, PubMed, Cochrane, Ovid, and ProQuest databases from 2012 to 2022. The self-care pooled score estimates through a fixed-effects meta-analysis using STATA 15. Also, I^2 statistic was used to determine heterogeneity across the articles.

Results Seventeen studies on the diabetes self-care activities were included in the meta-analysis. Variation in pooled mean attributable to heterogeneity for exercise and self-care is zero, while the variation for foot cares, general diet, and blood testing were estimated to be 53, 20.6, and 81.4%, respectively ($p < 0.01$). The random effects based pooled mean was estimated as follows: foot care (FC) 2.02 (95% CI: 1.05, 2.98), general diet (GD) 3.91 (95% CI: 3.21, 4.60), and blood testing (BT) 1.82 (95% CI: 0.64, 3.01). As well as based on the fixed effects, the pooled mean of exercise (E) and the total score of self-care (TSC) were obtained 2.12 (95% CI: 1.77, 2.47) and 3.35 (95% CI: 2.96, 3.74), respectively.

Conclusion The overall level of self-care was moderate and far from ideal. Dimensions of foot care, exercise, and blood glucose testing were also below average. Based on this evidence, policies to prevent diabetes should be directed toward educating patients on preventive activities. On the other hand, it is necessary to ensure that patients' interpretations are in line with doctors' recommendations.

Keywords Diabetes self-care activities · SDSCA · Type 2 diabetes · Systematic review · Meta-analysis

Introduction

Diabetes is on the list of the most common chronic diseases nowadays [1]. Type 2 diabetes is the most common type and usually involves adults. Type 2 diabetes is a condition in which the body does not produce enough insulin or becomes insulin resistant. About 422 million people worldwide suffer from diabetes, most of whom live in low- and middle-income countries. Diabetes is the cause of 1.5 million deaths annually. All countries of the world, regardless of income level, have witnessed a significant increase in the prevalence of type 2 diabetes over the past 30 years

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[2]. The global prevalence of diabetes is estimated to grow by about 2% by 2045. Diabetes prevalence is also expected to be greater in urban areas and middle- and upper-income countries. Global diabetes health spending was about $966 billion in 2021 and it is expected to reach $1054 billion by 2045 [2]. At the same time, what causes irreversible complications is that one in two patients suffering from diabetes is unaware of the disease [1]. Diabetes is a critical health issue because its complications directly affect the quality of life and can lead to death [3]. Stroke, cardiovascular disease, and peripheral artery disease (PAD) are the macrovascular complications of diabetes [1]. Studies have shown that the patient's knowledge about the disease and its health-related behaviors is the most important factor in controlling it [4]. A person's beliefs and knowledge about illness determine his or her health-related behaviors and self-care activities [5]. The concept of belief and knowledge can improve the health and safety of the patient and thus reduce the costs of treatment and care [6]. Therefore, one of the most widely used concepts in this field is diabetes self-care activities. A reliable tool entitled "diabetes self-care activities" has been used in studies around the world that include the dimensions of foot care, diet, exercise, and blood glucose testing [7]. The important role of prevention in controlling chronic diseases such as diabetes, the major impact of self-care behaviors as preventive behaviors, and the lower cost of self-care measures should be considered. Therefore, this study was conducted to systematically review and meta-analyze the status of diabetes self-care activities status in type 2 diabetic patients worldwide.

Materials and methods

Study design

This systematic meta-analytical review studies diabetes self-care activities in countries around the world. The search was conducted for 10 years (from 2012 to February 2022). A PRISMA-based (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) literature review was performed [8].

Search strategy

The main domains of the search included type 2 diabetes, self-care activities, and foot care. Keywords were also obtained from Medical Subject Heading (MeSh), literature review, and statements of experts in the field. It was then concluded with a pilot search. Keywords were "diabetic foot," "diabetic feet," "diabetes self-care activities," "SDSCA," "self-care," "self-care behavior," "attitude to health," "health behavior," "foot care," and "care behavior." AND, OR, and NOT Booleans used to bound the search. The search strategy was developed by two experts in the field of health services management who are well-experienced in patients' health behavior (OKh and AMa) and a specialist in systematic review and meta-analysis (BAh). The sample search strategy is shown below.

TITLE-ABS-KEY ("diabetic foot" OR "diabetic feet") AND TITLE-ABS-KEY ("diabetes self-care activities" OR "SDSCA" OR "self-care" OR "self-care behavior" OR "attitude to health" OR "health behavior" OR "foot care" OR "care behavior") AND (LIMIT-TO (PUBYEAR, 2021) OR LIMIT-TO (PUBYEAR, 2020) OR LIMIT-TO (PUBYEAR, 2019) OR LIMIT-TO (PUBYEAR, 2018) OR LIMIT-TO (PUBYEAR, 2017) OR LIMIT-TO (PUBYEAR, 2016) OR LIMIT-TO (PUBYEAR, 2015) OR LIMIT-TO (PUBYEAR, 2014) OR LIMIT-TO (PUBYEAR, 2013) OR LIMIT-TO (PUBYEAR, 2012)) AND (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (LANGUAGE, "English")).

Databases

Keywords were searched in Scopus, Web of Science, Google Scholar, PubMed, Cochrane, Ovid, and ProQuest databases. Springer and SAGE journals and some other key journals in the field were also searched manually. To enhance search scope, pre-prints, references, citation lists, and relevant papers were also examined.

Inclusion criteria

(1) Articles that had type 2 diabetic patient samples
(2) Articles that examined the dimensions of foot care, general diet, exercise, and blood testing of diabetes self-care activities
(3) Articles in which the mean and standard deviation of diabetes self-care activities (SDSCA) and its dimensions were reported or calculable
(4) Articles in which the patients studied had diabetic foot ulcers
(5) Peer-reviews, time-series, and cross-sectional studies

Exclusion criteria

(1) Articles related to type 1 diabetes and gestational diabetes

(2) Non-English articles
(3) Articles considering other questionnaires
(4) Articles out of sufficient information to enter the meta-analysis
(5) Secondary documents such as editorials and conference abstracts
(6) Mathematical modeling articles
(7) Meta-analyses and reviews

Review process

First, papers were compiled in EndNote X8. Next, duplicates and disjointed titles were skipped. The three researchers (AMa, SYo, ZMo) individually evaluated the titles and abstracts in terms of adherence with the inclusion and exclusion criteria. A list of the remaining papers was prepared to review the full texts. Two (AMa and SYo) researchers individually read the full texts of the remaining papers and adjusted them to the research question. If there was controversy in the above cases, two other researchers (OKh and BAh) would also examine the argument. Ultimately, two researchers extracted data from the papers into the data assemblage table. The table retains the author's name, year of publication, country, mean, and standard deviation. This table has been adjusted based on pilot testing on five articles.

Quality assessment and critical appraisal

By finishing the searching process, the included papers were assessed by three researchers (AMa, SYo, and ZMo) using the Newcastle–Ottawa Scale. It is a tool applied for the quality assessment of non-randomized studies included in a meta-analysis or systematic review. This tool retains three classifications and 8 items. Classifications include study group selection, group comparability, and determination of intervention or outcome for case–control or cohort studies, respectively. Qualitative evaluation is done by rewarding stars to each item [9]. Papers acquire between 0 and 10 points. Ultimately, articles that acquired a score below the average score were omitted. The remaining papers were selected and data including author name, year of publication, country, sample size, and mean and standard deviation of each dimension were documented in a data extraction template based on the PRISMA guidelines for systematic reviews.

Data analysis

Diabetes self-care activities (SDSCA) and its dimensions pooled score (PS) were approximated by random-effects meta-analysis, using STATA 15. The analysis results were demonstrated at a 95% confidence interval. I^2 statistics were used to investigate the possibility of heterogeneity across the publications ($I^2 \geq 50\%$ indicates heterogeneity). A forest plot was applied to show the results. An Egger test was executed to assess the publication bias.

Results

A total of 2061 papers were obtained from the search, of which 563 were duplicates. After removing duplicates, the title and abstract of 945 papers were matched with inclusion and exclusion criteria and inconsistencies were removed. Five hundred thirty-six full texts were rejected due to lack of quality after evaluation. Finally, 17 articles on diabetes self-care activities were meta-analyzed (Fig. 1). The characteristics of the articles included in the study are reported in Table 1.

Table 2 shows the degree of heterogeneity between studies based on the studied variable. The results of this table reveal that variation in pooled mean attributable to heterogeneity for exercise and self-care is zero, while the variation for foot cares, general diet, and blood testing were estimated to be 53, 20.6, and 81.4%, respectively ($p < 0.01$). However, general diet heterogeneity was not statistically significant.

The pooled mean (PM) and the 95% confidence interval for each of the study variables based on the estimation model are presented in Table 2. The pooled mean for foot care, general diet, and blood testing are different in the two models. There has been variation attributable to heterogeneity for these variables. Therefore, the random effects based pooled mean is acceptable to us. According to the results of Table 3 and Figs. 2 and 3, the pooled mean for these variables was estimated as follows: foot care (FC) 2.02 (95% CI: 1.05, 2.98), general diet (GD) 3.91 (95% CI: 3.21, 4.60), and blood testing (BT) 1.82 (95% CI: 0.64, 3.01). As well as based on the fixed effects, the pooled mean of exercise (E) and the total score of self-care (TSC) were obtained 2.12 (95% CI: 1.77, 2.47) and 3.35 (95% CI: 2.96, 3.74), respectively.

As the information in Table 4 displays, the bias coefficient is statistically significant only for the total self-care score and general diet ($p < 0.05$). Other dimensions of self-care (foot care, exercise, and blood testing) did not suffer from publication bias.

The funnel plots in Fig. 4 illustrate the result of the assessment of the publication bias for the self-care visually. The results of these graphs also confirm the existence of a publication bias for self-care at the 95% level.

Figure 5 illustrates the forest plot of the sensitivity analysis of the pooled mean of the self-care score. As it is known,

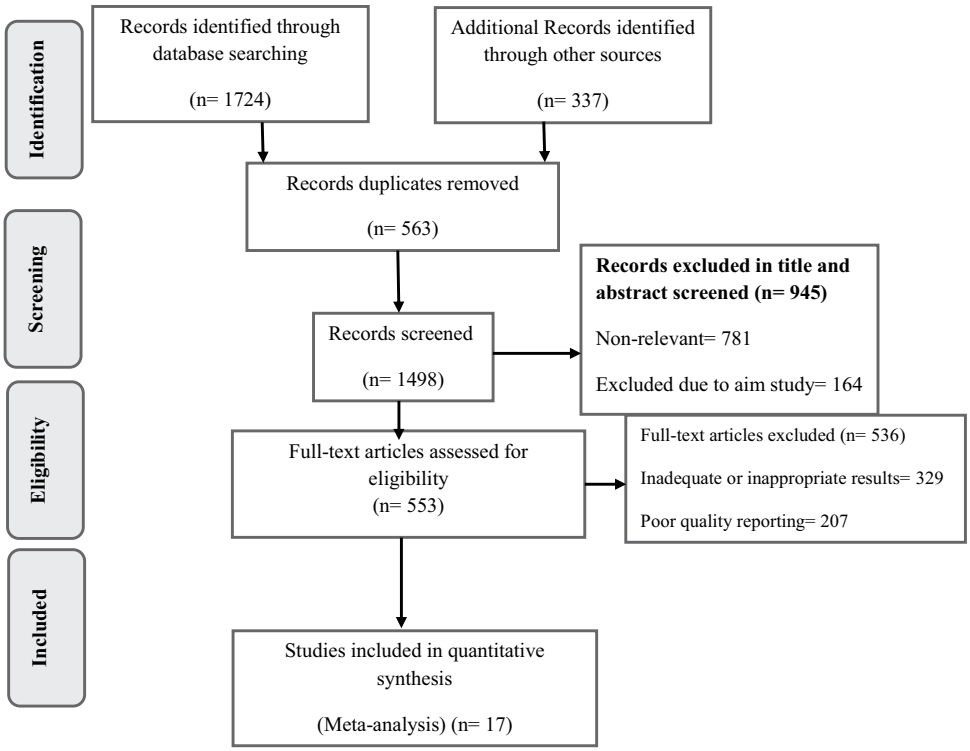


Fig. 1 The flow diagram of the literature search

the aggregated average is not sensitive to the results of individual studies. That is, removing the results of individual studies has no effect on the significance of the pooled mean score of self-care.

Discussion

This study measured the global pooled mean of diabetes self-care activities and its dimensions including foot care, diet, exercise, and blood glucose testing. The pooled mean of self-care activities in type 2 diabetic patients was moderate (3.36). Among the self-care activities, adherence to the diet, although slightly above average (4.84), was the strongest dimension. Blood glucose testing (2.40), foot care (2.37), and exercise (2.13) were below average, and with a small difference, exercise was the weakest dimension.

Regarding to diet dimension, Malaysia has the highest (5.20) and Korea the lowest (0.92) mean. This dimension includes the number and type of meals received during the day. The relatively higher average of this dimension can be due to more education about diet and the existence of nutritional guides on food labels. Rajput et al. demonstrated the critical role of diet in controlling type 2 diabetes in their review [27]. A study by Degefa et al. in the line with present study showed that the total mean of diet adherence is moderate and even low in African countries. Many factors influence the way in which diet-related behaviors occur [28]. These factors include beliefs, lifestyle, affordability, family support, access to healthy food, fruits, and vegetables, enough nutrition knowledge, and a correct understanding of medical advice [28, 29]. Many studies showed that lack of access to healthy food due to seasonal or economic barriers, as well as lack of knowledge in the field of healthy nutrition, also undermines the diabetic diet [11, 30, 31].

Regarding the blood sugar testing dimension, Italy has the highest (4.32) and Korea the lowest (0.14) mean. This dimension includes the number of blood sugar tests. In confirmation of the present study, a systematic review by Stephani et al. showed that this dimension has a low level, especially in poor countries [32]. On the other hand, these tests are more common in people who have access to a home glucometer [32–35]. Patients who inject insulin are more knowledgeable and skilled in performing constant blood sugar testing [29]. Many factors play a role in determining this dimension, including financial affordability, attitude toward testing, knowledge about the importance of testing, testing skills, social support, doctor's advice, and insurance coverage.

Regarding to foot care dimension, Iran has the highest (4.56) and Lebanon the lowest (0.33) mean. This dimension

Table 1 Characteristics of the descriptive cross sectional studies included in the meta-analysis

Ref	Author	Setting	Place	Year	Sample	Foot care		General diet		Exercise		Blood testing		SDSCA	
						Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
[10]	I.L. Jackson, et al	Nigeria	The University of Uyo Teaching Hospital, Akwa Ibom State	2021	226	2.5	2.1	3.95	1.15	2.4	1.2	1	1.1	2.76	1.34
[11]	Victor Mogre, et al	Ghana	Three hospitals in the Tamale Metropolis	2019	187	2.86	2.16	4.4	1.52	4.78	2.09	2.15	0.65	3.54	1.8
[12]	Ola Sukkarieh, et al	Lebanon	Lebanese American University, Byblos	2016	140	1.19	2.11	3.63	2.37	1.37	2.1	2.5	2.48	2.46	2.28
[13]	Ali Hassan Alhaiti, et al	Saudi Arabia	King Fahad Medical City (KFMC)	2020	247	3.28	1.69	2.57	1.74	2.14	2.01	4.18	2.43	3.04	1.96
[14]	Najwa S. ElGerges, et al	Lebanon	The primary healthcare center (PHC) diabetes clinic	2020	50	0.33	0.51	3.03	1.66	1.79	2.01	0.53	1.07	2.48	0.77
[15]	Sana Taher Ashur, et al	Libya	The National Centre for Diabetes and Endocrinology (NCDE) in Tripoli	2016	523	2.3	2.6	2.9	2.6	2.5	2.3	1.2	1.9	2.22	2.35
[16]	Sofia Akritidou, et al	Greece	Aretaeus Diabetes Center	2017	22	1.57	1.31	3.43	1.29	1.82	1.68	4	1.78	2.705	1.515
[17]	Hamdiye Arda Sürücü, et al	Turkey	The university hospital or the training and research hospital in the southeast of Turkey	2017	220	0.96	0.82	1.72	1.05	0.88	0.79	1.12	0.79	1.17	0.86
[18]	Marilia Braga Marques, et al	Brazil	Primary Health Care Units of Fortaleza/Ceará	2019	50	4.5	3.11	3.81	2.35	1.82	2.47	1.38	2.32	2.8775	2.5625
[19]	Kathleen Mulligan, et al	UK	UK National Health Service organizations and mental health and diabetes charities	2018	77	1.7	1.8	3.85	2.05	2.4	2.1	3.8	2.7	3.12	2.14
[20]	Azar Tol, et al	Iran	Omolbanin center, an outpatient diabetic center in Isfahan	2012	140	4.56	2.14	3.7	1.31	2.11	1.89	2.12	2.12	3.23	1.75
[21]	Anfal N. Al-Mallah, et al	Iraq	The Leila Qasm Diabetic Centre, Erbil	2017	50	4.2	2.8	3.15	1.55	2.6	2	1.6	2.1	2.8875	2.1125
[22]	Yee Cheng Kueh, et al	Malaysia	The Diabetes Clinic of the Hospital University Sains Malaysia (HUSM) in Kelantan	2016	266	3	2.65	5.2	2.15	2.5	2.34	1.2	1.81	2.97	2.23
[23]	Renu Bala, et al	India	The outpatient department (OPD) of Regional Research Institute for Homoeopathy, Imphal	2020	108	0.39	1.42	3.86	1.39	3.95	2.29	0.14	0.39	2.085	3.06
[24]	Young Mi Kang, et al	Korea	Chungnam National University Hospital	2018	23	1.14	3.31	0.92	3.48	0.59	1.05	0.33	2.4	0.745	2.56
[25]	Kyung Suk Shin, et al	Korea	Three community health centers in South Korea	2017	71	1.98	2.4	3.42	2.26	4.45	2.24	0.88	1.81	2.6825	2.1775
[26]	Davide Ausili, et al	Italy	Hospital ASST Sette Laghi, Varese	2017	302	3.32	0.3	4.97	0.12	2.2	0.2	4.32	0.3	3.7025	0.23

Measuring tool: the summary of diabetes self-care activities (SDSCA)

Studies' population: patients suffering diabetes type 2

Table 2 Heterogeneity and significance statistics of pooled mean

Statistics	Variables				
	FC	GD	E	BT	TSC
Heterogeneity I^2 (%)	53.3	20.6	0.0	81.4	0.0
Heterogeneity χ^2 (d.f. = 16)	34.26 $p < 0.01$	20.15 $p = 0.214$	8.47 $p = 0.934$	86.25 $p < 0.01$	12.25 $p = 0.726$
PM = 0	$z = 10.55$ $p < 0.01$	$z = 42.05$ $p < 0.01$	$z = 11.87$ $p < 0.01$	$z = 12.22$ $p < 0.01$	$z = 16.94$ $p < 0.01$

FC foot care, *GD* general diet, *E* exercise, *BT* blood testing, *TSC* total self-care, *PM* pooled mean

refers to the frequency of foot care and shoe inspection. In line with this meta-analysis, foot care activities are weak in many studies [28, 29, 36–39]. At the same time, patients who suffer from foot ulcers are more likely to seek foot care than patients who do not yet have foot ulcers. According to a study by Alosaimi et al., these activities were twice as common in patients with foot ulcers as in patients without foot ulcers [40]. A study by Stephani et al. showed that foot care is more common in women than men [32]. Reasons for not doing enough foot care activities can be lack of adequate training and medical advice, negative attitude toward these activities, lifestyle and how people use their feet, lack of time to take care of feet, too much work, lack of communication with the doctor, lack of support, and time-consuming and abnormal care activities.

Regarding to exercise dimension, Ghana has the highest (4.78) and Korea the lowest (0.59) mean. This dimension refers to the number and duration of physical activity. According to the present study, physical activity has a small share in diabetes self-care behaviors [28, 41–44]. Lack of physical activity may be due to lifestyle, lack of time, lack of adequate training and advice, physical condition, and cultural differences. Thus, the lack of access to exercise facilities and the lack of motivation lead to reducing this behavior, and explaining the importance and promotion of knowledge in this field strengthens this dimension [28].

Financial barriers, deficit knowledge and awareness of self-care, lack of specific protocols to guide diabetic patients in the field of self-care, difficulty interacting between patient and service provider, deficit knowledge of healthy lifestyle, hardship abandoning bad habits, lack of confidence, lack of patient self-esteem and self-efficacy, unilateral training, patients' lack of time, patients' job barriers, patients' disappointment, and lack of motivation are obstacles that undermine self-care. Social norms, people's attitudes toward diabetes, social stigma, lack of community or family and peer support, family conflicts, cultural beliefs, self-treatment and use of herbal remedies, believing that diabetes is caused by negative spiritual forces, and deliberate non-compliance are other decisive factors that weaken the various dimensions of self-care [11, 30, 31, 45–48].

The low pooled mean of diabetes self-care activities and its dimensions indicate that there is a lack of knowledge and education in this field. Given that the best way to cope with diabetic foot ulcers is to prevent them, it is necessary to strengthen all dimensions of self-care. Adherence to diet, physical activity, and frequent blood sugar tests are all necessary to prevent diabetic foot ulcers. Simultaneously, direct foot care activities are important, especially for people who suffer from foot ulcers.

Some measures that can be taken to enhance self-care include (1) improvement of patient interaction and service providers, (2) encourage family and community to support patients with diabetes, (3) improvement and facilitate the process of service delivery, (4) facilitating access to healthy food, (5) creating environments compatible with patients with diabetes, (6) motivating patients and their family, (7) modification of the attitudes, norms, and values of the society toward diabetes, and (8) use of technologies (such as cell phones) for education, reminder, the

Table 3 Pooled mean of variables based on fixed and random effects model

Model	Variables									
	FC		GD		E		BT		TSC	
	PM	95% CI	PM	95% CI	PM	95% CI	PM	95% CI	PM	95% CI
Fixed effect	2.37	1.93, 2.81	4.83	4.61, 5.06	2.12	1.77, 2.47	2.40	2.02, 2.79	3.35	2.96, 3.74
Random effect	2.02	1.05, 2.98	3.91	3.21, 4.60	2.12	1.77, 2.47	1.82	0.64, 3.01	3.35	2.96, 3.74

FC foot care, *GD* general diet, *E* exercise, *BT* blood testing, *TSC* total self-care, *PM* pooled mean, *CI* confidence interval

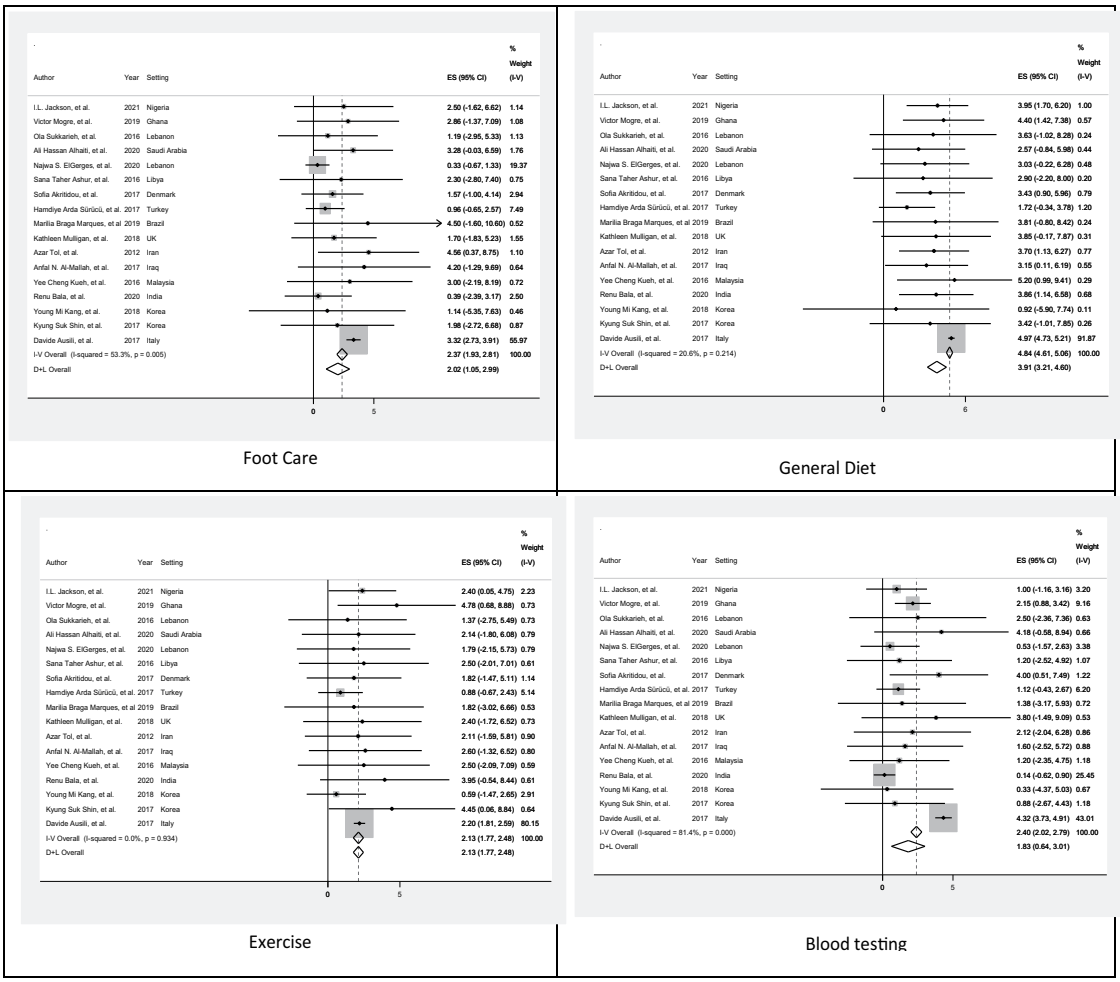


Fig. 2 Forrest plot for self-care dimensions

Fig. 3 Forrest plot for total self-care

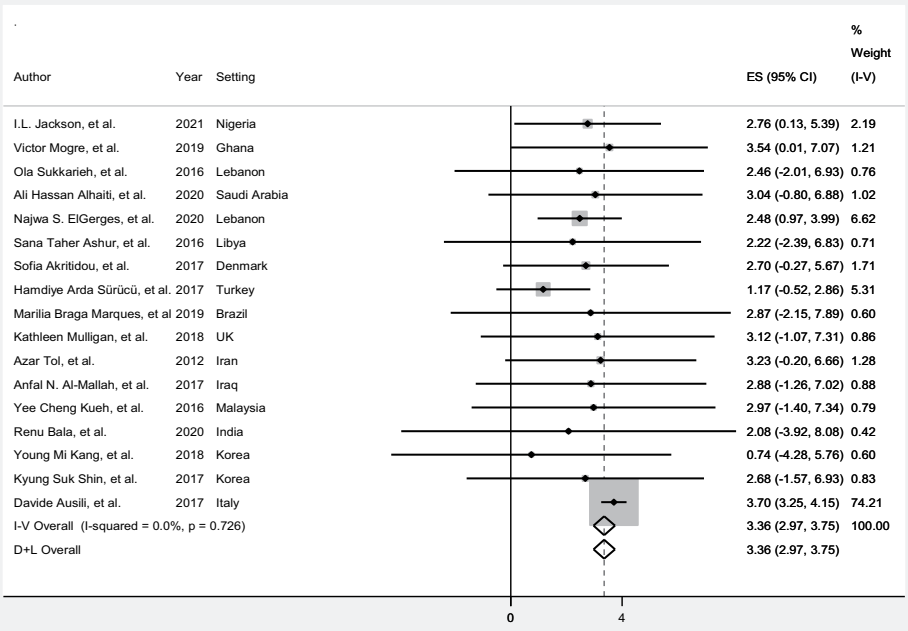


Table 4 Egger's test for assessing the publication bias

Variables	Bias Coef	SE	*t*	$p > \|t\|$	95% CI
FC	−0.315	0.519	−0.61	0.552	−1.422, 0.791
GD	−1.006	0.195	−5.12	0.001	−1.417, −0.584
E	0.051	0.231	0.22	0.831	−0.443, 0.544
BT	−0.839	0.852	−0.98	0.340	−2.655, 0.977
TSC	−0.675	0.232	−2.91	0.011	−1.171, −0.181

FC foot care, *GD* general diet, *E* exercise, *BT* blood testing, *TSC* total self-care

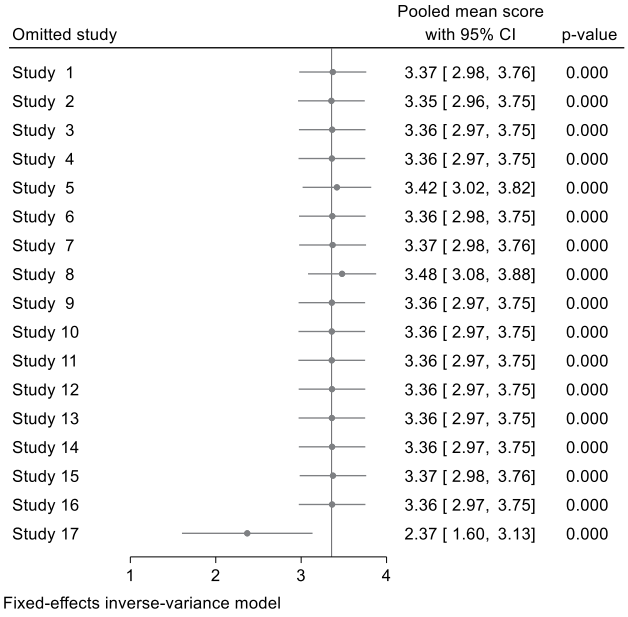


Fig. 5 Leave one out meta-analysis of diabetic foot self-care score mean

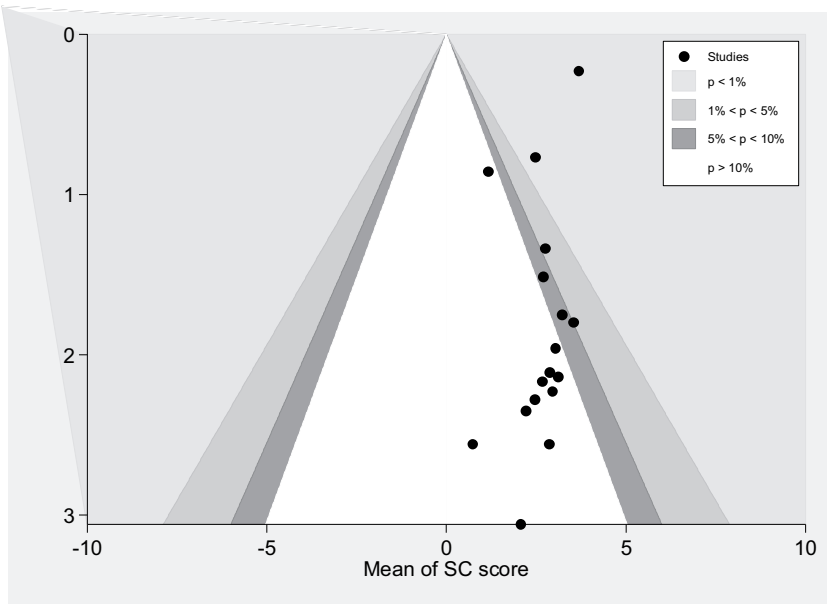
interaction of patients and service providers and changing patients' bad behaviors [30, 45–47].

This study also has some limitations. The first constraint is that in order to decrease the amount of heterogeneity, we only pooled the SDSCA and its dimensions' scores and excluded studies that used other methods such as semantic priming task, evaluative priming task, affect misattribution procedure, and extrinsic affective Simon task. The second constraint is that only English articles were contained in the analysis. It should be mentioned, however, that we endeavored to overwhelm this constraint by systematically reviewing the most popular databases.

Conclusion

This study obtained the pooled mean of diabetes self-care activities including diet, foot care, exercise, and blood glucose testing in different countries for the first time. The overall level of self-care was moderate and far from ideal. Dimensions of foot care, exercise, and blood glucose testing were also below average. Based on this evidence, policies to prevent diabetes should be directed toward educating patients on preventive activities. On the other hand, it is necessary to ensure that patients' interpretations are in line with doctors' recommendations. The capacity of skilled nurses can be used for self-care training and follow-up so that the shortage of physicians does not prevent continuous monitoring of self-care activities.

Fig. 4 Funnel plot for self-care



Data availability Not applicable.

Declarations

Ethics consideration The present study has ethical approval from the ethics committee of Qazvin University of Medical Sciences (ethics code IR.QUMS.REC.1401.329).

Competing interests The authors declare no competing interests.

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REVIEW ARTICLE

Effect of extracorporeal shockwave therapy on adhesive capsulitis in patients with type 2 diabetes mellitus: A systematic review

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Abstract

Objective To investigate the effectiveness of extracorporeal shockwave therapy (ESWT) on pain, range of motion (ROM) and disability on adhesive capsulitis (AC) in patients with type 2 diabetes mellitus (DM).

Method Clinical trials assessing the effectiveness of extracorporeal shockwave therapy on adhesive capsulitis in diabetes mellitus patients were included. Academic databases of Pubmed, Scopus, Web of Science, Cochrane Library, Science Direct, PEDro and grey literature sources such as Clinical trials.gov and Google Scholar were searched from the year 2000 to 2021. Physiotherapy Evidence Database (PEDro) scale was used to determine overall quality of each included article.

Result The search yielded eight studies evaluating the effectiveness of ESWT plus shoulder exercises and mobilization on AC in type 2 DM patient population. Compared to control treatment groups in majority of included studies ESWT appeared to be effective in managing pain, ROM and disability and showed large to very large treatment effects. Two of included studies reported positive effects of ESWT on inflammatory markers and blood glucose levels.

Conclusion Findings from this review suggested that ESWT combined with shoulder exercises and mobilization may be a useful treatment modality in shoulder AC for type 2 DM patients for pain reduction, disability and ROM improvement. There was insufficient data to confirm the findings of ESWT on blood glucose and inflammatory markers in diabetic patients. Therefore, future studies are needed to evaluate its effectiveness on these outcomes.

Trial registration number: #CRD4202127949.

Keywords Extracorporeal shockwave therapy · Adhesive capsulitis · Frozen shoulder · Periarthritis shoulder · Diabetes mellitus · Shoulder pain

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by an increase in blood glucose levels due to impaired insulin production and disturbed insulin effect on the body organs [1]. According to the International Diabetes Federation (IDF) atlas 2021 the global prevalence of DM was 10.5% (536.6 million people) in 20 to 79 years old individuals for both males and females estimated to rise by 12.2% (783.2 million people) by the year 2045 [2].

Adhesive capsulitis (AC) of the shoulder is an inflammatory and fibrosing disorder of musculoskeletal tissue that affects the Glenohumeral joint capsule, in which pain and progressive restriction of both active and passive shoulder ranges leads to functional disability and increased health care costs [3]. It can be classified as primary when the underlying cause is not known and secondary when the aetiological factors are identified such as trauma, surgery, arthritis and systemic diseases like diabetes, thyroid dysfunction or other known cause [4]. A meta-analysis showed that there is a strong relationship between DM and AC and the overall prevalence of AC is higher, which goes up to 13.4% in DM patients as compared to 2% in non-DM patients. Whereas no significant difference found in the prevalence of shoulder AC between type 1 DM and type 2 DM. Also, diabetic patients are five times more prone to develop AC [5].

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This association between AC and DM is part of DM-related chronic inflammation [6]. In DM the free fatty acids (FFAs) secreted from adipocytes enhance the production of pro-inflammatory mediators and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) which exacerbate the inflammatory process and contribute to synovitis and capsular thickening of the shoulder joint [7]. Additionally, the chronic hyperglycemic state in DM results in the formation of some stable and irreversible end products between body proteins and blood glucose causing thickening and cross-linking of collagen fibers of soft tissues [8].

MRI studies revealed that a thickness of ≥ 7 mm for rotator interval and ≥ 4 mm for coracohumeral ligament (CHL) was highly suggestive of the diagnosis of AC [9]. Clinically the patient exhibits a gradual onset of pain with restricted active and passive range of motion (ROM) in a capsular pattern i.e., most restriction in external rotation followed by the abduction and internal rotation [10]. Recently, Lyne et al. (2022) conducted a qualitative study that concluded that AC patients main concern was an early and effective pain management over the loss of ROM [11].

Both conservative and surgical treatment options are available for shoulder AC. Conservative treatment includes medication, therapeutic exercises, local corticosteroid injections, hydro-distension, mobilisation, electrotherapy, therapeutic ultrasound, laser therapy, hot fomentation, splinting, and diathermy used in various permutations and combinations to manage pain, ROM, and disability [12]. Surgical treatment includes arthroscopic capsular release, open capsular release, or manipulation under anesthesia(MUA) [7]. While treating surgically, for AC, there is a risk of getting iatrogenic complications with a high rate of recurrence in DM patients. Thus, most of these patients prefer a conservative approach which should be the first line of treatment. However, there is a lack of consensus regarding the best treatment strategy for AC of the shoulder [13].

Extracorporeal shockwave therapy (ESWT) has recently gained attention as a new therapeutic modality that has proved to be efficacious in reducing both pain and disability in various musculoskeletal and bony conditions like knee osteoarthritis, lateral epicondylitis, rotator cuff tendinopathy, chronic low back ache, osteonecrosis and fracture healing [14–18]. The shockwaves trigger soft tissue healing and cause pain relief by increasing blood flow, accelerating angiogenesis, hyper-stimulation of nociceptive nerve fibers and produce endogenous opioids like substance P at the treatment site [19]. In recent years, the efficacy of ESWT has been studied on AC in non-diabetic patient population [20–22]. To the best of our knowledge, this is the first systematic review done regarding the effectiveness of ESWT on AC in type 2 DM patients. Therefore, the purpose of this systematic review was to assess and examine the effectiveness of ESWT on pain, ROM, and disability in AC with type 2 DM patient population. We hypothesized that ESWT would be effective in AC associated with type 2 DM.

Methodology

Protocol and registration

This systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) under registration number #CRD4202127949. However, after registration, we fine-tuned the inclusion criteria by removing one observational study and increasing the period to last 20 years to include more studies. The review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines 2020 [23].

Eligibility criteria

The PICO framework (Population, Intervention, Comparison, and Outcome) was followed.

A study must meet the following inclusion criteria to be selected in this review:

Population

The study population had to be older than 18 years regardless of their ethnicity and gender with a clinical diagnosis of type 2 DM and suffering from shoulder AC with painful and restricted ROM from last ≥ 2 months. Participants without DM, uncontrolled type 2 DM, primary AC, history of glenohumeral dislocation, proximal humeral fracture, cervical radiculopathy, post-surgery stiffness, rotator cuff tear, any neurological disorder (stroke, Parkinson's disease, multiple sclerosis, etc.), cognitive impairment, any diagnosed psychiatric disorder, pregnant women were excluded.

Intervention

Extracorporeal shockwave therapy in which shock waves are provided on the most tender points in and around the shoulder joint. Any other joint was not involved in this study.

Comparison

There can be any type of comparison or intervention either alone or in combination (for example pulsed electromagnetic field therapy, phonophoresis, therapeutic ultrasound, intra-articular corticosteroid injection, therapeutic exercise program, joint mobilisation or manipulation, etc.).

Outcome measure

Studies having pain, range of motion, and disability as their outcome measures which would be measured by any validated scale or methodological tool were included.

Study design

English language studies including randomized clinical trials (of any type like parallel, pre-test, post-test design) and prospective comparative studies were included.

Information sources

A systematic literature search was performed on the databases: MEDLINE (assessed by PubMed), CENTRAL (Cochrane Library Central Register of Controlled Trials), Web of Science, PEDro, Scopus, and Science direct with basic and advanced search strategy thoroughly from January 2000 to November 2021. Grey literature sources such as the US National Institutes of Health database (clinical trials.gov) and Google scholar were also searched for the available studies published to manually add the missed studies. The reference list of the published systematic review, as well as meta-analysis, was also screened for studies.

Search strategy

The combination of the following keywords was used ((Extracorporeal shockwave therapy) OR (shockwave therapy)) OR (shockwave lithotripsy) AND (((adhesive capsulitis) OR (frozen shoulder)) OR (periarthritis shoulder)) OR (shoulder pain) AND ((diabetes mellitus) OR (type 1 diabetes mellitus)) OR (type 2 diabetes mellitus).

Selection process

Two authors independently executed the process of search and selection of studies. All the records that were found on different databases and grey literature sources were exported to the Zotero reference manager and duplicates were removed. The title and abstract of each included article were further screened and irrelevant articles were excluded based on exclusion criteria. After that, the remaining included articles were closely reviewed for full-text eligibility based on inclusion criteria. Figure 1 shows the flowchart of our search strategy in accordance with PRISMA guidelines 2020. The screening process was further discussed with other independent reviewers. Any disagreement between the authors was resolved by discussion and mutual consensus.

Data collection process and data items

The principal author conducted data extraction which was rechecked by one other researcher. The information extracted from each included trial on author's name, year of trial conduction, design of the study, subject characteristics (age, gender, duration of type 2 DM, number of participants), type of intervention including extracorporeal shockwave therapy; its parameters like type of ESWT, frequency, impulses, energy flux density, sessions per week, follow up or versus another intervention, comparison, outcome measures, and

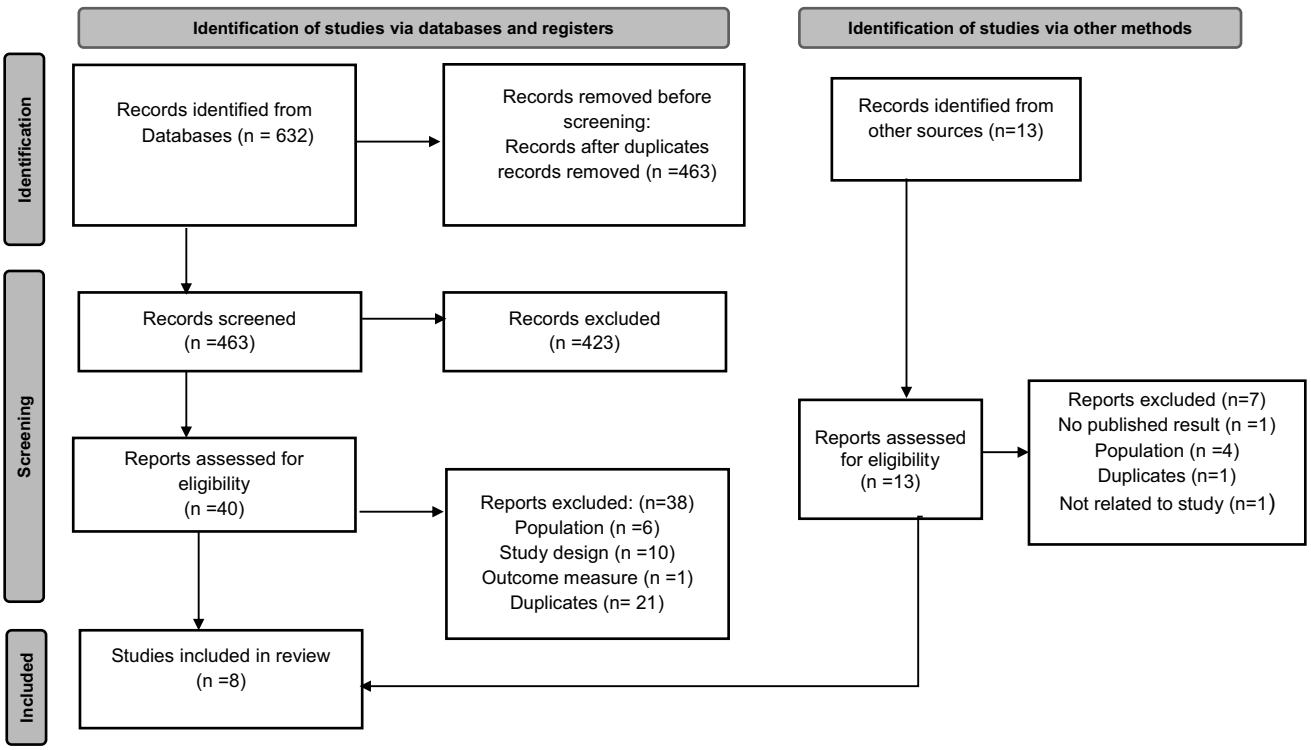

Fig. 1 PRISMA flow diagram for identification and selection of studies

result. In case of a mismatch between the results of the two researchers, the matter was discussed and solved amicably (Table 1).

Quality assessment

Physiotherapy Evidence Database (PEDro) scale was used to assess the methodological quality of each included study. The total score is calculated between 0 to 10 points except for criteria 1.This criterion (criterion 1) was related to the external validity of the studies and is not included in the calculation. Criteria 2–9 talk about the internal validity of the studies and criteria 10–11 help to determine whether studies have enough statistical information to make an interpretable result. Therefore, 10 out of 11 criteria were used to assess the quality of each article. Each criterion was rated as 'yes' (scored as 1) if it is fulfilled and 'no' (scored as 0) if it is not fulfilled. The maximum score was calculated by adding all the responses (maximum score = 10) [24]

Effect size

Effect size (Cohen's d) for each of the selected studies was also calculated (as shown in Table 2) to determine the significant difference between the post-treatment values of the experimental group (EG) and control group (CG) [25].

Results

Search results

A total of 632 studies were identified from various databases including (Pubmed: 242, Web Of Science: 92, Scopus: 11, CENTRAL:123, Science Direct: 159, PEDro: 5. An additional, grey literature sources including US National Institutes of Health database (clinical trials.gov) and Google scholar were also searched. After duplicates were removed 463 articles remained. Followed by title and abstract screening 423 articles were further excluded. Once the title and abstract were screened, 40 full-text articles were retrieved along with 13 additional articles identified from grey literature sources for full text screening, out of which eight articles met the inclusion criteria and were finally included for this review (7 RCTs and 1 is a prospective comparative pre-test, post-test study design) (shown in Fig. 1).

Study characteristics

Characteristics of the included studies are mentioned in Table 1. Studies included an overall 391 patients. Out of 366 were clinically diagnosed with type 2 DM and AC of the shoulder joint from the last ≥ 2 months (duration of AC symptoms mentioned in Table 1). The sample size ranged from 20 to 103 and the age of the patients lied in between 40 to 69 years. Follow up period for the included studies ranged from 0 to 12 weeks. Visual analogue scale (VAS), shoulder pain and disability index (SPADI), quick disabilities of arm shoulder and hand index (qDASH), disability of the arm shoulder and hand index (DASH), shoulder ROM (flexion, abduction, external rotation, internal rotation) were measured before and after the treatment in the included studies. Additional outcome measures assessed by some of the included studies (mentioned in Table1) are glucose triad (fasting and postprandial blood glucose levels (FBG, PPBG), HbA1c, global rating of change (GROC), pressure pain threshold, inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6). Duration of type 2 DM was reported in four studies ranged from 5 to 10 years, see Table 1. [26–29]. Method used to diagnose shoulder AC in the included studies mentioned in Table 3.

Quality assessment

PEDro criterion of scoring was used to assess the methodological quality of the included studies shown in Table 2. The PEDro score ranged from 5 to 9 and the average score was 5.2. The study was classified as poor if it scored less than 4, fair (scored 4–5), good (scored 6–8), and excellent quality if scored more than 8. Among them 5 studies were of good quality, 2 studies were fair while 1 of the study was of excellent quality.

Extracorporeal shockwave therapy specifications

Extracorporeal shockwave therapy specifications mentioned in Table 1. Radial type of ESWT was used for intervention in three studies [31, 33]. Two studies reported the use of a focused type of ESWT [26, 34].The type of ESWT was not reported in three studies [27–29]. In three studies [27, 28, 33] shockwave was applied on the most tender point closer to the insertion of the rotator cuff at the greater tuberosity of the humerus. One study [32] applied shockwaves on the anterior midline of the shoulder joint and two studies [29, 31] covered both anterior and posterior sides of the shoulder joint. While the patient was in a seated position when shockwave was applied [26–28, 31, 33]. In the rest of the three studies[29, 32, 34] position of the patient was not reported for shockwave application. The frequency of ESWT in the included studies ranged from 1 to 20 Hz in which two studies used frequency ranged from 1–15 Hz [27, 33], one study used 16–20 Hz [26], while the rest used 3 Hz [34], 8 Hz [32], 10 Hz [31] and 20 Hz [28]. Frequency was not reported in one study [29]. All studies used 2000 shockwave impulses except two studies in which one study used 1200 shockwaves [29] and the other study used 5 shockwave impulses [26].

Table 1 Characteristics of the included studies

Study	Description of study	Intervention and Comparison	Outcome measures	Key Findings	Effect Size
El Naggar et al. 2020 (Randomized clinical trial) [26]	n = 103 Type 2 diabetic subjects with AC for more than 3 months (6-30 months) Age(years) EG:55.9 ± 6.7; CG: 57.9 ± 9.0 M=24 ; F = 79 Duration of DM: NR Any method used to show AC pathological recovery after treatment: NR	CG: (n = 51) Single ultrasound-guided low-dose intra-articular corticosteroid Injection EG: (n=52) rESWT was applied for 12 weeks Frequency=10 Hz; EFD=NR; Impulses=2000; Sessions/week= 4 (1week apart) Both groups had to perform home based exercise protocol which includes passive stretching and pendulum exercises for shoulder.	qDASH VAS Flexion Abduction ER [Follow up: 0,4,8,12 weeks]	EG showed significant improvement in VAS (p = 0.025) and qDASH (p = 0.048). For ROM no significant difference (p > 0.05) was observed between both the groups. Is any adverse effect observed in EG after treatment: No adverse effect was observed	0.77 0.81 0.29 0.18 0.31
Elerian et al. 2021 (Randomized controlled trial) [27]	n = 48 Type 2 diabetic subjects with shoulder AC. Duration of symptoms not given. Age(years) EG:52.13 ± 3.06; CG: 51.33 ± 4.01 Duration of DM: NR Any method used to show AC pathological recovery: NR	CG: (n=24) Intra articular corticosteroid injection was given at 0,4,8th week) EG: (n=24) rESWT was given once a week for 12 weeks Frequency=8 Hz; EFD=0.06-0.14 mJ/mm2; Impulses=2000; Sessions/week=1 Both groups received traditional physiotherapy exercises and mobilization.	SPDI Flexion Abduction FBG (mg/dL) PPBG (mg/dL) HbA1C (mg/dL) [Follow up: 0,4,8,12 weeks]	Both groups improved (p < 0.001) after 8 & 12 weeks for all outcomes. Also FBG & PPBG decreased significantly (p < 0.001) in ESWT group. ESWT was more effective. Is any adverse effect observed in EG after treatment: No adverse effect was observed Adverse effects assessed through FBG, PPBG and HbA1c levels which gets improved in EG group.	2.57 3.69 2.76 2.20 3.73 2.10
Ebadh et al. 2021 (Randomized controlled trial) [28]	n = 60 Elderly type 2 diabetic males with mild to moderate AC. Duration of symptoms not given. Age(years) EG: 67.33 ± 1.78 CG:67.40 ± 1.56 M=60; F=0 Duration of DM: 10 years Any method used to show AC pathological recovery: NR	CG: (n=30) 20 minutes of PEMF was applied for 4 weeks EG(n=30) ESWT was applied on most tender point on affected shoulder twice a week for 4 weeks Frequency=20 Hz; EFD=NR; Impulses=2000; Sessions/week=2 Both groups received a therapeutic exercises and shoulder mobilization.	SPDI (pain scale part) SPDI (disability) Pressure pain threshold Abduction ER IR [Follow up: 0,4 weeks]	All outcomes improved significantly in both groups (p=0.0001) post treatment. However ESWT was more effective than PEMF Is any adverse effect observed in EG after treatment: NR	1.43 1.34 0.44 0.62 0.24 0.28
Hamed and Rahman 2006 (Randomized clinical trial) [29]	n= 40 Type 2 diabetic subjects with AC from past 3 months Age(years) : 50 ± 3.4 M= 12 ; F=28 Duration of DM: 10 ± 2.7 years Any method used to show AC pathological recovery: NR	CG: (n=20) 3 MHz ultrasound therapy was given. EG: (n=20) Therapeutic piezoelectric shockwave was given. Both groups received shoulder mobilizing exercises. Frequency=16-20 Hz; EFD=NR; Impulses=5; Sessions/week=3	VAS Flexion Abduction ER [Follow up: 0,8 weeks]	All outcomes showed significant improvement after treatment in both groups (p < 0.0001). EG has great effect on improving ROM & CG been more effective at pain reduction. Is any adverse effect observed in EG after treatment: NR	0.28 4.79 1.97 1.15

Table 1 (continued)

Study	Description of study	Intervention and Comparison	Outcome measures	Key Findings	Effect Size
Muthukrishnan et al. 2019 (Randomized controlled trial) [31]	n = 20 Type 2 diabetic subjects with AC from past 2 months. Age(years) EG:43.70 ± 10.4; CG: 45.5 ± 14.3 M=7 ; F=13 Duration of DM:NR Any method used to show AC pathological recovery: NR	CG: (n=10) Traditional conservative PT program (hot pack, ultrasound, mobilization and shoulder exercises) was given thrice a week for 4 weeks EG: f ESWT was given once a week for 12 weeks along with joint mobilisation and exercises Frequency=3 Hz; EFD=NR; Impulses=2000; Sessions/week=1	DASH. VAS GROC Flexion Abduction ER [Follow up: 0,12 weeks]	After treatment both groups showed significant improvement ($p < 0.05$) in all outcomes. But between group analysis showed significant ($p < 0.05$) improvement only in VAS in favor of ESWT. Is any adverse effect observed in EG after treatment: No adverse effect was observed	1.78 2.22 0.22 0.39 0.50 1.54
Ahmed et al. 2019 (Prospective comparative study, a pre- and post-test design) [32]	n= 50 Type 2 diabetic and non diabetic patients with AC from past 2 to 9 months Age(years) EG: 52.7 ± 7.8; CG: 52.4 ± 7.1 M=23 ; F=27 Duration of DM: 5 years Any method used to show AC pathological recovery: Blood samples were collected pre and post treatment to measure the effect of ESWT on inflammatory markers related to shoulder AC (CRP and IL-6)	Both groups (diabetic: n=25; non-diabetic: n=25) received ESWT once a week for 4 weeks along with therapeutic exercise program and shoulder mobilization. Frequency=1-15 Hz; EFD=0.22mJ/mm2; Impulses=2000; Sessions/week=1	SPDI VAS Flexion Abduction IR CRP(mg/L) IL-6(ng/ml) [Follow up: 0,4 weeks)	Significant changes was seen within the groups in all measured variables post treatment (p<0.05) but, non-significant difference was observed between the groups ($p > 0.05$). With greater improvement in non-diabetic group. Is any adverse effect observed in EG after treatment: NR	0.51 1.08 0.56 0.29 0.87 2.19 1.18
Adel et al. 2013 (Randomized clinical trial) [33]	n = 40 Type 2 diabetic subjects with AC from past 2 to 9 months. Age(years) EG:45.33 ± 8.64; CG: 46.26 ± 8.05 M=19 ; F=21 Duration of DM: NR Any method used to show AC pathological recovery: NR	CG: (n=20) 5 minutes phonophoresis was given along with general exercise program with shoulder mobilization. EG: (n=20) Similar exercise program along with rESWT for 4 weeks Frequency=1-15 Hz; EFD=0.22mJ/mm2; Impulses=2000; Sessions/week=1	SPDI VAS Flexion Abduction IR [Follow up: 0,4 weeks]	Between groups analysis showed significant improvement post intervention more in favor of EG. VAS (p = 0.006), SPADI (p = 0.01), ROM ($p < 0.05$). rESWT was more effective than control treatment Is any adverse effect observed in EG after treatment: NR	1.01 1.08 1.35 1.26 1.05
Seyam et al. 2018 (Randomized clinical trial) [34]	n = 30 Type 2 diabetic subjects with AC from past 6 months Age(years) EG:45.34 ± 8.7; CG: 46.26 ± 8.1 M=19; F=11 Duration of DM: 10 years Any method used to show AC pathological recovery: NR	CG: (n=15) 3MHz ultrasonic therapy, Infrared therapy EG: (n=15) ESWT was given twice a week for 5 weeks Frequency=NR; EFD=0.2mJ/mm2; Impulses=1200; Sessions/week=2 Both groups received therapeutic exercises	SPADI Flexion Abduction ER [Follow up: 0,5 weeks)	Significant improvement for SPADI ($p < 0.01$) and ROM ($p < 0.05$) after treatment between the groups. ESWT with therapeutic exercises was better than CG Is any adverse effect observed in EG after treatment: NR	1.007 1.34 1.26 1.05

CG Control group; *EG* Experimental group; *PEMF* Pulsed electromagnetic field therapy; *r ESWT* Radial Extracorporeal shockwave therapy; *f ESWT* Focused Extracorporeal shockwave therapy; *SPDI* Shoulder pain and disability index; *q DASH* Quick disability arm, shoulder and hand; *VAS* Visual analogue scale; *AROM* Active range of motion; *GROC* Global rating of change; *CRP* C- reactive protein; Interleukin-6; *M* male; *F* female; *DM* diabetes mellitus; *AC* adhesive capsulitis; *NR* not reported

Table 2 Quality Assessment Pedro scale

Author	Eligibility criteria	Randomization	Concealed Allocation	Baseline Comparability	Blinding of subjects	Blinding of therapist	Blind assessment	Adequate follow up	Intention to treat analysis	Between Group comparison	Point estimates	Total Score	Quality
Naggar et al. 2020 [26]	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	8/10	Good
Elerian et al. 2021 [27]	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	9/10	Excellent
Ebadh et al. 2021 [28]	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	6/10	Good
Hamed and Rahman 2006 [29]	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	6/10	Good
Muthukrishnan et al. 2019 [31]	Y	Y	N	Y	N	N	N	Y	N	Y	Y	5/10	Fair
Ahmed et al. 2019 [32]	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5/10	Good
Adel et al. 2013 [33]	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	6/10	Good
Seyam et al. 2018 [34]	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	7/10	Good

Note: *Y* yes; N:

Energy flux density (EFD) of 0.22 mJ/mm2 was reported in three studies [27, 29, 33] and one study [32] used a range of 0.06–0.14 mJ/mm2. EFD was not reported in four studies [26, 28, 31, 34]. No adverse effect was observed after ESWT treatment in three studies [26, 27, 31]. While in rest of the five studies any information about adverse effects of post treatment was not reported. The follow up period and number of sessions per week in included studies was mentioned in Table 1.

Result of individual studies

Effect on pain

All the included articles assessed pain. Four studies (Adel et al., 2013; Hamed & El-Rahman, 2006; Muthukrishnan et al., 2019; Seyam et al., 2018) [26, 29, 33, 34] compared ESWT with ultrasound therapy. Out of these four studies, three studies used VAS (Visual analogue scale) and one study (Seyam et al., 2018) used a total score of SPADI (shoulder pain and disability index) to assess pain outcome. Three studies (Adel et al.,2013; Seyam et al., 2018;Muthukrishnan et al.,2019) [29, 33, 34] reported large to very large treatment effects on pain by Cohen's d = 1.08, 1.007, and 2.22 respectively. Whereas one study by Hamed & El-Rahman. (2006) [26] showed small to medium treatment effects for ESWT application compared to ultrasound therapy (Cohen's d = 0.28).

In two studies El Naggar et al. (2020) and Elerian et al. (2021) [31, 32] compared the effectiveness of ESWT with intra-articular corticosteroid injections and found shockwave therapy group improved better in terms of pain relief after 12 weeks of intervention with large to very large treatment effects (Cohen's d = 0.81, 2.57). This was followed by the use of VAS and SPADI as a tool to assess pain.

Ebadh et al. (2021) compared ESWT with pulsed electromagnetic field therapy (PEMF) in sixty diabetic elderly patients followed by the estimation of pain severity assessed by the pain scale part of SPADI and pressure pain threshold was also assessed by the algometer. They found their results to have a very large treatment effects on pain severity (Cohen's d = 1.43) and a small to medium treatment effects (Cohen's d = 0.44) on pressure pain threshold in these patients. Ahmed et al. (2020) compared diabetic and non diabetic patients with AC and found that ESWT was effective in both the groups for pain reduction which was evaluated using VAS. Large improvement (Cohen's d = 1.08) was seen in non diabetic group [27].

Based on the results, the majority of the studies proved that ESWT is very effective in managing pain when compared to control treatment groups. Though only one study Hamed & El-Rahman. (2006)[26] showed small to medium treatment effects on pain. Thus, it was concluded that ESWT

Table 3 Method used for the diagnosis of AC

Study	Method used for the diagnosis of AC.
Naggar et al. 2020 [26]	AC was diagnosed on the basis of history, physical examination and radiographic evaluation present with shoulder pain and restricted ROM.
Elerian et al. 2021 [27]	Diagnostic criteria described by Lebiedz and Kay 2010 [30] as the patient present with painful restricted ROM mainly in abduction and external rotation and HbA1c levels in either type of DM not related to the presence of shoulder AC.
Ebadh et al. 2021 [28]	AC was diagnosed by physical examination and MRI by an orthopedic surgeon. Patients recruited from outpatient clinic.
Hamed and Rahman 2006 [29]	Patients were diagnosed on the basis of X-ray (antro-posterior and lateral views) and clinical examination (pain evaluation by VAS and shoulder ROM by manual goniometer for shoulder flexion, abduction and external rotation).
Muthukrishnan et al. 2019 [31]	Diagnosed on the basis of MRI findings and physical examination for pain and restricted ROM. Patients recruited from outpatient clinic.
Ahmed et al. 2019 [32]	AC diagnosed on the basis of clinical features including severe limitation in joint ROM during passive and active movement examination, exaggerated pain particularly during evening time.
Adel et al. 2013 [33]	AC was diagnosed on the basis of clinical examination and radiological findings by an orthopedic surgeon
Seyam et al. 2018 [34]	Method of diagnosis for shoulder AC is not reported in this study. Patients recruited from outpatient clinic.

is effective in reducing pain in diabetic patients with shoulder AC. Refer to Table 1.

Effect on range of motion

Among the included studies, four studies (Adel et al.,2013; Ahmed et al., 2020; Elerian et al., 2021; Seyam et al., 2018) [27, 29, 32, 33] used electro-goniometer, three studies (Ebadh et al.,2021; Hamed & Rahman,2006; El Naggar et al.,2020)[26, 28, 31] used universal goniometer as a tool to assess shoulder ROM while one study Muthukrishnan et al. (2019) [34] did not mention the type of tool used to assess ROM. Five studies evaluated flexion, abduction and external rotation (ER) in which four studies (Muthukrishnan et al.,2019; Seyam et al., 2018; Elerian et al.,2021; Hamed & Rahman, (2006) [26, 29, 32, 34] displayed medium to very large clinically important treatment effects in the experimental groups (Cohen's d = 0.39, 0.50, 1.54; 1.34,1.26, 1.05; 3.69, 2.76; 4.79, 1.97, 1.15). Whereas one study, El Naggar et al. (2020) [31] reported small to medium treatment effect (Cohen's d = 0.29, 0.18, 0.31) respectively.

In the studies done by Ahmed et al. (2019) and Adel et al. (2013)[27, 33] both utilized electro goniometers to assess flexion, abduction, and internal rotation (IR) ROM of the shoulder. The result from the data of these studies showed medium to large and very large treatment benefits in ROM improvement and gave values (Cohen's d = 0.56, 0.29, 0.87; 1.35, 1.26, 1.05) respectively. Whereas, a study done by Ebadh et al. (2021) [28] assessed abduction, ER and IR showed medium to large effects in abduction (Cohen's d = 0.62) and small to medium treatment effects in ER and IR (Cohen's d = 0.24, 0.28).Therefore, based on the result, the majority of the studies showed an overall improvement in shoulder ROM after ESWT in diabetic patients with AC. Refer to Table 1.

Effect on disability

All the included studies assessed disability except one study Hamed & El-Rahman. (2006) [26]. Five out of seven studies (Ebadh et al.,2021; Elerian et al., 2021; Ahmed et al., 2019; Adel et al.,2013; Seyam et al., 2018) [27–29, 32, 33] used SPADI. Among these studies, Ebadh et al. (2021) [28] and Elerian et al. (2021) [32] reported very large treatment effects of the intervention with Cohen's d = 1.34 and 2.57 respectively. While Adel et al. (2013) and Seyam et al. (2018) [29, 33] showed large treatment effects (Cohen's d = 1.01 and 1.007). Study done by Ahmed et al.(2020) [27] reported medium effects (Cohen's d = 0.51).Two studies Muthukrishnan et al. (2019) and El Naggar et al. (2019) [31, 34] used DASH and qDASH as their outcome measure and displayed large to very large treatment effects on disability as evidenced by Cohen's d = 0.77, 1.78 respectively. Therefore, from the result, it can be concluded that ESWT is effective in reducing disability in AC associated with DM in the material of the outcome measures. Refer to Table 1.

Additional outcome measures

Elerian et al. (2021) [32] assessed FBG, PPBG, and HbA1c levels both in the experimental and control group and found a significant decrease ($p < 0.001$) in the levels of these outcomes in the experimental group with very large treatment effects (Cohen's d = 2.20, 3.73, 2.10) after 8 and 12 weeks of

intervention. While comparing type 2 diabetic and non-diabetic patient Ahmed et al. (2020) [27] reported a significant decrease in levels of inflammatory markers (CRP, IL-1) after 4 weeks of ESWT administration in both the groups and displayed the respective values of Cohen's d = 2.19, 1.18. Whereas, Muthukrishnan et al. (2019) [34] also assessed GROC and showed a small treatment effect (Cohen's d = 0.2). Even though only a few studies showed improvement in outcomes related to glucose triad and inflammatory markers, ESWT appears to have some beneficial effects on glucose tolerance and inflammation in type 2 diabetic patients with AC. In all the included studies along with ESWT, both groups (EG and CG) received physiotherapeutic exercise programs and manual mobilization for shoulder joints except one study El Naggar et al. (2020) [31] in which, patients in both the groups performed a home-based exercise program without manual mobilization. Refer to Table 1.

Discussion

The purpose of this systematic review was to investigate the effectiveness of extracorporeal shockwave therapy (ESWT) on adhesive capsulitis (AC) in type 2 diabetes mellitus patient population concerning the improvement in shoulder pain, ROM, and disability. Findings from this review directed us more toward the improvement in outcomes measures assessed after ESWT administration in most of the included studies.

Of the eight included articles, seven had proved that ESWT is beneficial in reducing pain as reported by their large to very large treatment effects except for one study Hamed & Rahman. (2006)[26] provided small to medium treatment effects which may be due to the difference in ESWT dosage used. One study by Ebadh et al. (2021) [28] also assessed pressure pain threshold in diabetic elderly patients and showed small to medium treatment effects which may be related to, two important factors i.e., age of the patients and hyperglycemic state in DM as explained by Morley.(1998) as these two factors are associated with each other for a decreased pressure pain threshold [35].The analgesic effects of ESWT comes in agreement with Simplicio et al. (2020) who reported that shockwaves induced pain relief by the over-stimulation of the pain-carrying nerve fibers so that, inhibiting pain impulse conduction along with physical and chemical alterations in pain receptors and neurotransmitters [36]. Schmitz. (2010) found that, when ESWT was used, it leads to a sufficient decrease in substance P in the treatment area and decrease the formation of this molecule in dorsal horn cells of the spinal cord [37].

For ROM and disability, the result from the majority of the included studies indicates medium to large and very large treatment effects except for El Naggar. (2020), Ahmed et al. (2020), and Ebadh et al. (2021) [27, 28, 31] showed small to medium treatment effects. This disparity might be due to the difference in the type of outcome measures or assessment tool used. In study done by El Naggar. (2020) patients performed a home based exercise program. In shoulder AC a decreased ROM and functional disability is a consequence of pain and stiffness, therefore we might extrapolate that the reduction in pain after ESWT combined with shoulder exercises and mobilization was responsible for the improvement in shoulder ROM and disability. This assumption has been supported by a qualitative study [11] conducted on shoulder AC patients, where they stated that if the pain was treated effectively, these patients were better able to recover with their reduced ROM, and functional disability and engage more with physical therapy.

Various other systematic reviews and meta-analysis also reported positive effects of ESWT on pain and functional disability in the different population such as knee osteoarthritis [38], chronic low back pain [18], calcific tendinitis of the shoulder [39, 40], subacromial impingement syndrome, lateral and medial epicondylitis of the elbow [41], various tendinopathies of the lower limb [42], spasticity [43], diabetic foot ulcers [44], osteonecrosis and fracture healing [15, 45]. The articles rated in most of these reviews were of moderate to good quality. Our study also included articles of good quality, though one article included was of fair quality and one reported as excellent quality according to PEDro scoring. During our thorough search of databases, we came across two recent systematic reviews and meta-analysis [21, 22] which evaluated the efficacy of ESWT on primary AC of the shoulder in non-diabetic patients and showed clinically meaningful treatment effects in managing pain and shoulder ROM. Whereas, Cao et al. (2019) [20] is a registered protocol of a systematic review of randomized controlled trials (RCTs) with the unpublished result. However, the population used in our systematic review was different since we used only type 2 DM patient population.

Reduction in inflammatory markers in diabetic patients reported in one of the study Ahmed et al. (2020) [27] with large to very large treatment effects after ESWT application, may be explained by some recent animal studies [46–48] who suggested that shockwaves improves blood flow in the treatment area, stimulates growth factor, improves the elastic property of collagen fibers, reactivates healing and thereby decrease inflammatory markers and cytokines. Similarly, the reduction in FBG, PPBG, and HbA1c levels reported by Elerian et al. (2021) [32] in the experimental group was supported by the findings of a recent animal study [47] which claimed that shockwaves had some direct physiological effects on insulin production, blood glucose levels, and HbA1c in the treatment area where the shockwaves was applied.

Up to the best of our knowledge, this is the first systematic review that investigated the effectiveness of ESWT on

shoulder AC in the type 2 DM patient population. Also, this review focused on the clinically meaningful outcome measures related to pain, disability, and shoulder ROM. A thorough comprehensive literature search was performed on six internationally esteemed academic databases along with grey literature sources. Any disagreement was settled by dialogue. The methodological quality of included studies was assessed using the PEDro scale. PRISMA 2020 guidelines were followed and PROSPERO registration was done to ensure authenticity.

Most included trials lack long-term data that could only provide short-term (up to 12 weeks) effects in all the comparisons. The included studies assessed the combined effect of ESWT with therapeutic exercises and shoulder mobilization along with the absence of blinding of participants and therapists except in one study by Elerian et al. (2021)[32] therefore it is difficult to conclude as the combined treatment protocols were given.

More trials need to be done on the effectiveness of ESWT on shoulder AC focusing on type 2 DM patient population and other types of diabetic patient population like type 1 DM should also be considered in future studies. Elerian et al. (2021) and Ahmed et al. (2019) [27, 32] reported improvement in blood glucose levels and inflammatory markers associated with AC after treatment with ESWT and found a significant difference between their results, therefore to check the direct effects of ESWT, future studies focus on these outcomes should also come up. Finally, well-designed, long term follow up trials were needed to determine the sustainability of intervention effects with a standard ESWT protocol.

The findings of this review revealed that, type 2 diabetic patients with shoulder AC can take a clinical advantage of ESWT as a safe and non invasive treatment modality without any adverse treatment effects. Due to the limited number of trials included in this review and their heterogeneity in ESWT parameters (frequency, shockwave impulses, energy flux density and number of sessions per week) it is therefore difficult to come up with a standard protocol. But as per the findings of this review six out of eight studies used 2000 shock wave impulses. Frequency of ESWT in the included studies ranged from 1 to 20 Hz and EFD ranged from 0.06 to 0.22 mJ/mm2. Number of sessions per week was ranges from 1 to 4. Most of the type 2 diabetic patients in the included studies are in their painful phase (> 2 months; see Table 1) of shoulder AC. Applying the findings of this review in clinical practice, ESWT can be an effective treatment modality for shoulder AC in type 2 DM patients for short terms goals (4 to 12 weeks) within the given ranges as the result shows significant clinical improvements. Findings of this review high-lightened that ESWT proved to be beneficial in pain reduction followed by disability and ROM improvement with large to very large treatment effects in type 2 DM patient population.

Conclusion

Based on the findings of the present review, ESWT appears to be effective in shoulder AC for type 2 DM patient population in terms of pain relief, disability, and ROM improvement within the given ranges. Owning to heterogeneity regarding ESWT parameters the conclusion remained blurred for a standard ESWT protocol. Also, the evidence is not enough to confirm the effectiveness of ESWT on blood glucose levels and inflammatory markers in type 2 diabetic patients (it was only reported by two studies). Further, studies of high methodological quality with a standard ESWT protocol are needed to elucidate the clinical effectiveness of ESWT on these outcomes in type 2 DM patient population with shoulder AC.

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POSITION STATEMENT

Diabetic retinopathy screening guidelines for Physicians in India: position statement by the Research Society for the Study of Diabetes in India (RSSDI) and the Vitreoretinal Society of India (VRSI)-2023

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Abstract
Diabetic retinopathy (DR) is a leading cause of blindness among working-age adults worldwide. India is the diabetes capital of the world and one in five adults is said to have diabetes in India. With the increase in diabetes, there is an increasing burden of diabetic retinopathy (DR). All patients with diabetes have a risk of losing vision due to DR. The prevalence of diabetic retinopathy is 12.5%; out of which, 4% are said to have vision-threatening diabetic retinopathy (VTDR) The early stages of DR are symptomless, necessitating a proactive screening for an early detection of DR in all people with diabetes before they develop VTDR. This is a position statement jointly developed by RSSDI (Research Society for the Study of Diabetes in India) and VRSI (Vitreo Retinal Society of India) to provide guidelines for Physicians on DR screening in India. These guidelines emphasize the need for regular DR screening of all people with diabetes. It is recommended that the Physicians establish an effective DR screening model in their clinics, eg., a non-mydriatic fundus camera utilizing artificial intelligence (AI) algorithms for fundus photography to identify referral or non-referral DR. This will facilitate early detection and timely referral to an ophthalmologist thereby preventing VTDR. The need to create public awareness regarding blindness due to DR and a collaboration between Physicians and ophthalmologists for the management of diabetes, opportunistic screening of DR, and timely management of DR may play a crucial role in decreasing the burden of blindness secondary to diabetes.

Keywords Diabetic retinopathy · Screening guidelines · Physician · India

Introduction

India has 101 million people with diabetes, and these numbers are predicted to increase to 125 million by 2045 [1, 2]. It is estimated that one in five adults will have diabetes in India [2]. Majority are said to have type-2 diabetes during their working age impacting their work and family and causing an economic burden on the country.

Diabetes being a chronic disease has several associated systemic complications including DR which affects the

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vision. DR can be asymptomatic in the early stages, and if not detected and managed on time, it may progress to VTDR. The prevalence of diabetic retinopathy is 12.5%; out of which, 4% are said to have VTDR [3]. In order to timely identify patients with VTDR, we are required to actively screen all the people with diabetes. This is a huge task in a low-income country like ours where the resources are limited. Thus, there is a need for opportunistic screening for DR done at Physician clinics, which are the first point of contact for any patient with diabetes. Therefore Physicians can play an extremely important role in spreading awareness regarding various complications of diabetes including retinopathy and also do an early screening for DR. This manuscript provides guidelines for DR screening by the Physicians. The diagnosis of DR is possible with imaging and thus the various models available to the Physicians to develop the facility of DR screening in their own clinics are also highlighted.

Who are at risk of developing DR and the timing of the first screening for DR?

Individuals with diabetes are at risk of developing diabetic retinopathy (DR). The onset and progression of DR can be influenced by the duration of diabetes. It is important to note that people with type 2 diabetes may not be aware of the exact duration of their condition. Therefore, it is recommended that individuals with type 2 diabetes undergo screening for DR at the time of diagnosis.

According to the International Society of Pediatric and adolescent diabetes (ISPAD) guidelines in type 1 diabetes, it is recommended to start DR screening after 11 years of age with 2–5 years of duration of diabetes This timeframe allows for the identification of any early signs of retinopathy [4].

For women with known diabetes who are planning to conceive, it is crucial to assess their risk of developing DR. Therefore, it is recommended that they undergo their first screening for DR prior to conception. Additionally, it is advised for these women to have a follow-up screening during the first trimester of pregnancy to monitor any changes in their retinal health.

Why patients with diabetes have to be screened for diabetic retinopathy?

Patients with diabetes should be screened for diabetic retinopathy (DR) for several important reasons. The SMART India Study was conducted in 10 Indian States and one union territory, involving over 6,000 patients with diabetes aged 40 years and above who had gradable retinal images. The study found that 12.5% of people with diabetes had any grade of DR, and 4% had VTDR. This translates to approximately 3 million people in India who are at immediate risk of vision loss due to DR [3].

Unfortunately, the early stages of DR are asymptomatic, and once vision loss occurs, it is usually not completely reversible. Therefore, it is crucial to screen for DR after 2–5 years of type-1 and at the time of diagnosis of diabetes type-2, in order to detect it at an early stage and prevent the development of VTDR.

Role of Physicians in DR screening

Physicians have a critical role in the sensitization and screening for DR as they are usually the first healthcare providers that people with diabetes encounter. Physicians can play an extremely important role in creating awareness of the risk of vision loss due to DR. It needs to be highlighted that DR being asymptomatic in early stages may progress silently and thus the need for regular screening for DR. It is important to educate the diabetics visiting the clinic of the Physicians regarding the various complications of diabetes such as heart failure, neuropathy, nephropathy including retinopathy requiring regular screening to avoid permanent loss of vision by putting up charts, providing patient education pamphlets and having counselors in the clinic.

Patients being asymptomatic are reluctant to go to an ophthalmologist for a preventive eye check-up when they have good vision and go only when they start losing vision secondary to VTDR [3]. Physician clinics can provide an opportunistic screening for DR, just like how they would do a blood investigation for their diabetic patients. This will be a very important value addition to the services offered to the patients. This will ensure that patients having DR will be identified for an early referral and also will avoid unnecessary visits to an ophthalmologist when eyes have no evidence of DR. This will also help to reduce the unnecessary overload of patients with no DR being referred to an ophthalmologist.

DR screening guidelines for a Physician

It is important for Physicians to be aware of the screening guidelines for DR. Any patient with diabetes should undergo at least one eye exam every year irrespective of the glycemic control and the duration of diabetes [4]. The DR screening guidelines depend on the type of diabetes and the stage of DR at the time of screening (Table 1). This is based on the International clinical DR and diabetic macular edema (DME) severity scale [5]. However, the screening interval

Table 1 Diabetic retinopathy screening guidelines for a Physician

Type 1 diabetes mellitus	Initial eye examination recommended 5 years following diagnosis of type 1 diabetes mellitus
Type 2 diabetes mellitus	Initial eye examination is recommended at the time of diagnosis of type 2 diabetes mellitus
Pregnancy in patients with diabetes mellitus	• Eye examination prior to conception and early during 1st trimester • Follow-up should be individualized based on severity and recent changes in retinopathy
Diabetic retinopathy severity	
No apparent DR	Annual fundus photo screening at Physician clinic
Any evidence of DR or DME	Immediate referral to an ophthalmologist

DR diabetic retinopathy, *DME* diabetic macular edema

should be individualized based on the patient's systemic status including glycaemic control, blood pressure, lipid levels and other comorbidities (Fig. 1).

DR screening models for Physician clinics

Developing a DR screening model at a Physician's clinic has become easy and economical. DR diagnosis can be achieved through image analysis or AI-based algorithms which requires basic resources such as a fundus camera, a trained technician to capture high-quality retinal images, and an internet connection.

In India, options are available to enhance your skill in fundus examination and fundus photography through certified courses on DR, e.g., courses offered by Indian Health Outcomes,Public Health and Economics Research Centre (IHOPE) [6].

Screening models for diabetic clinics where there is no facility for screening to be done by an ophthalmologist

Direct ophthalmoscopy by the Physicians

Physicians and primary health care providers may use a direct ophthalmoscope (DO) for DR screening. Most of the doctors are trained in this technique during their undergraduate training [7].

Tele-screening using portable or non-portable fundus cameras

A retinal fundus camera is used to take photos of the retina through an undilated (non-mydriatic) pupil that can be used to capture retinal images at Physician clinics. Details of the different types of fundus cameras available in the market utilized for DR screening are provided in Table 2. With simple

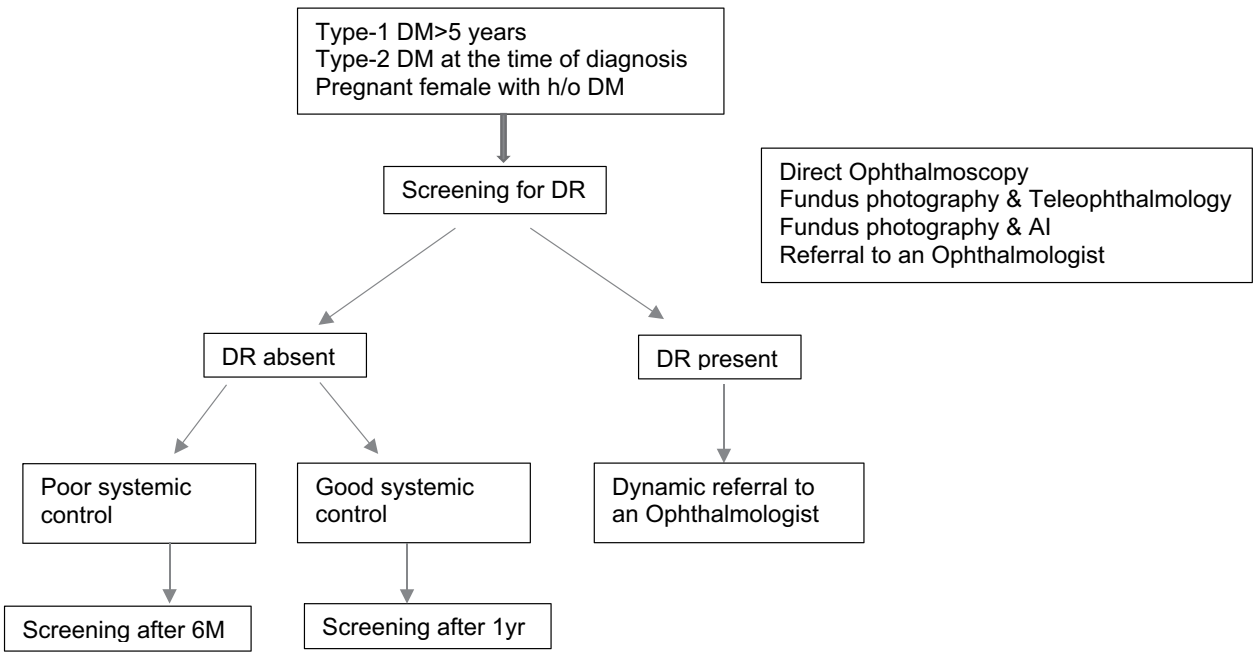

Fig. 1 Flow chart for DR screening at a Physician clinic

Table 2 Different types of Fundus cameras utilized for the DR screening

Fundus cameras	Advantage	Disadvantage
Mydriatic desktop fundus cameras	30 to 45° FOV, can cover up to 75° FOV with montage, stereo images of various fields, excellent resolution, seven-field stereo photography, easy to use with training, good image quality, good for hospital usage, better sensitivity and specificity for detecting DR by ETDRS fundus photograph grading than DO or IDO exam	Requires mydriasis, expensive, time-consuming procedure for training photographers and graders
Commercially available cameras	Carl Ziess FF 450 plus, 3 Nethra-Forus (Model: Flora)	
Non-mydriatic Fundus camera	45° FOV, Autofocus, auto alignment, user interphase software, task automation, storage of images, and export of images to database and facility to share retinal images through a network or internet connection.	Ungradable photographs and low sensitivity particularly in Indian eyes with dark iris, in cases of cataract or small pupil size
Commercially available	Zeiss Ciruss 600, Crystal Vue NFC-700, 3 Nethra-Forus (Model: Classic), Canon CR-2 &AF, Centre Vue DRS, Topcon TRC NW 400, AFC-230, Nidek, gamagori, Japan	
Ultra-wide field Fundus camera	Very wide field of view up to 200° without dilatation, can detect peripheral DR lesions	Very expensive, cannot be used for mass screening unless the cost gets reduced
Commercially available	Optos, Optomap/Daytona Staurenghi lens (Ocular Staurenghi 230 SLO Retina Lens), Ocular Instruments Inc, Bellevue, WA, USA)' Pomerantzeff camera, Retcam (Clarity Medical Systems, Inc., Plea santon, CA, USA), ZEISS Clarus 500 (Zeiss, Carl Zeiss Meditech, Inc., Dublin, USA)	
Handheld	Handheld digital cameras are portable, require less space, minimum power consumption and less skills and training	Image quality may be inferior without mydriasis, especially for older people with cataracts
Commercially available cameras	Zeiss Visuscout 100 (Carl Zeiss, Jena, Germany), Smartscope Pro (Optomed, Oulu, Finland) Volk Pictor Plus (Volk Optical, Mentor, OH, USA), VersaCam TM DS-10 (Nidek, Gamagori, Japan), Horus DEC 200 (MiiS, Hsinchu, Taiwan), Genesis-D (Kowa, Nagoya, Japan) and Optomed Aurora	
Smartphone-based fundus camera	Smartphones are universally available and these smartphone-based fundus cameras are cost-effective alternative options for traditional fundus cameras.	Require mydriasis
Remidio fundus on phone (FOP)(Remidio Innovative Solutions,Bangalore,India)	US FDA-approved validated retinal imaging system, mydriatic and non-mydriatic and anterior imaging module, 45° FOV (M) with ± 20D adjustment with 12x optical magnification, 40° FOV (NM) and 10X magnification, Tabletop model or handheld mode and can also be attached to a smartphone	
Made in India retinal camera(MII Retcam, India)	Capable of visualizing even peripheral regions of the retina up to pars plana along with the posterior pole, provides video and still image	Require mydriasis
Other Smartphone-based validated fundus cameras are available	Peek Retina(Nesta, London, UK), PanOptic + iExaminer (Welch Allyn, Skaneateles Falls, NY), D-EYE, Padova, Italy	Peek vision device requires mydriasis, PanOptic and D-Eye and have limited FOV 25° and 20

training, any clinic assistant or optometrist (if available) can capture retinal images using these fundus cameras. These images can be stored and forwarded online to remote ophthalmologists or trained graders (today very few are available) [8]. The retina specialist will review the images, grade them for DR severity and generate a report to be sent back to the Physician's office. This whole process may take a few hours to a few days depending on the resources available (Fig. 2).

Tele-screening using portable or non-portable fundus cameras using AI

Artificial Intelligence (AI) is playing a major role in DR screening. AI is able to grade the retinal images equivalent to a retina specialist and can identify referable DR and not referable DR. More importantly, it can grade and generate a report almost instantaneously. The patient will be able to get his eye report within a few minutes and the Physician can advise the patient accordingly [9].

The sensitivity and specificity of a few commercial automated DR grading software are given in Table 3 [10–13].

The report is provided to the patient along with a disclaimer that it is a screening test with a specific sensitivity and specificity and cannot be used for any medicolegal purpose. The main purpose of the screening test and the automated report is to alert the patients in case any evidence of DR is found so that he or she can be timely referred for a detailed evaluation and further management by an ophthalmologist. This may ensure better compliance for DR screening and reduce the risk of vision loss due to DR.

Physician clinics with an ophthalmology referral facility can ensure timely diagnosis and management of DR.

DR screening as a part of diabetes care

– Incorporation of DR screening as a standard of care for diabetes with regular referral to an ophthalmologist for DR screening.
– The second option is that instead of sending every patient to the Ophthalmologist for DR screening, an in-clinic screening can be done using a fundus camera with AI or without AI using teleophthalmology.
– If the patient has a referral DR, then is sent for further evaluation and management to the in-house ophthalmologist.

Community-based DR screening

DR screening during diabetes screening

Non-Governmental Organizations (NGOs), diabetic clinics and corporate offices conduct annual health check-up programs or diabetes screening programs annually [14]. DR screening can be included as a part of the package. These days, corporate screening programs are also implemented in offices where a sedentary lifestyle and work stress increase the risk of diabetes and DR at a younger age, and these camps can help in opportunistic screening for DR.

Risk-based DR screening

Risk-based screening (age, duration of diabetes, blood sugar levels, cholesterol levels, blood pressure levels) would be feasible, cost-effective, and safe, and the screening can be individualized based on the risk score.

The All India Ophthalmological Society (AIOS) task force committee and Vitreo Retinal Society India (VRSI) recommends diabetic retinopathy (DR) screening for individuals with known diabetes who are receiving treatment and have a random blood sugar (RBS) level of ≥ 200 mg/dl (≥ 11.1 mmol/l), or have glycosylated hemoglobin (HbA1C) levels of > 6.5% (48 mmol/l). Screening is also necessary for females with type-1 diabetes and gestational diabetes when they first report to a healthcare provider [15].

Fig. 2 DR screening using Teleophthalmology

Table 3 Sensitivity and Specificity of the commercial automated DR grading algorithms

Few commercial automated DR grading software	Fundus images involved	Sensitivity and specificity
IDx-DR with Topcon Fundus camera (US FDA-approved AI algorithm, on April 2018)	819 participants' fundus images	The sensitivity and specificity of the technology were 87.2% and 90.7% respectively for detecting more than mild DR.
Retmarker	102,856 fundus images	Compared to arbitrated human grading results. The sensitivity for retmarker in detecting DR was 73.0% for any retinopathy, 85.0% for referable retinopathy and 97.9% for proliferative retinopathy, and false positive rate was 47%,
EyeArt (Eyenuk Inc., based in Los Angeles, USA), (US FDA approved in 2020)	A total of 915 participants' fundus images	96% sensitivity and 88% specificity for detecting mild DR and 92% sensitivity and 94% specificity for detecting vision-threatening DR
Google AI (Google Inc.)	Training-103,634 images Validation of 5764 images from both sites	In INDIA, two centers dataset was used: Sankara Nethralaya Sensitivity, 92.1%; Specificity, 95.2%; AUC, 0.980; Aravind Eye Hospital Sensitivity, 88.9%; Specificity, 92.2%; AUC, 0.963

PM-JAY DR guidelines for a Physician

Ayushman Bharat Pradhan Mantri Jan Arogya Yojana (AB PM-JAY) is a Centre and state co-sponsored health insurance/assurance scheme providing healthcare services as per predefined packages to about 500 million population based on specific eligibility. This scheme is implemented by the National Health Authority (NHA) in partnership with state health agencies through 28,000 empaneled facilities (both government and private) across India except 3 states (Delhi, West Bengal, and Odisha).

In April 2022, NHA released the HBP 2022 which includes the DR screening package including—refraction, fundus photo and optical coherence tomography (OCT). This package has a cross-speciality model with reimbursement applicable to screening both by a Physician and an ophthalmologist [16].

Role of metabolic control in the management of DR

Good metabolic control including blood sugar level, blood pressure and dyslipidemia retards the progression of DR

- Higher initial levels of HbA1c increase the risk of DR. Intensive glycemic control (HbA1c < 7%), especially in the early stages of onset of diabetes, has a profound impact on the progression of DR and reduces the risk of developing DR by 27% [17].
- Control of high lipids reduced the risk of developing hard exudates and decrease the associated vision loss [18].
- In type-2, there is a decrease in DR by 31% for every 1% decrease in HbA1c and a decrease in vitreous hemorrhage by 11% for every 10 mmHg decrease in systolic blood pressure [19]. It is recommended that diabetics with hypertension regularly monitor their blood pressure and keep it below 140/80 mm Hg.

Quality assurance standards for DR screening

Quality assurance should be a part of any DR screening program in order to achieve maximum benefit [20].

1. Informed consent before capturing the retinal images
2. A good quality, affordable, sleek, easy-to-operate fundus camera that can provide high-resolution images with a wide field of view (130–200°) would be ideal.
3. Two images of the retina per eye (one macula-centered and one optic disc-centered image) would be useful to ensure that DR is not missed. An additional image of the anterior segment of the eye would provide additional information regarding media opacity/cataract.
4. Although non-mydriatic fundus cameras are used for screening, mydriasis using dilatation eye drops improves the gradeability of the images and reduces the number of ungradable images.
5. The grading of the retinal images should be done by certified human graders.
6. AI software used should be validated and approved for DR detection [21]. A disclaimer should be there with the report that it is a screening test and not for medicolegal purposes. AI grading of DR can be utilized as

an assistive tool, with the final grading of DR to be determined by a doctor.
7. The DR screening reports should be promptly available to the patient when having a follow-up check-up with the Physician to ensure a timely referral to an ophthalmologist.
8. Proper backup and storage of the retinal images and DR diagnosis data is essential for follow-up. Preferable if there is an inbuilt alert system in case a patient misses his or her screening visit.
9. There should be a recall system where individuals with diabetes who have been screened the previous year are called back for DR screening/retinal imaging through a reminder call/SMS.

Creating public awareness for DR

Every opportunity should be used to spread awareness of blindness due to DR. A Physician should insist on the report of DR screening at the time of consultation. Patient education posters in patient waiting areas and pamphlets with pictures can help spread awareness about DR. DR screening should become a part of the standard of care for diabetes.

Summary of the position statement

- Any individual with diabetes is susceptible to developing DR and the onset is earlier in those with the risk factors
- The occurrence of DR is influenced by the duration of diabetes, applicable to both type-1 and 2 diabetes.
- The initial stages of DR are symptomless, necessitating proactive annual screenings for early detection in all diabetes patients.
- Advanced stages of DR can lead to irreversible vision loss, and despite the advanced treatment procedures, including complex vitreoretinal surgeries, the visual prognosis may remain poor.
- Optimal management of systemic factors like blood sugar, blood pressure, and lipid profile (LDL cholesterol < 70 mg%) is pivotal in slowing down DR progression.
- Physicians, as the primary healthcare providers for diabetes patients, have a vital role in raising awareness about DR.
- Physicians should establish an effective DR screening program in their clinics, facilitating early detection, timely referrals to ophthalmologists, and prevention of VTDR.
- Non-mydriatic cameras utilizing AI algorithms in fundus photography can aid in identifying referral or non-referral cases of DR.
- Collaboration between Physicians and ophthalmologists is imperative in the screening process for DR in diabetes patients.
- Public awareness campaigns through clinic posters, patient education materials, and media initiatives can enhance understanding of the risks of blindness associated with DR.

Conclusion

India faces a significant burden of diabetes, resulting in a rise in diabetic retinopathy (DR) cases and preventable blindness. Lack of awareness and asymptomatic early stages of DR contribute to patients not seeking eye screenings, leading to the development of VTDR and permanent vision loss.

In conclusion, the position statement by RSSDI and VRSI serves as a crucial guide for fostering collaboration between Physicians and ophthalmologists in India. By working together, they can effectively combat the burden of diabetic retinopathy (DR) and prevent unnecessary blindness. The statement emphasizes the importance of raising awareness, implementing early screening measures and utilizing cost-effective models to detect and manage DR at it's early stages. Through this collaborative effort, we can strive towards a future where preventable blindness due to DR becomes a rarity in India.

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ORIGINAL ARTICLE

The association between thyroid hormone changes within the normal range and bone mineral density in patients with type 2 diabetes mellitus

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Abstract

Objective The objective was to investigate the association between thyroid hormone changes within normal range and bone mineral density (BMD) in patients with type 2 diabetes (T2DM).

Materials and methods A total of 661 T2DM patients with normal thyroid function were included in our study, and they were classified into three groups: normal group, osteopenia group, and osteoporosis group. One-way ANOVA was used to compare the differences in variables between the three groups. Pearson's correlation analysis was performed to assess the association between clinical parameters and BMD, and multivariate logistic regression analysis was conducted to determine the potential risk factors for osteoporosis. $p < 0.05$ was considered statistically significant. Evaluation of the sensitivity and specificity of thyroid hormone by ROC curves to determine more reliable thresholds for experimental indicators.

Results The three groups showed significantly different population groups (male and female groups) ($p < 0.05$). Age, fasting blood glucose (FBG), and glycosylated hemoglobin (HbA1C) were higher in the osteoporosis group ($p < 0.05$). In contrast, body mass index (BMI), serum uric acid (SUA), and free triiodothyronine (FT3) were lower in osteoporosis group ($p < 0.05$). Thyroid peroxidase antibodies (TPOAb) and anti-thyroid globulin antibodies (TgAb) positive proportion was significantly higher in osteoporosis group. BMD of lumbar vertebrae was positively correlated with BMI, SUA, and FT3 ($p < 0.05$), while it was negatively correlated with age and FBG ($p < 0.05$). Multivariate logistic regression analysis indicated that TPOAb positive and TgAb positive levels were associated with an increased risk of osteoporosis ($p < 0.05$), while higher SUA and FT3 levels were associated with a decreased risk of osteoporosis ($p < 0.05$). The ROC curves demonstrated that FT3, FT4, and TSH had a good discriminative ability, with AUC values of 0.840, 0.602, and 0.522.

Conclusion The circulating concentration of FT3 was positively correlated with BMD. Maintaining normal and slightly higher levels of FT3 and SUA may prevent the reduction of BMD and the incidence of osteoporosis in T2DM patients. Besides, T2DM patients with TPOAb positive and TGAb positive levels should be routinely checked for BMD to prevent osteoporosis development.

Keywords Type 2 diabetes · Osteoporosis · Bone mineral density · Thyroid hormone

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Introduction

Osteoporosis is prevalent in patients with type 2 diabetes (T2DM), especially the elderly, which places a significant financial and social burden on society [1–3]. The previous study indicated that diabetes is an independent risk factor for low-energy subtrochanteric and diaphyseal fractures [4]. Many factors could influence T2DM complicated with osteoporosis. T2DM patients have many changes in their endocrine system and metabolism. Metabolic products directly or indirectly could lead to the occurrence and development of osteoporosis through various signaling pathways.

Many studies have shown that thyroid hormone is closely associated with bone metabolism and excess T3 shortens bone turnover and leads to poor bone mineralization [5]. As a result, the effects of hyperthyroidism and hypothyroidism on bone mineral density have attracted much attention [6, 7]. However, thyroid hormone levels are often normal in T2DM patients with osteoporosis. Moreover, it is unknown whether thyroid hormone fluctuation within the normal range would impact BMD [8]. Therefore, further studies on T2DM patients with normal thyroid function are necessary to clarify the effect of thyroid hormones on bone mineral density.

Our study aimed to explore the relationship between thyroid hormone fluctuation within the normal range and BMD in T2DM patients. Moreover, this study further assessed those potential risk factors for BMD, which would provide clinical prevention strategies for osteoporosis development in T2DM patients.

Materials and methods

Study population

This study was a hospital-based cross-sectional study, and all participants were consecutively included from the Department of Endocrinology in Chongqing Red Cross Hospital between October 2020 and March 2021. We used strict inclusion and exclusion criteria. The inclusion criteria were (1) two endocrinologists with a joint diagnosis of T2DM, not in a state of hyperosmolar hyperglycemia, and no diabetic ketoacidosis, (2) normal thyroid function, and (3) all receive bone mineral density testing. Exclusion criteria were (1) incomplete data, (2) previous history of hospitalization, (3) T2DM with acute complications, (4) abnormal liver and kidney function, (5) secondary osteoporosis, (6) treated with drugs that affect bone metabolism, such as steroid hormones, vitamin D, and derived calcium bisphosphonates and TZDS hypoglycemic agents, (7) hyperthyroidism and hypothyroidism, and (8) patients who have used thyroid hormone or anti-thyroid medication. Finally, 384 postmenopausal females and 277 males with T2DM, who were 50 years and older were included in this study.

This study involving human data was in accordance with the Declaration of Helsinki and was approved by Ethics Committee of Chongqing Red Cross Hospital. The informed consent was completed from all patients.

Sample size calculation

We reformulated the sample size calculation by using PASS 11.0, (estimated the means-one mean-confidence interval-confidence intervals for one mean in the left hand menu bar). We set it to a two-sided test with $\alpha = 0.05$,1-$\alpha = 0.95$, a tolerance error of 0.1, a standard deviation of 0.73, and all other defaults. A sample size of 122 was calculated, and a minimum of 153 patients were required assuming a 20% lost-to-review rate. This study included 611 patients, which met the calculated sample size.

Data collection

In this study, the patients' baseline information, including age, sex, and BMI, were obtained. Biochemical and BMD analyses were also collected, and the same machine was used for estimating BMD for every participant. BMD was measured by dual-energy X-ray absorptiometry, which was used in the X-ray tube through a certain device to obtain two kinds of energy, low-energy and high-energy photon peaks. After penetrating the body with photon peaks, the received signals in scanning system would send to the computer for data processing. Bodyweight and height were measured when patients wore light clothing and without shoes. BMI was defined as the weight (kilograms) divided by height (meters). Serum biochemical tests included the determination of FBG, HbA1C, SUA, blood calcium, blood phosphorus, and 25-hydroxyvitamin D levels. In this study, the thyroid-related indicators, including FT3, free thyroxine (FT4), thyroid-stimulating hormone (TSH), thyroid peroxidase antibody (TPOAb), and anti-thyroglobulin antibodies (TgAb) were determined. Blood samples were collected after overnight fasting for at least 8 h. A dual-energy X-ray absorptiometry was used to detect the BMD for each patient at the total lumbar area.

Diagnostic criteria

The T2DM patients were diagnosed using the diagnostic criteria [9] established by the 1999 WHO Diabetes Expert Committee. Moreover, T2DM were indicated by

diabetes symptoms, plasma glucose ≥ 11.1 mmol/L, or FPG ≥ 7.0 mmol/L, or OGTT 2hPG ≥ 11.1 mmol/L.

Trained technicians used a dual-energy X-ray bone densitometer (Hologic, USA) to measure bone density in the low median region of the pelvis on the left forearm. Osteoporosis was defined by [10] the diagnostic criteria recommended by WHO in 1994. T was calculated as follows: measured BMD minus peak bone density and then divided by the standard difference of patient's bone density. $T \leq -2.5$ indicated osteoporosis, $-2.5 < T < -1$ indicated bone loss, and $T \geq -1$ indicated normal bone mass. In this study, the patients were divided into the normal group ($n = 272$), osteopenia group ($n = 238$), and osteoporosis group ($n = 151$).

Statistical analysis

Normally distributed data were presented as mean ± standard deviation ($x \pm s$). One-way ANOVA was used for multicomponent comparison. The χ^2 test was conducted for count data case (percentage) comparison between groups. Pearson correlation analysis was conducted to assess the correlation between BMD and related clinical parameters. Multivariate logistic regression analysis was conducted to evaluate the risk factors for osteoporosis. $p < 0.05$ was considered a statistically significant difference. SPSS 19.0 software and R software version 4.0.5 (R Project for Statistical Computing, Vienna, Austria) was performed for all statistical analyses. Picture drawing was done with GraphPad Prism 8 software.

Results

Cohort characteristics

Data were retrospectively collected from 661 patients with T2DM and normal thyroid function and were screened according to strict inclusion and exclusion criteria. All patients underwent BMD testing between October 2020 and March 2021. Please see Fig. 1 for the inclusion and exclusion table of patients.

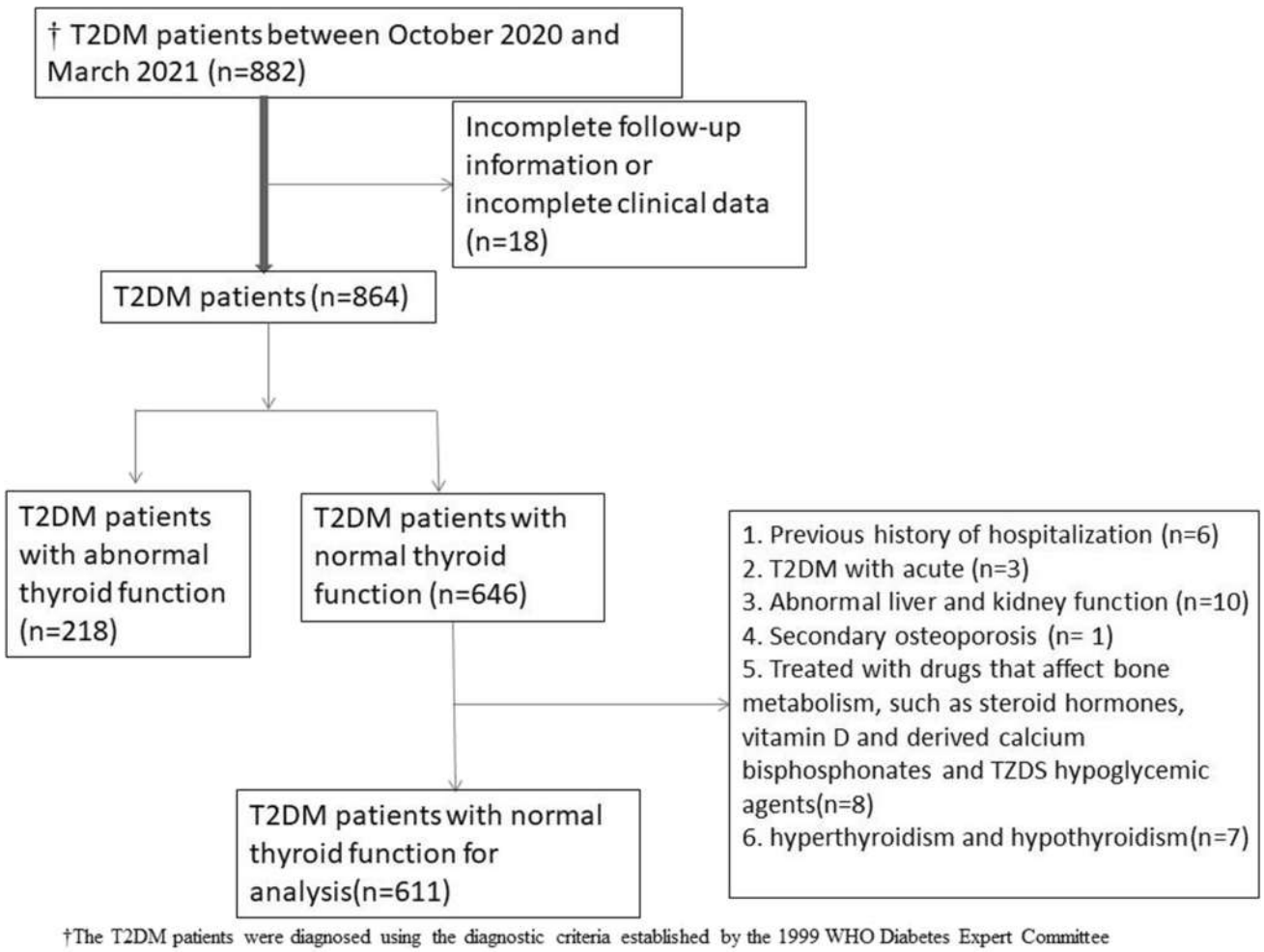

Fig. 1 Inclusion and exclusion table of patients

Comparison of baseline clinical parameters in three groups

There were significant differences in age, sex, BMI, FBG, HbA1C, SUA, blood phosphorus, and FT3 among the normal group, osteopenia group, and osteoporosis group ($p < 0.05$). The proportion of TPOAb positive and TgAb positive were significantly higher in osteoporosis than the osteopenia group and normal BMD group (Table 1), Violin plots were also drawn to depict the differences in FT3 and FT4 concentrations between the three groups; FT3 concentrations were lower in patients with osteoporosis than in patients with normal bone mass and were statistically significantly different ($p = 0.012$) (Fig. 2A). For FT4 concentrations, there was no significant difference between the three groups of patients (Fig. 2B).

Correlation analysis between BMD and biochemical results

Pearson correlation analysis showed that lumbar BMD was positively associated with BMI, SUA, and FT3 ($r = 0.357$, 0.219, 0.089; $p < 0.05$), while negatively association with age, FBG, and blood phosphorus ($r = -0.156, -0.044, -0.084$; $p < 0.05$). The results of Pearson correlation analysis were presented in (Table 2).

Multivariate logistic regression analysis for osteoporosis

The presence or absence of osteoporosis was used as the dependent variable, and statistically significant variables in correlation analysis were used as independent variables. Logistic regression analysis showed that FBG, TPOAb positive, and TgAb positive levels were independently associated with osteoporosis, and BMI, SUA, and FT3 levels were protective factors for osteoporosis development ($p < 0.05$) (Table 3).

ROC curve based on FT3, FT4, and TSH in patients with normal thyroid function and the area under the curve

The ROC curves were plotted according to the continuous variables of FT3, FT4, and TSH to distinguish osteoporosis/reduction and normal, and the area under the ROC curves for the three cases were 0.840, 0.602, and 0.522. Please see Fig. 3.

Predictive performance of the thyroid hormone

The optimal cut-off value is based on the value corresponding to the maximum Jorden index. Accuracy, sensitivity, specificity, PPV, and NPV for the data at the best cut-off values of FT3 and FT4 are 0.781, 0.838, 0.792, 0.704, and 0.889 and 0.582, 0.618, 0.604, 0.531, and 0.607. Please see (Table 4).

Table 1 Comparison of clinical characteristics in three groups ($x \pm s$)

	OP group ($n = 272$)	Osteopenia group ($n = 238$)	normal bone mass group ($n = 151$)	F/χ^2	p value
lumbar vertebra BMD	−3.24 ± 0.55	−1.79 ± 0.39	−0.04 ± 0.74	1651.516	0.000
Gender (male/female)	92 (180)	102 (136)	83 (68)	62.000	0.000
Age (yrs)	66.30 ± 7.89	64.10 ± 10.47	62.61 ± 10.59	7.946	0.000
BMI (kg/m^2)	22.76 ± 2.44	24.35 ± 3.15	25.40 ± 3.70	40.730	0.000
FBG (mmol/L)	9.60 ± 3.68	8.92 ± 2.97	8.59 ± 2.77	4.441	0.048
HbA1C (%)	9.47 ± 2.02	8.98 ± 2.02	8.21 ± 2.14	10.312	0.016
SUA (μmol/L)	302.09 ± 85.76	327.07 ± 89.38	349.59 ± 89.61	14.789	0.000
Blood calcium (mmol/L)	2.34 ± 0.14	2.32 ± 0.12	2.34 ± 0.12	1.727	0.179
Blood phosphorus (mmol/L)	1.21 ± 0.20	1.17 ± 0.13	1.18 ± 0.14	4.315	0.014
25-(OH)vitD (ng/ml)	21.37 ± 6.03	21.78 ± 6.38	20.37 ± 5.00	2.616	0.074
FT3 (pmol/L)	4.95 ± 0.63	5.02 ± 0.73	5.14 ± 0.87	3.148	0.044
FT4 (pmol/L)	10.91 ± 1.81	10.85 ± 1.98	10.92 ± 1.60	0.080	0.923
TSH (μIU/ml)	2.56 ± 1.86	2.46 ± 1.87	2.50 ± 1.76	0.184	0.832
TPOAb positive (%)	97 (35.7)	55 (23.1)	37 (24.5)	165.012	0.003
TgAb positive (%)	81 (29.8)	48 (20.2)	33 (21.9)	138.239	0.029

Abbreviation: *BMD*, bone mineral density; *BMI*, body mass index; *FBG*, fasting blood glucose; *SUA*, serum uric acid; *FT3*, free triiodothyronine; *FT4*, free thyroxine; TSH, thyroid-stimulating hormone; *TPOAb*, thyroid peroxidase antibodies; *TgAb*, anti-thyroid globulin antibodies

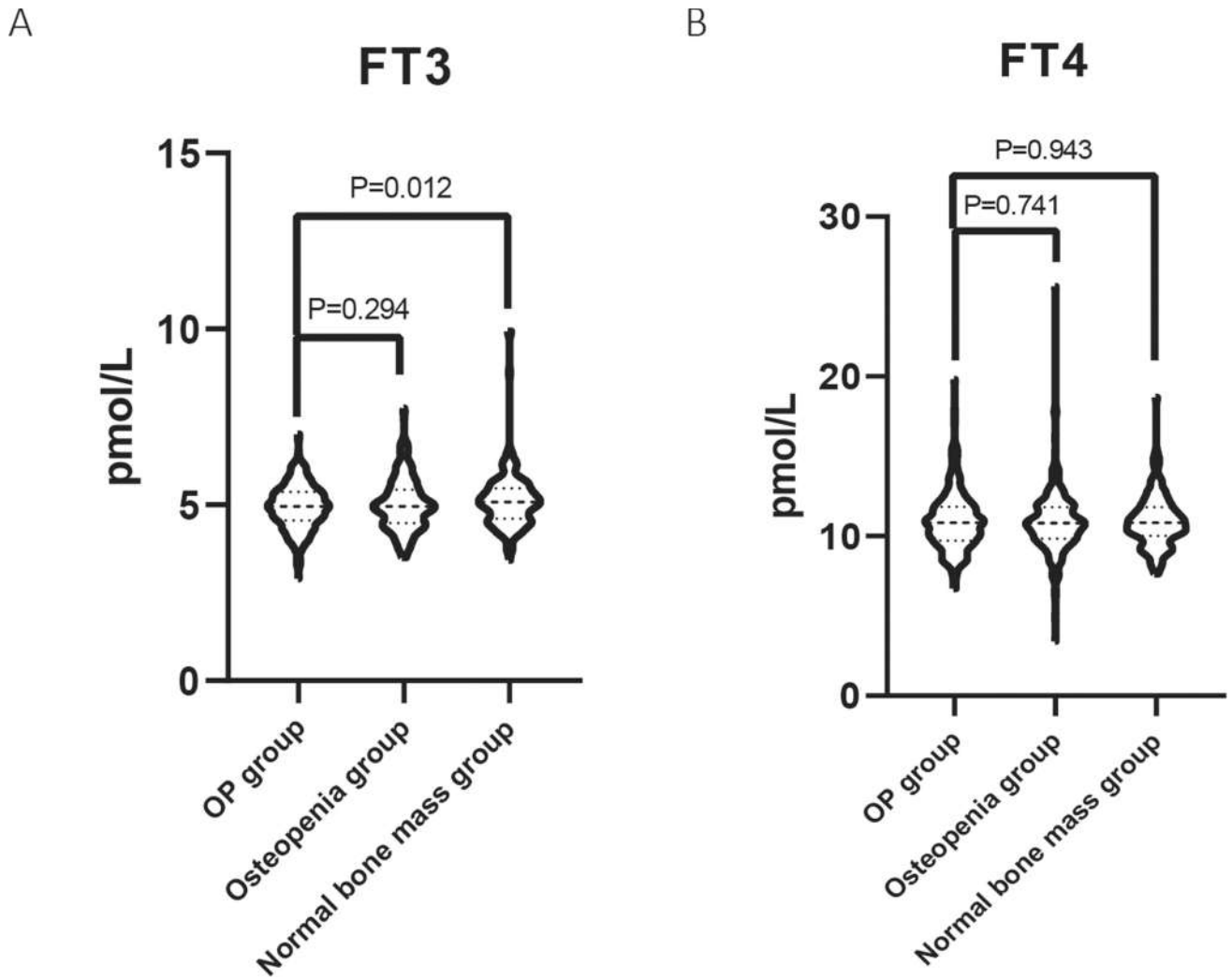

Fig. 2 FT3 and FT4 concentrations between the three groups

Discussion

Although the circulating concentrations of T3 and T4 and their uptake and local activation and inactivation by target tissues determine the intracellular levels of T3 and its action in local tissues, excess T3 shortens bone turnover and leads to poor bone mineralization. Our study included 661 participants with normal thyroid function from the Department of Endocrinology in Chongqing Red Cross Hospital between October 2020 and March 2021. Patients with T2DM who have low FT3 and SUA levels should be aware of the risk of osteoporosis. There is a need to routinely check BMD in TPOAb positive and TGAb positive T2DM patients. However, given the inherent limitations of this study, a large cohort study is needed to further determine the role of thyroid hormones on BMD in T2DM patients.

Osteoporosis is usually diagnosed after a fracture, and the quality of life for patients could be affected by the condition of brittle fracture. Several studies have reported that T2DM patients have a significantly higher fracture risk than the general population [11, 12]. Thus far, few studies have looked into the BMD changes in T2DM patients and the pathogenesis of diabetic osteoporosis [13, 14]. The vertebral body is a site of systemic stress concentration, and the decreased mechanical strength, low energy damage, and accumulated damage during long-term load could make it a potential location for osteoporotic fractures. The incidence rate for vertebral fractures is higher than hip fractures [15]. Therefore, this study based on lumbar BMD to analyze data in T2DM patients in order to obtain more practical data. Besides promoting tissue growth, development, and differentiation, thyroid hormones disturb sugar, fat, and protein metabolism. In recent years, the effect of thyroid function on bone metabolism has been gradually appreciated. Moreover, both clinical hyperthyroidism and hypothyroidism could reduce BMD and increase the occurrence of fractures [7,

Table 2 Pearson correlation analysis for the risk factors of BMD in type 2 diabetes patients

	Lumbar vertebra BMD	
	r value	*p* value
Age (yrs)	−0.156	0.000
Diabetic duration (y)	−0.034	0.388
BMI (kg/m^2)	0.357	0.000
FBG (mmol/L)	−0.044	0.013
HbA1C (%)	0.053	0.171
SUA (μmol/L)	0.219	0.000
Blood calcium (mmol/L)	0.010	0.805
Blood phosphorus (mmol/L)	−0.084	0.030
25-(OH)vitD (ng/ml)	−0.051	0.190
FT3 (pmol/L)	0.089	0.022
FT4 (pmol/L)	−0.005	0.890
TSH (μIU/ml)	−0.025	0.525
TPOAb	−0.035	0.366
TgAb	−0.028	0.465

Abbreviation: *BMD*, bone mineral density; *BMI*, body mass index; *FBG*, fasting blood glucose; *SUA*, serum uric acid; *FT3*, free triiodothyronine; *FT4*, free thyroxine; *TSH*, thyroid-stimulating hormone; *TPOAb*, thyroid peroxidase antibodies; *TgAb*, anti-thyroid globulin antibodies

Table 3 Multivariate logistic regression analysis for the risk factors of osteoporosis in type 2 diabetes patients

	β	SE	OR	95% CI	*p* value
BMI	−2.487	0.607	0.213	0.119 ~ 0.278	0.001
FBG	2.192	0.639	2.292	1.798 ~ -2.586	0.014
SUA	−0.005	0.001	0.014	0.003 ~ 0.017	0.001
FT3	−2.475	0.629	0.554	0.246 ~ 0.706	0.022
TPOAb positive	1.528	0.254	2.224	1.783 ~ 2.878	0.012
TgAb positive	1.156	0.279	2.375	1.955 ~ 2.801	0.007

Abbreviation: *BMI*, body mass index; *FBG*: fasting blood glucose; *SUA*, serum uric acid; *FT3*, free triiodothyronine; *FT4*, free thyroxine; *TPOAb*, thyroid peroxidase antibodies; TgAb: anti-thyroid globulin antibodies

16]. Although several studies in China and some countries have reported the relationship between thyroid-related hormones within normal range and bone metabolism, the results still showed to be controversial. A meta-analysis by Aubert et al. [17] reported that lower TSH and higher FT4 levels were associated with an increased risk of hip fracture in adults with normal thyroid function, suggesting that TSH fluctuations within the normal range could affect bone density and would alter the risk of fracture. This is relatively consistent with our findings, while a retrospective study by Deng et al. showed that circulating concentrations of TSH and BMD were positively correlated with normal thyroid function in men, in patients with normal thyroid function.

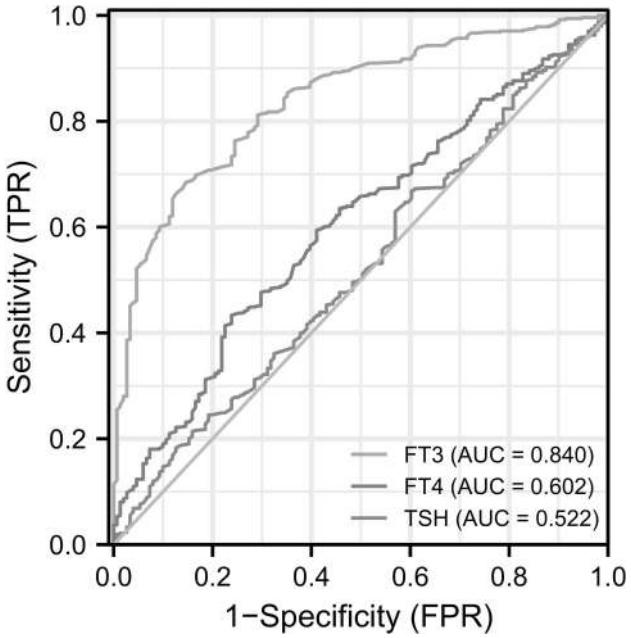

Fig. 3 ROC curves for the three cases

Herein, the FT3 level was lower in the osteoporosis group than the osteopenia and normal groups. Moreover, FT3 was positively associated with lumbar BMD in T2DM patients with normal thyroid function ($p < 0.05$), which showed consistent results with Baqi et al.'s findings [18]. Thyroid hormones could influence bone metabolism through osteoblasts and osteoclasts. For instance, T3 mainly enhances bone formation by directly binding to osteoblast nuclear receptors and indirectly binding to osteoclasts [19]. FT3 was identified as a protective factor of osteoporosis in T2DM patients. Therefore, maintaining FT3 within the normal range could prevent BMD loss, osteoporosis, and osteoporotic fractures development in T2DM patients. However, the relationship between osteoporosis and serum TSH is unclear. In this study, TSH and FT4 were not associated with lumbar BMD. Similarly, Svare et al. [20] also found that serum TSH is not correlated with the incidence of fracture in the forearm or hip among postmenopausal women. Moreover, in Leader et al.'s [21] study, TSH was not associated with hip fracture incidence, and this study also investigated 13,325 healthy subjects (65 years or older) in 1994 and found that lower TSH levels within the normal range are associated with an increased risk of hip fracture in patients with the normal thyroid function in women rather than men. Similarly, no significant difference was found in the TSH concentration among our study's three groups. The ROC curve presented in our cases also showed an area under the curve for TSH of 0.522, which is essentially non-discriminatory. This remains consistent with the results of our study. Therefore, TSH and FT4 levels within the normal range are not associated with

Table 4 Predictive performance of the FT3/FT4

Variables	AUC	Accuracy	Sensitivity	Specificity	PPV	NPV
FT3	0.840 (0.775–0.892)	0.781	0.838	0.792	0.704	0.889
FT4	0.602 (0.544–0.678)	0.582	0.618	0.604	0.531	0.607

Abbreviation: *FT3*, free triiodothyronine; *FT4*, free thyroxine; *AUC*, areas under the receiver operating characteristic curve; *PPV*, positive predictive value; *NPV*, negative predictive value

BMD, probably due to the reason that the included middle-aged and aged patients were not grouped based on sex.

The association between thyroid autoantibodies and osteoporosis has gradually become a study hotspot due to the appearance of bone immunology [22]. For instance, studies have suggested that thyroid autoantibodies could affect bone metabolism. Polovina et al. [23] found that autoimmune thyroid disease is associated with reduced spine and hip bone density and increased risk of fracture incidence in postmenopausal women with normal thyroid function. They also showed that TPOAb is a potential marker for the increased fracture risk in these patients. Another study reporting 335 postmenopausal women with normal thyroid function also suggested that positive TPOAb and positive TgAb may increase the fracture risk [24]. Similarly, Snezana et al. [25] reported that autoimmune thyroid disease is associated with decreased bone density of the spine and collum femoris in postmenopausal women, indicating that positive TPOAb is a predictor for fracture risk in postmenopausal women. In summary, TPOAb positivity and TgAb positivity affect bone metabolism, which is consistent with our findings, and we also consider both positivities as independent risk factors for the development of osteoporosis in patients with normal thyroid function ($OR > 2$, $p < 0.05$).The immune system and the skeletal system could interact under physiological or pathological conditions. Moreover, immune mediators may be involved in the process that promotes bone loss [26]. Abnormal immune system activation could directly affect bone remodeling, which could lead to pathological bone loss. Chronic autoimmune diseases could cause disorders of osteoclast activity and induce osteoclast-mediated bone resorption, thus increasing the risk of osteoporosis and fracture [27].

The results in this study provide an important reference for clinical osteoporosis prevention in T2DM patients since this study had a large sample size. However, several limitations still should be taken into consideration in our study. It did not group the patients based on gender and age. Moreover, this was a single-center, retrospective cross-sectional study. Therefore, further large-scale prospective studies are needed to further confirm our results.

In this study, hyperglycemia, TPOAb positive, and TGAb positive were identified as osteoporosis risk factors in T2DM patients. Besides, maintaining normal and slightly higher levels of FT3 and SUA could prevent excessive reduction of BMD and the incidence of osteoporosis in T2DM patients. Therefore, normal or slightly higher levels of FT3 could promote osteoporosis prevention, especially for T2DM patients with positive TPOAb and positive TgAb. Furthermore, the bone density of T2DM patients should be routinely examined for early intervention to achieve an improved clinical outcome.

Conclusion

Results from this cross-sectional study showed that maintaining normal and slightly higher levels of FT3 and SUA could prevent the reduction of BMD and the development of osteoporosis among T2DM patients. Additionally, T2DM patients with positive TGAb and TPOAb levels should be regularly monitored for BMD to prevent subsequent osteoporosis.

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

Declarations

Ethics approval and consent to participate This retrospective study was approved by the ethics committees of Chongqing Red Cross Hospital. All patients provided written informed consent.

Consent for publication The patient provided written informed consent for publication of this research and the associated images.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Bone mineral density and its predictors in a cohort of adults with type 1 diabetes attending a tertiary care institute in North India

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Abstract

Background The incidence of type 1 diabetes (T1D) is showing a rising trend all over the world, and these patients are living longer than before. With increasing longevity, they are at increased risk of chronic complications including fractures. However, the bone mineral density (BMD) data in T1D patients are conflicting and variable across the studies. Here, we aimed to study the BMD in adult patients with T1D and delineate its predictors.

Material and methods We recruited 40 T1D patients and equal number of age- and sex-matched control subjects. Clinical, biochemical, hormonal, and densitometric assessments were performed for each of the participants and compared between the two groups.

Results The median age of T1D and control subjects was 23.5 (21–27.75) years and 24 (22–26) years, respectively. The median duration of diabetes in T1D patients was 12.5 (9–15.75) years with a mean HbA1C of $8.7 \pm 1.9\%$. The serum corrected calcium, phosphorous, alkaline phosphatase, creatinine, and plasma 25-hydroxyvitamin D and iPTH levels were comparable between the groups. In T1D subjects, the mean lumbar spine and left hip BMD were 0.876 ± 0.154 gm/cm^2 and 0.780 ± 0.112 gm/cm^2, respectively, which corresponded to mean *Z*-scores of -1.5 ± 1.4 and -1.4 ± 0.9, respectively, which were significantly lower compared to control group. Multiple linear regression analyses showed that BMI and serum albumin were significant determinant of lumbar spine and hip BMD, respectively, in patients with T1D.

Conclusion Patients of T1D have apparently reduced BMD, which is being influenced by BMI and serum albumin level.

Keywords Type 1 diabetes · Bone mineral density · Body mass index · Albumin

Introduction

Type 1 diabetes mellitus (T1D) is characterized by autoimmune destruction of pancreatic β-cells with consequent absolute insulin deficiency and hyperglycemia. It affects more than one million children and adolescents worldwide, and the incidence is progressively rising [1, 2]. Since the discovery of insulin, there has been a sharp decline in the acute complication-related mortality associated with T1D. Further, with the advent of newer form of insulin analogs having better pharmacokinetic and pharmacodynamic profile and development of newer technology in this field, patients with T1D are living longer and hence inadvertently are at an increased risk of long-term complications [2].

Adult patients with T1D have a six-fold increased fracture risk [3]. Recent studies where dual X-ray absorptiometry (DXA) had been used revealed lower bone mineral density in adolescents and adults with T1D as compared to control population [4, 5]. However, data is not consistent across all the studies in this regard [6]. In a meta-analysis, Vestergaard et al. observed that the increased risk of hip fracture in T1D patients could not be explained by BMD alone [7]. In a more recent study on patients with long standing T1D, the authors did not find any substantial differences in *T*-score between cases and matched controls [8]. Despite having higher BMD at lumbar spine, the female patients of T1D in the same study cohort showed higher rate of fragility fractures compared to controls. Hence, there must be additional factors, which might be contributing to the fracture risk in T1D.

Consistent with the international trend, the prevalence of T1D in India is also rising and the current prevalence reported in various studies varies from 3.7 to 10.2 per 100,000 populations [6]. There is paucity of data regarding

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bone health in patients of T1D from India. Here, we aimed to study the bone mineral density in adult patients with T1D in India and tried to find out its determinants in the same cohort.

Material and methods

This prospective study was conducted in Endocrinology department of Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh. Consecutive patients of T1D of age ≥ 18 years of either sex, visiting Endocrinology OPD of PGIMER, Chandigarh, during the year 2017 were included. Patients who had concurrent hypothyroidism were on stable doses of L-thyroxine therapy for at least 3 months with T_4 level within normal range at the time of recruitment. Patients with all other comorbidities such as celiac disease, malignancy, chronic kidney disease (eGFR < 60 ml/min), and pregnant women were excluded. Patients who had received glucocorticoids, complementary and alternative medications, bisphosphonate, teriparatide, or any other medicines that may affect bone turnover were excluded. All the patients were examined for pubertal development, and those with less than Tanner stage 5 were also not included in the study. All the female patients included in the study were eumenorrheic. We also included equal number of age- and sex-matched healthy adult controls from the Chandigarh Urban Bone Epidemiological Study (CUBES) [9–12].

Clinical and demographic details of these patients were evaluated by detailed interviews. Past and family history of fragility fractures was inquired of. The patients were thoroughly examined, and details including height, weight, pubertal staging, and bony deformities were documented. Height was estimated thrice by a stadiometer, and the mean was considered as the final height. Weight was also measured thrice with a digital weighing machine, and the mean value was taken. The formula, weight (in kg)/height (in meter^2), was used to calculate body mass index (BMI).

Laboratory parameters

Blood samples were drawn from the study participants after an overnight fast. These samples were processed for hemoglobin, creatinine, total calcium, inorganic phosphorous, alkaline phosphatase, glycated hemoglobin (HbA1C), triiodothyronine (T_3), tetraiodothyronine (T_4), thyroid stimulating hormone (TSH), 25-hydroxyvitamin D (25(OH)vitamin D), intact parathyroid hormone (iPTH), and IgA tissue transglutaminase (IgA TTg) antibody. Serum total calcium level was corrected for albumin level. Hemoglobin level was measured with Coulter LH 780 Automated Analyzer (Beckman Coulter, Inc., Brea, CA, USA). Serum creatinine, albumin, total calcium, and inorganic phosphorous were estimated by Modular P800 Analyzer (Roche Diagnostics, Mannheim, Germany). HbA1c was estimated by Bio-Rad D10 analyzer (DCCT standardized). Plasma T_3, T_4, TSH, 25(OH) vitamin D, and iPTH were estimated using electrochemiluminescence (ECLIA) using Elecsys 2010 Analyzer (Roche Diagnostics, Mannheim, Germany). IgA-tTg antibody was estimated by using fluoroenzyme assay.

DXA

All the T1D and control subjects underwent dual energy X-ray absorptiometry (DXA) scan using the HOLOGIC Discovery A (QDR 4500; Hologic Inc., Bedford, MA) scanner for assessment of bone mineral density and *Z*-scores at the lumbar spine (L1-L4) and left hip. All DXA scans were performed by a dedicated, International Society of Clinical Densitometry (ISCD)-certified technician. Quality control procedures were carried out in accordance with the manufacturer's recommendations.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 23. The normality of data was checked using the Shapiro–Wilk test. Normally, distributed data were expressed as mean ± standard deviation (SD), and non-normally distributed data were expressed as median (interquartile range, IQR). The comparison between two normally distributed data was performed using Independent Samples *t*-test, and for non-parametric data, Mann–Whitney *U* test was used. Correlations between BMD and demographic/biochemical variables (continuous) were performed using Pearson/Spearman correlation based on the normality of data. Following univariate analysis, the continuous variables were considered for multiple linear regression analysis. A p value < 0.05 was considered significant.

Results

We recruited 40 T1D and equal number of age- and sex-matched healthy adult controls. The median age of T1D subjects was 23.5 (21–27.75) years and 18 were male. The median age of control subjects was 24 (22–26) years with equal male to female ratio. The demographic, clinical, and biochemical data of these patients are described in Table 1. Although T1D group had comparable mean height compared to control group (158.82 ± 12.19 cm and 159.84 ± 10.54 cm, respectively, $p = 0.693$), the mean weight and BMI were more in the latter group (50.97 ± 9.90 kg

Table 1 Clinical and biochemical parameters of T1D and control group

Parameters	T1D ($N=40$)	Control ($N=40$)	p value
Age (year)	23.5 (21–27.75)	24 (22–26)	0.946
Height (cm)	158.82 ± 12.19	159.84 ± 10.54	0.693
Weight (kg)	50.97 ± 9.90	59.73 ± 11.88	0.001
BMI (kg/m^2)	20.10 ± 3.07	23.29 ± 3.56	0.000
Duration of DM (years)	12.5 (9–15.75)	-	
Urea (mg/dl)	24.2 (19.3–29.49)	23.0 (18.0–31.0)	0.764
Creatinine (mg/dl)	0.69 ± 0.16	0.72 ± 0.15	0.361
Ca (mg/dl)	9.09 ± 0.47	9.18 ± 0.40	0.377
Corrected Ca (mg/dl)	8.89 ± 0.52	8.83 ± 0.48	0.611
P (mg/dl)	3.57 ± 0.51	3.67 ± 0.53	0.398
ALP (U/L)	136.96 ± 92.28	105.18 ± 36.93	0.054
Albumin (mg/dl)	4.23 ± 0.39	4.43 ± 0.41	0.029
PTH (pg/ml)	66.20 ± 98.46	52.44 ± 22.54	0.466
25(OH)vitamin D (ng/ml)	13.5 (8.99–23.11)	11.46 (6.7–20.6)	0.259
HbA1c (%)	8.67 ± 1.94	5.34 ± 0.39	0.000
T3 (ng/ml)	1.20 ± 0.27	1.32 ± 0.24	0.049
T4 (μg/dl)	7.65 ± 1.14	7.80 ± 1.68	0.644
TSH (mIU/ml)	2.51 (1.49–3.98)	2.76 (2.13–4.47)	0.268

vs. 59.73 ± 11.88 kg, $p=0.001$ and 20.10 ± 3.07 kg/m^2 vs. 23.29 ± 3.56 kg/m^2, $p=0.000$; respectively).

The median duration of diabetes in the T1D patients was 12.5 (9–15.75) years with a mean HbA1C of 8.7 ± 1.9%. Among the T1D patients, two had fragility fracture, while none of the subjects in the control group had similar history. Among these two patients, one had fracture at right 4th and 5th metacarpal, and the remaining one sustained fracture at supracondylar region of left humerus. None of the siblings of T1D patients had history of fragility fracture. Six T1D patients had hypothyroidism for which they were receiving stable dose of L-thyroxine. The serum corrected calcium, phosphorous, alkaline phosphatase, creatinine, and plasma 25(OH) vitamin D and iPTH level were comparable between the two groups (Table 1). Although serum albumin and plasma T3 level were significantly lower in T1D group compared to control (4.23 ± 0.39 mg/dl vs. 4.43 ± 0.41 mg/dl, $p=0.029$ and 1.20 ± 0.27 ng/ml vs. 1.32 ± 0.24 ng/ml, $p=0.049$, respectively), T4 and TSH level were comparable between both the groups. In T1D subjects, the mean lumbar spine and left hip BMD were 0.876 ± 0.154 gm/cm^2 and 0.780 ± 0.112 gm/cm^2, respectively, which corresponded to mean *Z*-scores of -1.5 ± 1.4 and -1.4 ± 0.9, respectively. The BMD and *Z*-scores at lumbar spine and left hip in T1D subjects were significantly lower as compared to those in the control group (Table 2).

In patients with T1D, there were no gender differences in BMD at the lumbar spine ($p=0.996$) or at the hip ($p=0.981$). In this subgroup of subjects, there were significant positive correlations between lumbar spine BMD and BMI ($r=0.480$ and $p=0.002$) and serum albumin ($r=0.427$ and $p=0.006$). Similarly, there were significant positive correlations between hip BMD and BMI ($r=0.440$ and $p=0.005$) and serum albumin ($r=0.416$ and $p=0.008$). Notably, there were no significant correlations between lumbar spine and hip BMD with age, T1D duration, HbA1c, serum calcium, phosphate, or 25(OH) vitamin D. Multiple linear regression analyses showed that BMI (unstandardized coefficient $\beta=0.019$ and $p=0.014$) was significant determinant, while serum albumin (unstandardized coefficient $\beta=0.119$ and $p=0.050$) was trending to be significant determinant of lumbar spine BMD. On the contrary, albumin (unstandardized coefficient $\beta=0.110$ and $p=0.029$) was found to be a significant determinant of hip BMD, while BMI was trending to be significant for the same (unstandardized coefficient $\beta=0.011$ and $p=0.073$) in patients with T1D.

Table 2 DXA parameters in T1D and control group at lumbar spine and left hip

Parameters	T1D ($N=40$)	Control ($N=40$)	p value
Lumber spine BMD (gm/cm^2)	0.876 ± 0.154	0.945 ± 0.107	0.022
Lumber spine *Z*-score	-1.5 ± 1.4	-0.9 ± 0.9	0.019
Left hip BMD (gm/cm^2)	0.780 ± 0.112	0.902 ± 0.108	0.000
Left hip *Z*-score	-1.4 ± 0.9	-0.5 ± 0.8	0.000

Discussion

In this prospective cohort study, we observed apparently lower BMD and *Z*-scores at both lumbar spine and hip in T1D subjects as compared to control. The BMI and serum albumin were also lower in the former group. In patients with T1D, there were no gender differences as far as the BMD is concerned. In the same subgroup of patients, BMI and serum albumin were significant determinant of lumbar spine and hip BMD, respectively.

Although fracture risk increases in long standing T1D patients by almost four to six folds, BMD in these patients is variable with inconsistent results across the studies. In a meta-analysis, Shah et al. reported that spine and femoral BMD was modestly lower in T1D adults as compared to controls [13]. Similarly in another meta-analysis, Vestergaard and colleagues observed lower BMD at the hip and spine in patients with T1D as compared to controls [7]. On the contrary, Pan and colleagues noted that the pooled differences in the lumbar spine BMD were not different between T1D subjects and controls [14]. Similarly, Leidig-Bruckner

et al. did not observe any difference in femoral neck BMD between T1D subjects and controls [15]. These were further reinforced by observation of Novak D and colleagues, who reported comparable femoral and lumbar spine areal BMD between T1D and control subjects [16]. In a lone study reported from India, Joshi et al. found lower BMD of total body and at lumbar spine in patients with T1D as compared to the control population [17]. Similar to most of these studies, we also noted apparently lower BMD and *Z*-score in patients with T1D compared to healthy controls.

Although HbA1C was significantly higher in T1D group compared to control, it did not show any correlation with lumbar spine and hip BMD. Duration of diabetes also did not appear to have significant influence on BMD. Similar to our finding, Leidig-Bruckner et al. also reported diabetes-specific variables including HbA1C and duration of diabetes were not significant predictors of the corresponding BMD [15]. Roggen et al. noted the absence of correlation between HbA1C and duration of diabetes with forearm volumetric apparent mineral density in T1D patients [18]. Karaguzel and colleagues also did not observe any correlation of BMD with duration of diabetes and HbA1C level [19]. However, Joshi et al. from India had shown that lower BMD at total body and at lumbar spine was associated with higher HbA1C [17].

We observed lower serum T_3 level in patients with T1D as compared to control. Six of our T1D patients had hypothyroidism, and all of them were on stable doses of L-thyroxine. Isolated low T_3 in T1D subjects had been documented in the literature. Völzke and colleagues reported lower serum free T3 levels in T1D cohort compared to control subjects suggestive of a possible low T_3 syndrome in the former group [20]. Tahirović et al. noted existence of euthyroid sick syndrome in children and adolescents with T1D having poor glycemic control with lower T3 and elevated rT3 level compared to control group [21]. Radetti and colleagues observed higher serum cortisol along with lower T_3 level in T1D subjects as compared to controls [22]. Stress models demonstrating an interplay between the hypothalamus-pituitary-thyroid and -adrenal axes provide a possible explanation of low T_3 syndrome in T1D subjects [23, 24].

In our T1D study cohort, BMI and serum albumin were significant determinants of lumbar spine and hip BMD, respectively. Further, BMI and serum albumin were trending to be significant predictors for hip and lumbar spine BMD, respectively. Similar to our finding, Leidig-Bruckner et al. noted that BMI was significantly associated with lumbar spine and femoral neck BMD [15]. Roggen et al. found a correlation between trabecular BMD and BMI *z*-score in female patients of T1D [18]. It is an established fact that lower BMI may be associated with increased risk of osteoporosis due to lesser quantity of mechanical loading produced thereof [6]. Apart from this, the adipose tissue, through the secretion of various adipocytokines, also increases BMD. Patients of T1D usually have low BMI, and hence, they are at increased risk of developing osteoporosis [25].

We observed lower serum albumin level in T1D subjects compared to control group. Insulin is an important hormone involved in regulation of albumin synthesis. The correlation of insulin reserve and serum albumin level is evident from animal models of insulin deficiency where three days of insulin therapy normalized serum albumin level [26]. This had been further established by Feo et al. who observed impaired hepatic production of albumin in insulin deficient state with a 10% rise in albumin synthesis daily following insulin infusion in individuals with diabetes mellitus [27]. Afshinnia et al. in a large retrospective analysis of data of 21 121 patients observed an independent association of lower serum albumin level with frequency of osteoporosis [28]. Although, the exact pathophysiological mechanism that correlate low albumin values with a decreased BMD is not clear, hypoalbuminemia may be directly or indirectly related to nuclear factor-κB (NF-κB). NF-κB can induce osteoclastic activity and may suppress osteogenesis [29–31]. Other proposed mechanism includes reduced albumin deposition and enhanced efflux from spongiosal components of bone, given the fact that albumin is present throughout the bony matrix [28].

Our study is limited by a relatively small sample size. A prospective study with a larger population of T1D may be more useful in delineating significant correlation between individual variables. We took single HbA1C value of individual T1D patient during the study. However, we know that glycemic adverse effects on any organ are cumulative in nature. Hence, an average HbA1C value over a prolong period may be reflective of a true glycemic burden in an individual rather than single point of time measurement and ideally should have been done in these kinds of studies. We did not evaluate the bone microarchitecture, which may be altered in patients with T1D. A further study detailing the bony microarchitecture by HRpQCT or bone histomorphometry may be more informative in this regard.

Conclusion

To conclude, patients of T1D have apparently reduced BMD and BMD *Z*-score as compared to control population. While BMI was a significant predictor of lumbar spine BMD, serum albumin was appeared to influence hip BMD. Further, long-term follow-up is required to delineate whether these findings would translate into increased fracture risk in the same group of patients.

Declarations

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Free T4 is associated with exenatide-related weight loss in patients with type 2 diabetes mellitus and obesity

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Abstract

Background Factors regarding exenatide-related weight loss have been underrecognized. We aimed to reveal the association between free T4 (fT4) level and exenatide-related weight loss, and change in thyroid function with exenatide treatment in euthyroid adult patients with type 2 diabetes and obesity.

Methods We included euthyroid adult patients with type 2 diabetes and obesity whom exenatide was added to metformin treatment. We excluded those with contraindication to exenatide or history of thyroid dysfunction. We analyzed baseline demographic, clinical and laboratory features, and the change (difference between the last [6th month] and baseline levels) in body weight, body mass index (BMI), TSH, fT4, fasting blood glucose, HbA1c. We grouped them as Group A: weight loss-absent vs. Group B: weight loss-present (<10%/≥10%).

Results In total ($n=106$), TSH-change was -0.077(±1.10), and fT4-change -0.0123(±0.20) ($p=0.229$ and $p=0.908$, respectively). TSH decreased more in group A than in Group B ($p=0.018$). Baseline and the last fT4 levels were higher in group B ($p=0.010$ and $p=0.004$, respectively). ROC curve analysis indicated that baseline fT4 (cut-off:1.16 ng/dL, AUC:0.708, $p=0.010$) was associated with weight loss. The ratio of patients having higher baseline fT4 (≥1.16) was higher in group B ($p=0.016$). Baseline BMI (≥40 kg/m^2) and fT4 (≥1.16 ng/dL) levels were positive predictors for weight loss ($p=0.024$ and $p=0.013$, respectively). Decrease in BMI was negatively correlated with baseline BMI ($p=0.002$).

Conclusion Exenatide provides more weight loss in the patients with higher baseline BMI or fT4. Thyroid function remains unchanged during treatment.

Keywords Diabetes · Exenatide · Thyroid · Obesity · Weight loss

Introduction

Obesity is a frequent co-existing condition in the patients with type 2 diabetes. In one study, 59% of the patients with type 2 diabetes did have obesity, 31% were overweight [1]. Hence, antidiabetic medications providing weight loss has come into prominence in type 2 diabetes. Glucagon like peptide 1 receptor agonists (GLP1Ra) have been increasingly used in the patients with type 2 diabetes especially if weight reduction is desired [2]. Oldest GLP1Ra is exenatide which was shown to provide both antihyperglycemic effect and weight loss in type 2 diabetes [3, 4]. It exerts weight reducing effect via increasing satiety and energy expenditure, nausea and decreasing caloric intake [5, 6].

Thyroid dysfunction was known to cause weight oscillations, but, recently, studies analyzing the association of thyroid hormones with obesity in euthyroid subjects with type 2 diabetes have gained popularity. Baseline free thyroxine (fT4) and free triiodothyronine (fT3) were found to be correlated with diet-induced weight loss in euthyroid overweight or obese subjects [7, 8]. However, iatrogenic subclinical hyperthyroidism due to levothyroxine (LT4) suppression

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therapy in thyroid cancer was shown not to provide extra weight reduction [9].

The effect of exenatide on the occurrence of thyroid cancer is controversial [10–14]. However, there is a limited number of studies indicating the effect of exenatide on serum thyroid-stimulating hormone (TSH) levels or thyroid volume in euthyroid patients with type 2 diabetes [15, 16]. In previous studies, baseline HbA1c, body mass index (BMI) and duration of type 2 diabetes were shown to be important factors for exenatide-related weight loss [17, 18]. No study regarding the possible associations between fT4 and exenatide-related weight loss in type 2 diabetes has been conducted. We primarily aimed to analyze the associations between baseline fT4 level and exenatide-related weight loss in euthyroid adult patients with type 2 diabetes and obesity, and as a secondary objective to observe the effect of exenatide use on thyroid functions.

Materials and methods

Data collection

Adult patients who were referred to the Adult Endocrinology Clinics of the Kocaeli Derince Training and Research Hospital between May 2019 and May 2020 and exhibited obesity and type 2 diabetes were included in this study. This observational, retrospective cohort study was approved by the Ethics Committee of our institution (University of Health Sciences, Kocaeli Derince Training and Research Hospital; approval number 2020/73) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

Euthyroid patients with type 2 diabetes and obesity (BMI of $\geq$30 kg/m^2) were included. Patients younger than 18 years of age; those with a history of chronic illnesses other than type 2 diabetes, obesity, hypertension or hyperlipidemia were excluded. The patients with secondary obesity (Cushing's syndrome or hypothyroidism) or type 1 diabetes also were excluded. Those with any contraindication to exenatide, history of overt or subclinical thyroid dysfunction, history of thyroid malignancy or surgery, or who had been prescribed LT4 or antithyroid medications, or antidiabetic agents other than metformin or exenatide, and those for whom data were missing were excluded.

Clinical, radiological and laboratory evaluation

Basic demographic information (age, sex) and clinical characteristics (height, body weight [BW], BMI, duration of type 2 diabetes [months]) were recorded. BW (kg) and height (m) were measured with the patient barefoot and in light clothing. The BMI was calculated as the BW/square of height (kg/m^2).

Exenatide was prescribed to the patients who had been taking metformin for a while. The patients were evaluated for contraindications to exenatide (renal failure [estimated glomerular filtration rate $<$30 mL/min/1.73 m^2], known hypersensitivity to exenatide, history of pancreatitis, multiple endocrine neoplasia syndrome type 2, personal or family history of medullary thyroid cancer, history of drug-induced immune-mediated thrombocytopenia) use before the initiation of treatment with exenatide. Abdominal sonography was performed to exclude cholelithiasis. If there was no contraindication to exenatide, it was initiated at a dose of 5 mcg subcutaneously (sc) twice daily in the first month of the treatment, and the dose was increased to 10 mcg sc twice daily after the first month. All the patients were under metformin treatment, and lifestyle changes, such as diet and physical exercise, also were recommended.

We measured BW, BMI, TSH, fT4, fasting blood glucose (FBG), and HbA1c both before the initiation (baseline) and at 6th month (last) of the treatment with exenatide. The patients with baseline thyroid function tests (TSH or fT4) out of normal range at the initiation of treatment were excluded. We assessed the change (the difference between the last and baseline levels) in BW, BMI, TSH, fT4, FBG and HbA1c with treatment. Change in the parameters were calculated as following: (last level)-(baseline level). BMI-change(%) was also calculated: [(last BMI)-(baseline BMI)]$\times$100/(baseline BMI).

According to the change in BW, we grouped the patients as Group A: weight loss-absent and Group B: weight loss-present (Group B1: $<$10% *vs.* Group B2: $\geq$10% weight loss). We grouped them also according to baseline fT4 (according to ROC curve analysis), TSH change (decreased *vs.* increased), fT4 change (decreased *vs.* increased), and HbA1c change (decreased *vs.* increased).

Independent of the basal cortisol level, to exclude autonomous cortisol secretion or Cushing's syndrome, we performed the 1-mg overnight dexamethasone suppression test (DST) in the patients. We excluded the patients with cortisol levels $\geq$1.8 mcg/dL after DST.

Low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (Tchol), and triglycerides (TG), anti-thyroid peroxidase (ATPO) were measured before the initiation of treatment with exenatide.

We grouped the patients also according to ATPO (negative *vs.* positive), presence of hypertension (absent *vs.* present), hyperlipidemia (hypercholesterolemia or hypertriglyceridemia) (absent *vs.* present).

All laboratory measurements were performed in the morning after an overnight fast. FBG was measured as mg/dL (mmol/L), LDL mg/dL (mmol/L), HDL mg/dL (mmol/L), Tchol mg/dL (mmol/L), TG mg/dL (mmol/L),

TSH mIU/L (mIU/L), fT4 ng/dL (pmol/L), and ATPO IU/mL.

FBG was measured via a glucose oxidase method with the aid of an AU-2700 analyzer (Olympus Co. Ltd., Tokyo, Japan). TG, Tchol, and HDL were measured on the same analyzer, using enzymatic methods (Olympus Diagnostics, Hamburg, Germany) and LDL levels were calculated using Friedewald's equation. HbA1c levels were measured as "%" in National Glycohemoglobin Standardization Program (NGSP) and as "mmol/mol" units using high-performance liquid chromatography (HPLC). TSH, fT4, and ATPO levels were measured via chemiluminescence methods using a DxI 800 system (Beckman Coulter, Inc., Fullerton, CA, USA).

Statistical analysis

SPSS software (ver. 22.0; IBM Corporation, Armonk, NY, USA) was used for all analyses. The Shapiro-Wilk test was used to assess the normality of the data. Homogeneity of variance was evaluated using the Levene test. When comparing two independent groups in terms of quantitative measures, Mann-Whitney U-tests were used. When comparing more than two independent groups in terms of quantitative measures, Kruskal Wallis test was used. Pearson's chi-squared tests were used to compare categorical variables. To detect the relationship between the real classification of the procedure's success and the classification made by the cut-off values, sensitivity and specificity ratios, and positive and negative predictive values were expressed by ROC (Receiver Operating Characteristic) curve analysis with nonparametric method. To determine the risk factors regarding weight loss, we used multiple logistic regression analysis. Wilcoxon signed rank test was used to assess the change in BMI, TSH, fT4, FBG and HbA1c levels between first and the last measurements. To analyze the correlations of variables with each other, Spearman's (Rho) correlation was used. Quantitative variables are reported as the means (X) ± standard deviations (SDs) in the tables. Categorical variables are reported as numbers (n) and percentages (%), and p-values <0.05 were taken to indicate statistical significance.

Results

Of total 106 patients, mean age was 50.51 ± 9.93. Mean age and duration of type 2 diabetes were similar among groups. Only 13.20% ($n=14$) of the patients did have a diagnosis of type 2 diabetes longer than 12 months. 14.15% ($n=15$) of the patients did not lose weight (Group A). Baseline BMI was significantly lower in Group A than in group B ($p=0.010$), or Group B1 or B2 ($p=0.009$) (Table 1). BMI-change and BW-change were positive in Group A, but negative in Group B, B1, and B2. TSH-change was more negative in Group A than in Group B. Baseline and last fT4 were significantly lower in Group A than in Group B. However, fT4-change was similar among the groups. FBG, HbA1c, and lipid levels were similar among groups (Table 2).

BW, BMI, FBG and HbA1c were significantly reduced during treatment with exenatide ($p<0.001$), but TSH or fT4 did not change significantly in total ($p=0.229$ and $p=0.908$, respectively). Additionally, in Group A, there was a significant change in TSH ($p=0.041$) (Table 3).

ROC curve analysis indicated that baseline fT4 (cut-off:1.16 ng/dL, AUC:0.708, $p=0.010$) was associated with weight loss (Table 4 and Fig. 1).

Table 1 Comparison of demographic, baseline clinical and laboratory parameters regarding the presence of weight loss

Parameters	Group A ($n=15$)	Group B ($n=91$)			Total ($n=106$)	p1	p2
		Group B1 ($n=49$)	Group B2 ($n=42$)	Group B Total ($n=91$)			
	X(± SD)						
Age (year)	52.67(11.34)	49.35(9.51)	51.10(9.94)	50.15(9.70)	50.51(9.93)	0.282	0.460
Duration of T2D (months)	5.60(6.09)	5.96(6.05)	4.88(5.29)	5.46(5.71)	5.48(5.73)	0.830	0.651
Baseline BMI (kg/m^2)	38.89(6.17)	43.80(7.74)	46.38(7.61)	44.99(7.75)	44.13(7.81)	0.010	0.009
Baseline TSH (mIU/L)	2.32(1.50)	2.12(1.29)	2.16(1.20)	2.14(1.24)	2.17(1.27)	0.849	0.947
Baseline fT4 (ng/dL)	1.11(0.16)	1.24(0.22)	1.25(0.17)	1.25(0.20)	1.23(0.20)	0.010	0.031
Baseline FBG (mg/dL)	128.46(20.88)	146.48(45.52)	143.73(53.73)	145.21(49.22)	142.84(46.57)	0.404	0.579
Baseline HbA1c (%)	7.49(1.02)	7.32(1.26)	7.15(1.64)	7.24(1.44)	7.28(1.39)	0.233	0.240
LDL (mg/dL)	126.0(30.48)	112.91(29.83)	124.08(31.46)	118.06(30.94)	119.19 (30.85)	0.301	0.257
HDL (mg/dL)	43.46(10.34)	43.24(9.37)	43.05(12.11)	43.15(10.66)	43.20(10.57)	0.960	0.711
TG (mg/dL)	181.93(86.03)	180.20(82.11)	167.22(92.79)	174.21(86.95)	175.30(86.46)	0.888	0.678
TChol (mg/dL)	208.46(38.20)	191.26(32.50)	199.83(40.66)	195.21(36.54)	197.09(36.89)	0.187	0.358

p1: the difference between Group A and B. p2: the difference between Group A, B1 and B2

Table 2 Change in clinical and laboratory parameters during exenatide treatment

Parameters	Group A ($n=15$)	Group B ($n=91$)			Total ($n=106$)	p1	p2
		Group B1 ($n=49$)	Group B2 ($n=42$)	Group B Total ($n=91$)			
	X(± SD)						
Last BMI (kg/m^2)	39.44(6.33)	41.43(7.46)	38.41(6.56)	40.04(7.18)	39.95(7.04)	0.824	0.133
BMI-change	0.55(0.66)	-2.36(1.31)	-7.96(3.75)	-4.95(3.90)	-4.17(4.10)	<0.001	<0.001
BMI-change (%)	1.39(1.73)	-5.41(2.85)	-16.94(6.85)	-10.73(7.69)	-9.01(8.31)	<0.001	<0.001
Last TSH (mIU/L)	1.77(1.31)	2.08(1.22)	2.21(1.14)	2.14(1.18)	2.09(1.20)	0.171	0.336
TSH-change	-0.55(1.28)	-0.03(0.86)	0.04(1.25)	0.0003 (1.06)	-0.077(1.10)	0.018	0.049
Last fT4 (ng/dL)	1.09(0.17)	1.23(0.15)	1.24(0.15)	1.24(0.15)	1.22(0.16)	0.004	0.016
fT4-change	-0.0213(0.12)	-0.011(0.24)	-0.01(0.19)	-0.0108(0.22)	-0.0123(0.20)	0.586	0.768
Last FBG (mg/dL)	134.73(43.08)	129.83(36.62)	127.30(39.44)	128.67(37.75)	129.52(38.39)	0.583	0.604
FBG-change	6.26(47.43)	-16.65(34.99)	-16.42(34.03)	-16.54(34.36)	-13.32(37.09)	0.394	0.679
Last HbA1c (%)	7.08(1.13)	6.88(1.34)	6.66(1.33)	6.78(1.33)	6.82(1.30)	0.272	0.298
HbA1c-change	-0.41(0.79)	-0.43(0.86)	-0.49(0.98)	-0.46(0.91)	-0.45(0.89)	0.549	0.836

p1: the difference between Group A and B. p2: the difference between Group A, B1 and B2

Table 3 Change in clinical and laboratory parameters during exenatide treatment

Change in parameters	Total *p* value	Group A	Group B
BMI (kg/m^2)	<0.001	0.011	<0.001
TSH (mIU/L)	0.229	0.041	0.779
fT4 (ng/dL)	0.908	0.589	0.971
FBG (mg/dL)	<0.001	0.712	<0.001
HbA1c (%)	<0.001	0.018	<0.001

Sex, presence of hyperlipidemia, ATPO positivity, last HbA1c or HbA1c-change were similar among the groups. Hypertension was more frequent in Group A than in Group B1 and B2 ($p=0.024$). The ratio of patients whose TSH decreased was higher in Group A than in Group B ($p=0.017$), the difference was more prominent between Group A and Group B2 than the difference between Group A and B1. The ratio of baseline fT4 of <1.16 ng/dL was found as higher in Group A than in Group B ($p=0.016$) (Table 5).

Baseline free T4 and baseline BMI were used in the model of multiple logistic regression analysis. Baseline BMI (≥40 kg/m^2) and baseline fT4 (≥1.16 ng/dL) levels were found as positive predictors for weight loss (Table 6).

In total, baseline fT4 was negatively correlated with BMI-change (%) (Rho:-0.168; $p=0.086$). There were not any significant correlation besides this finding.

Discussion

We showed that BW, BMI, FBG and HbA1c were reduced, but TSH or fT4 did not change significantly with exenatide treatment. Weight loss was associated with higher baseline and last fT4, but not fT4-change. Higher baseline BMI and baseline fT4 were important predictors of exenatide-related weight loss.

In one study analyzing 97 obese patients under weight reduction intervention (lifestyle change), baseline fT4 or fT3 did not significantly change after 6 months of lifestyle changes, and also revealed that higher baseline fT4 was associated with more weight loss, but only in females [7]. In our study, baseline fT4 was associated with weight loss in both sexes. Similarly, in a randomized trial, higher baseline fT3 and fT4, but not baseline TSH or fT4-change, were shown to be associated with greater weight loss at 6 months of energy-reduced diets in overweight and obese euthyroid adult patients [8]. In that study, fT4 or fT3 levels were shown not to be associated with weight regain. We could not analyze

Table 4 ROC curve analysis indicating the cut-off values of the factors associated with weight loss

Dependent variable: weight loss	Cut-off	Sensitivity	Specificity	+PV	-PV	AUC ± SE	95% CI	*p* value
Baseline fT4 (ng/dL)	≥1.16	65.90%	63.35%	91.6	23.46	0.708 ± 0.068	0.574–0.842	0.010

ROC (Receiver Operating Characteristic) Curve Analysis (Honley&Mc Nell—Youden index J), AUC: Area under the ROC curve, SE: Standard Error, CI: Confidence Interval + PV: Positive Predictive Value, -PV: Negative Predictive Value

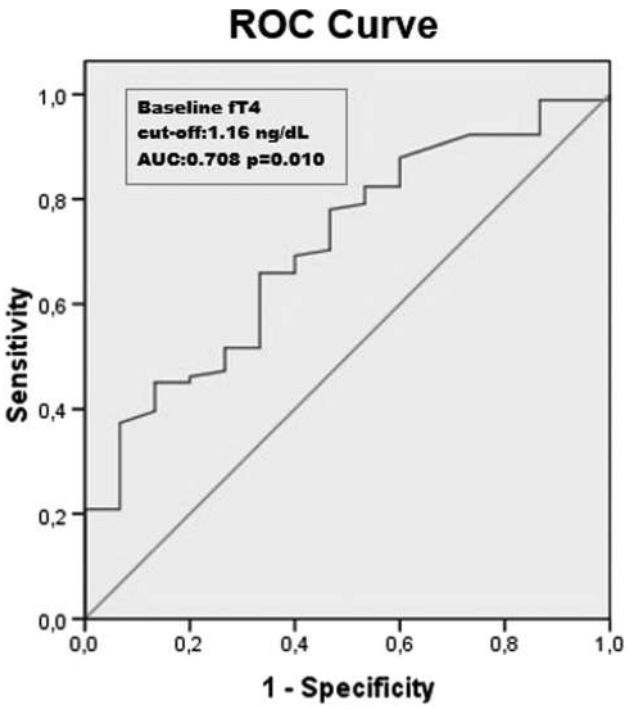

Fig. 1 ROC curve, the optimal cutoff value, sensitivity, and specificity of baseline free T4

the maintenance of weight change or weight regain after the treatment. In one study analyzing euthyroid patients who underwent bariatric surgery, those with lower baseline TSH did lose more weight after laparoscopic adjustable gastric banding [19]. Higher baseline TSH and fT3 were shown to be associated with substantial weight loss after lifestyle intervention also in children [20]. We found that TSH did not significantly change during exenatide treatment, and baseline TSH was not correlated with weight change.

Table 6 Multiple logistic regression analysis showing predictors of exenatide-related weight loss

Variables	Weight loss Adjusted odds ratio (95% CI)	*p* value
Baseline BMI (>40 kg/m^2)	3.953 (1.203–12.993)	0.024
Baseline fT4 (≥1.16 ng/dL)	4.667 (1.385–15.724)	0.013

Some studies revealed that fT4 or fT3 levels might be increased in obesity [21, 22]. Higher fT3 or fT4 was shown to correlate positively with sleeping metabolic rate and lipid oxidation, but negatively with weight gain [23–25]. We revealed that higher baseline fT4 predicted more weight loss. Higher fT4 is associated with higher metabolic rate, which may explain the association of baseline fT4 levels and the extent of weight-loss. However, contradictory results regarding the effect of thyroxine on resting energy expenditure were reported [26, 27]. In one study, short-term LT4 treatment was shown not to affect resting metabolic rate or exercise efficiency [28]. Iatrogenic subclinical thyrotoxicosis in the patients with thyroid cancer under LT4 suppression was shown not to provide any extra weight loss in comparison to euthyroid patients under LT4 replacement [9].

Elevated TSH in obesity was proposed to be a result of thyroid hormone resistance, decreased expression in TSH receptor, or elevated leptin secretion from adipose tissue [29–31]. In some studies, elevated thyroid hormones in obesity were demonstrated to decrease during weight loss by lifestyle intervention, and the decrease, in turn, was known to be related with weight re-gain [20, 32, 33]. However, in

Table 5 Comparison of categorical parameters between the groups

Parameters	Group A (*n* = 15)	Group B			Total (*n* = 106)	p1	p2
		Group B1 (*n* = 49)	Group B2 (*n* = 42)	Group B Total (*n* = 91)			
	n						
Sex (female/male)	9/6	36/13	31/11	67/24	76/30	0.278	0.554
Baseline BMI (<40/≥40 kg/m^2)	9/6	19/30	10/32	29/62	38/68	0.035	0.036
Hypertension (absent/present)	12/3	33/16	19/23	52/39	64/42	0.094	0.024
Hyperlipidemia (absent/present)	13/2	42/7	37/5	79/12	92/14	0.988	0.946
TSH-change (decreased/increased)	13/2	29/20	20/22	49/42	62/44	0.017	0.031
Baseline fT4 (<1.16/≥1.16 ng/dL)	10/5	19/30	12/30	31/60	41/65	0.016	0.034
fT4-change (decreased/increased)	8/7	23/26	15/27	38/53	46/60	0.402	0.394
ATPO (negative/positive)	14/1	45/4	36/6	81/10	95/11	0.611	0.557
HbA1c-change (decreased/increased)	13/2	39/10	31/11	70/21	83/23	0.396	0.559

p1: the difference between Group A and B. p2: the difference between Group A, B1 and B2

our study, TSH decrease was more prominent in the patients who did not have weight loss. We cannot explain this change, but it may be related with the sample size and the duration of follow-up.

Exenatide was known to provide weight loss in the patients with obesity and type 2 diabetes, but not shown to cause excess weight loss in the normal-weight patients with type 2 diabetes [2, 3, 17, 18]. It was also shown to lead weight loss also in obese subjects without type 2 diabetes [17]. In our study, although both groups (weight loss-absent or weight loss-present) did have obesity, baseline BMI was associated with exenatide-related weight loss. In one study including patients with newly diagnosed type 2 diabetes treated with exenatide, there was a nonsignificant trend from normal-weight to overweight to obese patients for weight reduction [18]. In various studies, higher baseline BMI was found to be associated with more weight loss in the patients with type 2 diabetes under exenatide treatment [4, 6, 17]. Some genetic factors, such as GLP1 receptor variants, might affect the extent of weight loss associated with exenatide in various BMI levels [34, 35].

In one study, exenatide treatment was shown to lead to a significant reduction in TSH, but no change in fT4, fT3 or thyroid autoantibody levels or thyroid volume in type 2 diabetes [15]. They did not analyze any association between weight loss and thyroid hormones. We showed no significant change in thyroid function tests with exenatide treatment, but baseline fT4 was an important predictor for weight loss in the patients with type 2 diabetes under exenatide treatment.

Strength and limitations

Our study is the first to investigate the association of fT4 levels with weight loss in the patients with type 2 diabetes under exenatide treatment. We could not measure and analyze fT3 levels. Our study was designed in a retrospective manner which might have difficulties in such an analysis of responders (weight loss) of exenatide in a population. It would be beneficial to compare euthyroid, subclinical hyperthyroid and hypothyroid, and overt hypothyroid patients with type 2 diabetes under exenatide treatment.

Conclusion

We concluded that weight loss occurred with exenatide in the majority of euthyroid patients with type 2 diabetes. Exenatide provides more weight loss in those with greater obesity, and higher baseline fT4. Thyroid function seems to remain unchanged during exenatide treatment.

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

The authors assure that this paper has not been published before nor has been submitted for publication to another scientific journal.

The abstract of our article was accepted and presented as an "Audio Electronic Poster" (AEP-223) with a heading of "Free T4 is associated with exenatide-related weight loss in patients with type 2 diabetes mellitus" in 2021 online congress of European Society of Endocrinology (e-ECE 2021) and as an oral presentation in Endokurs-5 2021 (S-22).

Declarations

Ethics approval and consent to participate This observational, retrospective cohort study was approved by the Ethics Committee of our institution (University of Health Sciences, Kocaeli Derince Training and Research Hospital; approval number 2020/73) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

Human rights Written informed consent was given by all the participants included in the study.

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

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ORIGINAL ARTICLE

Prospective study of clinical, biochemical, and radiological characteristics of diabetic Charcot neuroarthropathy at a tertiary care centre

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Abstract

Objective The aim of this study was to assess demographic, clinical, biochemical, radiological profile and treatment response in diabetic patients with Charcot neuroarthropathy (CN).

Methods This was a prospective study for screening of CN in patients with diabetes mellitus (DM) attending tertiary care centre over a period of 1 year. Acute CN (ACN) was diagnosed based on clinical features of local inflammation and temperature difference of > 2 °C from the normal foot after exclusion of other inflammatory causes. Chronic CN (CCN) was diagnosed when no inflammatory signs were present in a deformed foot with radiological findings supportive of diagnosis. In all these patients, demographic data, clinical features, biochemical investigations, X-ray, and MRI foot were done. The effect of offloading and customized foot wear in ACN, CCN were, respectively, studied over 1 year.

Results Out of 5049 DM patients screened for CN over 1 year, 25 patients (0.49%) were diagnosed to have CN, of which 12 had ACN (0.23%) and 13 had CCN (0.26%). CN patients had significantly higher mean body mass index (BMI) (27.9 vs. 26.2 kg/m^2; $p = 0.02$), longer duration of DM (12 vs. 9.6 years; $p < 0.001$), higher HbA1c (10.3 vs. 8.8%; $p = 0.001$), greater degree of peripheral neuropathy and retinopathy compared to controls. MRI could be able to detect 25% ACN cases where X-rays were non-diagnostic. The median duration of clinical resolution was 3 months in ACN patients.

Conclusions High index of suspicion is required for diagnosing CN in DM patients.

Keywords Diabetes mellitus · Acute Charcot neuroarthropathy · Chronic Charcot neuroarthropathy · Clinical resolution

Introduction

Diabetic Charcot neuroarthropathy (CN) is a not a rare but a serious complication of diabetes mellitus (DM) and is often missed in early stages leading to fractures, dislocations, and deformities. Various studies have suggested that CN in addition to foot outcomes also contributes to early and higher mortality independent of foot ulcer and other comorbidities [1, 2]. Early diagnosis and appropriate offloading in acute Charcot neuroarthropathy (ACN) and customized footwear for chronic Charcot neuroarthropathy (CCN) are cornerstones in the management of CN. Neurovascular and neurotraumatic theories have been proposed as the pathogenetic mechanisms for the development of diabetic CN [3, 4]. Increased vascularity due to autonomic neuropathy, repeated unnoticed trauma because of loss of protective sensation (LOPS), increased levels of cytokines (TNF-α, IL-6), and decreased secretion of calcitonin gene-related peptide (CGRP) contributes to progressive joint and bone destruction. TNF-α and IL-6 enhance osteoclast-mediated bone resorption, which is further facilitated by decreased CGRP, increasing the ratio of receptor activator of nuclear factor kappa-B ligand (RANKL) to osteoprotegerin (OPG) in favour of RANKL, thereby inducing osteoclastogenesis [5]. The prevalence of CN in diabetic population has recently been reported to be between 0.1 and 7.5% [6]. There are very few studies which comprehensively looked into clinical, biochemical, and radiological aspects in a prospective manner and the effect of offloading in CN patients in the Indian context [7].

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The present study plans to evaluate the demographic, clinical, and biochemical characteristics unique to CN patients compared to controls and prospectively evaluate the response to total contact cast (TCC) and customized footwear in ACN and CCN patients respectively over 1 year.

Materials and methods

This study was carried out in the Department of Endocrinology, MKCG MCH, over a period of 1 year from January 2021 to January 2022. All the patients with DM visiting the Department of Endocrinology were screened for CN. ACN was diagnosed to be present when there were features of inflammation like redness, pain, tenderness, warmth along with temperature difference of > 2 °C from the unaffected foot after the exclusion of other inflammatory conditions like gout, cellulitis, osteomyelitis, rheumatoid arthritis. CCN was diagnosed to be present when foot deformity was present in the absence of inflammatory signs after exclusion of other causes of foot deformity like trauma, previous fractures, congenital deformities, with radiological features suggestive of CCN. In all these patients, a detailed demographic data and medical histories were taken including age, sex, weight, height, duration of diabetes, antidiabetic medications, hypertension, personal history of occupation, smoking habit, alcohol consumption, and employment status.

Clinical assessment included features of inflammation in the foot and temperature of the foot by infrared thermometer (Otica Meditronix Co., with accuracy ± 0.2 °C and measurement range from 0 to 100 °C). The temperature difference from the unaffected foot was assessed, and a difference of > 2 °C was defined to be significant. Detailed neurological assessment of the feet was done to detect the loss of pain, touch, vibration sensation (128 Hz tuning fork) and loss of protective sensation with 10-g monofilament. Further clinical assessment was conducted to detect the presence of callus, anhidrosis, fissures, tinea pedis, active ulceration, cellulitis, oedema, and presence of amputation at enrolment. Vascular assessment of feet was done by examining all peripheral pulses and ankle brachial index (ABI). Peripheral arterial disease (PAD) was diagnosed if ABI was less than 0.9.

Biochemical investigations including HbA1c that was estimated by high-performance liquid chromatography (HPLC) method on Bio-Rad 10 analyser, fasting plasma glucose (FPG), 2-h post-prandial plasma glucose (2-h PPG) were estimated in Seimens AUTOPAK 300 auto analyser, and lipid profile, Sr. creatinine, and Sr. urea were estimated by TOSHIBA 120 FR automated analyser. Complete blood count with ESR was done in all cases. C-reactive protein (CRP) levels and urinary spot albumin creatinine ratio (ACR) were estimated by nephelometry in a protein analyser (MISPA-i_3). Diabetic nephropathy was defined by the presence of spot urinary ACR of ≥ 30 mg/g of creatinine on two different occasions.

Radiological investigations included X-ray of both feet anteroposterior (AP) and oblique views. The modified Eichenholtz classification [8], which relies on clinical and x-ray findings, was used for staging of Charcot foot. Stages 0 (prodromal phase) and 1 (development phase) are taken as ACN, and stages 2 (coalescence phase) and 3 (consolidation phase) are taken as CCN [9].

The anatomical location of CN distribution on the affected foot was done according to Sander's and Frykberg's classification system [10]. To determine the severity of deformity in Charcot feet, Meary's angle, calcaneal pitch, and cuboid height were calculated from X-ray of foot in oblique view [3]. Meary's angle is generally close to zero degree, and Calcaneal pitch normally lies between 20 and 30° [11].

MRI foot was done in all cases of suspected CN by a single radiologist for detection of earliest lesions. MRI protocol for Charcot foot included sagittal T1, transverse T1 foot images including short tau inversion recovery (STIR), and coronal T2 hindfoot. The presence of bone marrow oedema, soft tissue oedema, bone dislocations, fragmentation, and fractures was noted in all ACN cases.

Patients with ACN were offloaded with TCC and were followed every fortnightly till clinical resolution. Clinical remission of active CN was defined as absence of inflammatory signs and temperature difference < 2 °C between the affected foot and a similar site on the opposite foot on two successive follow-up visits 2 weeks apart [12].

During each follow-up visit of ACN patients on TCC, an average of three temperature recordings at the region of interest of foot was obtained after the removal of cast for 30 min. Inflammatory markers like ESR were done at clinical resolution. MRI of feet was repeated in cases of doubtful resolution. Blood investigations like FPG, 2-h PPPG, HbA1c were done to check for the glycemic status of patients and were treated accordingly. After clinical remission of active CN, the TCC was discontinued, and participants were provided with customized footwear for Charcot foot during subsequent follow-up. Patients were reviewed for 3 months with thorough foot examination for recurrence of CN.

Customized footwear was prescribed in all CCN patients. Patients with foot ulcer were followed at 2-week interval to look for healing of ulcer or development of any complications like new ulcer formation or osteomyelitis by clinical examination and necessary investigations like X-ray foot where required. Glycemic status and progression of foot deformities were checked every 3 months in all CCN patients.

Age- and gender-matched patients with DM and without CN who consented for the study were selected as controls in

the ratio of 5:1. The study was approved by the Institutional Ethics Committee of MKCG Medical College, Berhampur. Appropriate informed consent was obtained from all study participants, and confidentiality of data was maintained throughout the study.

Statistical analysis

Statistical analysis was carried out by using Microsoft Excel 2007 (Microsoft Corp, Redmond, WA). Descriptive statistics for the categorical variables were performed by computing the frequencies (percentages) in each category. For the quantitative variables, approximate normality of distribution was assessed by using Shapiro–Wilk test. Variables following normal distribution were summarized by mean and standard deviation (SD), and the remaining variables were summarized as median (inter-quartile range [IQR]). Continuous variables were compared using Student's *t*-test and Mann–Whitney U-test, and categorical variables were compared by using χ^2-test. A *p*-value of less than 0.05 was considered statistically significant.

Results

In the present study among 5049 diabetes patients screened for CN, 25 patients were detected to have CN. Out of 25 patients with CN, one had type 1 DM and the rest 24 patients had type 2 DM. Among the 25 CN patients, 12 patients had ACN (0.23%) and 13 patients had CCN (0.26%). The control group constituted 125 age- and sex-matched DM patients without CN (Fig. 1).

The comparison of baseline characteristics of cases and controls is shown in Table 1. The mean ages of cases and controls were 55.6 ± 8.8 years and 54.7 ± 8.5 years, respectively, and were not statistically different. The CN patients had significantly higher BMI (27.9 ± 3.2 vs. 26.2 ± 2.7 kg/m2), longer duration of DM (12 ± 3.9 vs. 9.6 ± 3.5 years) and higher HbA1c levels (10.3 ± 2.4% vs. 8.8 ± 1.9%) compared to the controls. Sensory neuropathy (100% vs. 73%) and retinopathy (68% vs. 46%) were significantly more common in CN patients than controls. There was no significant difference in the prevalence of hypertension, diabetic nephropathy (urine ACR), CAD, CVA, and PAD (Table 1).

Comparing clinical features between acute and chronic CN

The mean ages of ACN and CCN patients were 52.6 ± 7.6 years and 54.4 ± 4.8 years, respectively. Median delta temperature at presentation in ACN patients was 3 °C (IQR 2.7–3.1 °C). There was no significant difference between these two groups with respect to BMI (26.2 ± 7.8 vs. 27.1 ± 1.4 kg/m2), smoking, duration of DM, glycemic status (HbA1c), lipid profile, and prevalence of hypertension, neuropathy, retinopathy, nephropathy, CAD, CVA, or PAD. However, active foot ulcer (67% vs. 22%, $p = 0.03$),

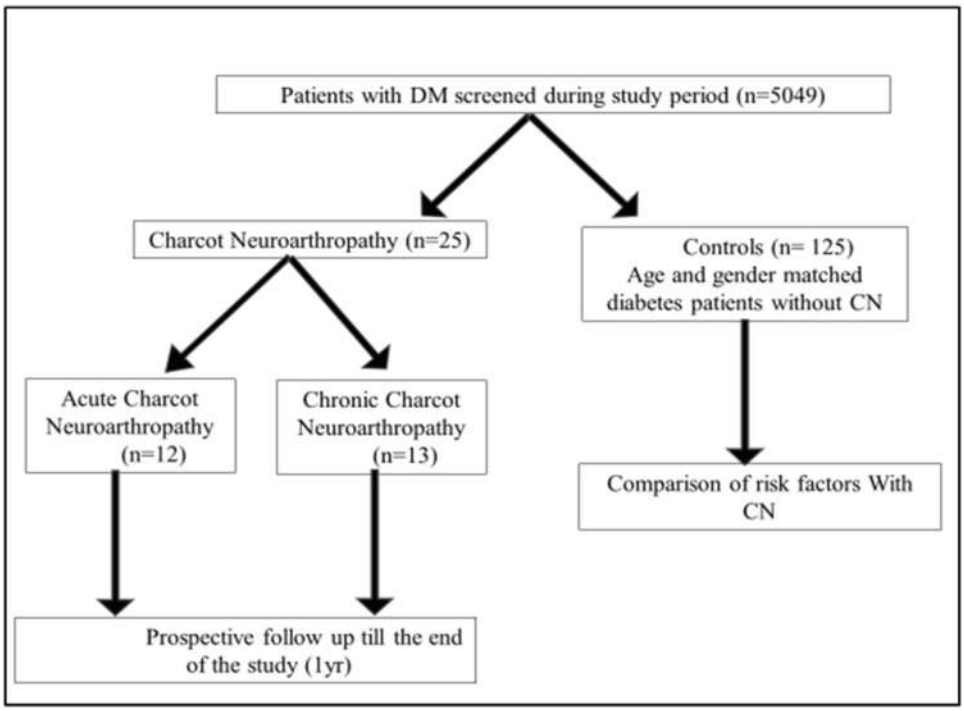


Fig. 1 Overview of study

Table 1 Comparison between CN patients and their controls at baseline

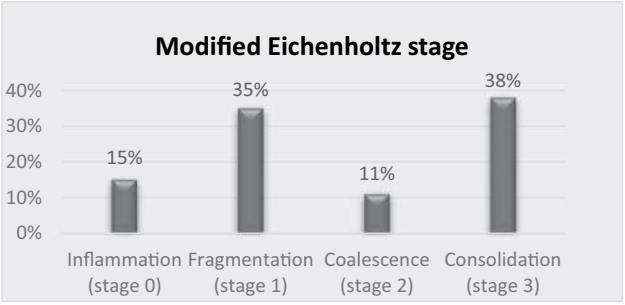
Variable	CN ($n=25$)	Controls ($n=125$)	p-value
Age (years)	55.6 ± 8.8	54.7 ± 8.5	0.84
Gender (males/females)	17/8	85/40	0.90
History of smoking, *n* (%)	8 (32)	36 (29)	0.74
BMI (kg/m^2)	27.9 ± 3.2	26.2 ± 2.7	0.02
Duration of DM (years)	12.0 ± 3.9	9.6 ± 3.5	0.001
FPG (mg/dL)	171 ± 45	154.8 ± 38.4	0.02
2-h PPG (mg/dL)	264.7 ± 17.2	209.2 ± 28.6	0.04
HbA1c (%)	10.3 ± 2.4	8.8 ± 1.9	0.001
Creatinine (mg/dL)	1.1 ± 0.5	1.0 ± 0.5	0.15
eGFR (mL/min/1.73 m^2)	76.9 ± 34.2	84.4 ± 30.9	0.50
UACR (mg/g)	63.8 ± 87.6	38.5 ± 46.9	0.17
Triglycerides (mg/dL)	161 ± 25.4	156.9 ± 33.3	0.63
LDL c (mg/dL)	124.6 ± 19.5	129.7 ± 28.3	0.23
HDL c (mg/dL)	42.2 ± 9.2	43.5 ± 8.8	0.43
Hypertension, *n* (%)	17 (68)	73 (58)	0.37
Neuropathy, *n* (%)	25 (100)	91(73)	0.001
Nephropathy, *n* (%)	5 (20)	16 (13)	0.34
Retinopathy, *n* (%)	17(68)	58 (46)	0.04
ABI	Rt:1.16 ± 0.21 Lt:1.20 ± 0.25	Rt:1.15 ± 0.22 Lt:1.17 ± 0.21	0.86 0.48
PAD, *n* (%)	2 (8)	24 (19)	0.18
CAD, *n* (%)	5 (20)	19 (15)	0.55
CVA, *n* (%)	3 (12)	10 (8)	0.51
On OAD only, *n* (%)	8 (32)	62 (50)	0.10
On insulin only, *n* (%)	11 (44)	43 (34)	0.36
On OAD with insulin, *n* (%)	6 (24)	20 (16)	0.33

CN, Charcot neuroarthropathy; *BMI*, body mass index; *DM*, diabetes mellitus; *FPG*, fasting plasma glucose; *eGFR*, estimated glomerular filtration rate; *UACR*, urinary albumin creatinine ratio; *LDLc*, low density lipoprotein cholesterol; *HDLc*, high density lipoprotein cholesterol; *ABI*, ankle brachial index; *PAD*, peripheral arterial disease; *CAD*, coronary artery disease; *CVA*, cerebrovascular accident; *OAD*, oral antidiabetic medication

clawing of toes (92% vs. 31%, $p=0.02$), and Rocker bottom feet deformity (77% vs. 8%, $p<0.001$) were significantly more common in CCN compared to ACN.

Radiological findings in CN patients

Of the 25 patients with CN, the right foot was involved in 14 patients and the left foot was involved in 10 patients and, in one patient, both feet were involved (total of 26 Charcot feet in 25 patients). Staging of CN according to Eichenholtz is given in Fig. 2. Out of 12 ACN patients, three patients were detected to be in Eichenholtz stage 0



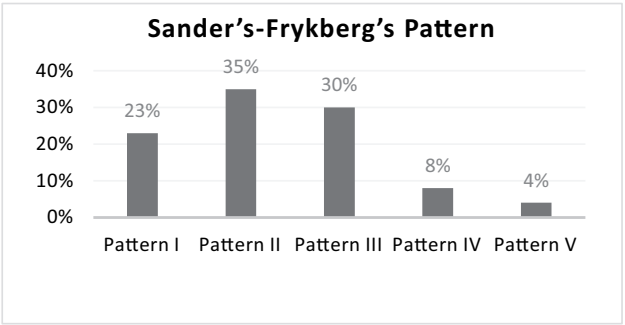


Fig. 2 Pattern of involvement of foot in CN patients

(one had bilateral feet involvement) and nine patients were in stage 1. Out of 13 CCN patients, three patients were detected to be in Eichenholtz stage 2 and 10 patients were in stage 3 (Fig. 3).

The pattern of involvement of joints in the foot in the present study according to Sanders and Fryberg classification (Fig. 2) shows pattern II (TMT joints) was the most common (35%) followed by pattern III (intertarsal joints, 30%) followed by pattern I (MTP and IP joints, 23%). Least involved was pattern V (calcaneum) (4%). In three patients, standard X-rays could not detect ACN with clinical features of inflammation was picked by MRI. Meary's angle was increased in 65% (17 feet), calcaneal pitch was decreased in 50% (13 feet), and cuboid height was decreased in 58% (15 feet) of all CN patients.

MRI findings

MRI foot was done in all cases of CN. Patients with Eichenholtz stage 0 ACN had bone marrow oedema and soft tissue oedema in sagittal STIR sequence. In the present study, three patients who were in stage 0 with normal X-ray findings were detected by MRI with bone marrow oedema. MRI findings in feet, which were in stage of fragmentation, were bony destruction with cortical fractures and dislocations with bone marrow oedema and soft tissue oedema in STIR sequence. MRI findings of CCN (Eichenholtz stages 2 and 3) were fractures, dislocations, with subchondral cysts with

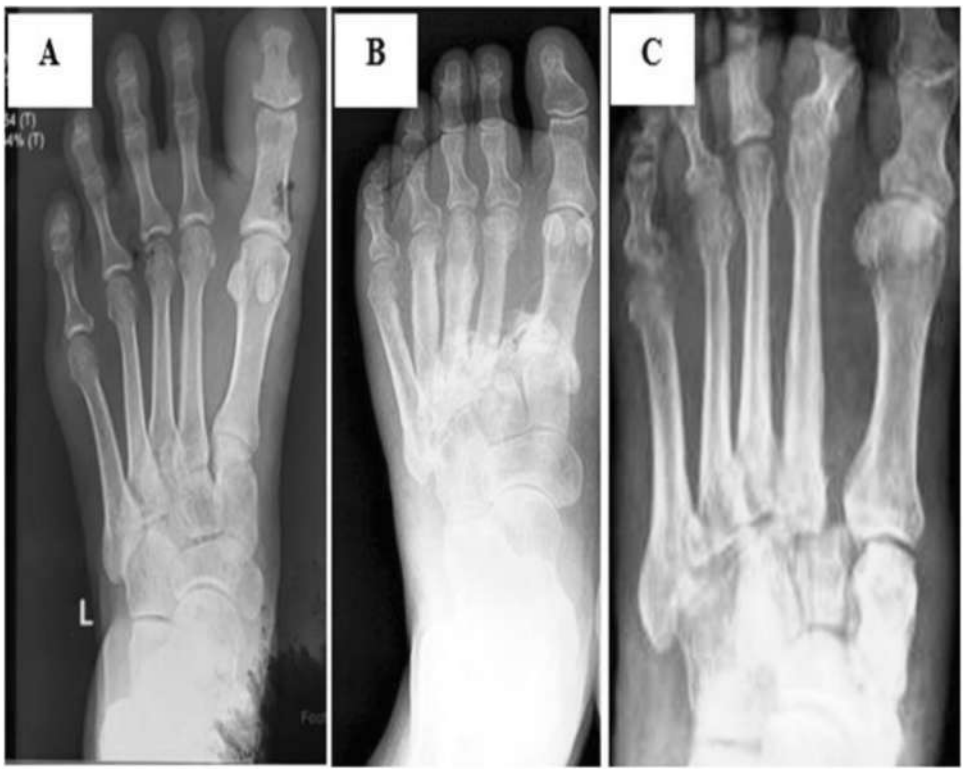


Fig. 3 **A** Radiograph of the left foot AP view showing no abnormalities (stage 0); **B** lateral subluxation of 2nd to 5th metatarsal bases with fracture at the base of 2nd metatarsal and dislocation of medial Lisfranc joint and obliteration of Lisfranc joint space (stage 1); **C** fusion and coalescence of larger fragments and sclerosis of bones (stage 2)

intraarticular bodies with gross deformity of feet without bone marrow oedema in STIR sequences. Two patients of CCN had osteomyelitis, which was detected by MRI by the ghost sign (bones that disappear on T1-weighted images and reappear after contrast or on T2W images).

Comparison of systemic inflammatory parameters in acute and chronic CN at baseline

To look for the systemic inflammation, CRP, ESR, and TLC were done in cases of CN. Patients with ACN had significantly higher median (IQR) CRP compared to patients with CCN [21 mg/L (IQR 15.5–26) vs. 8 mg/L (IQR 5–9); $p = 0.04$]. There were no statistically significant differences with respect to ESR and TLC between acute and chronic CN patients.

Follow-up of CN patients

Follow-up of acute CN patients

The median duration of follow-up in the ACN patients after clinical resolution was 4 months (IQR 3.5–4.7 months), and the total duration of follow-up was 7.7 months (IQR 6–9.7 months). At the end of the study, 11 out of 13 Charcot feet had complete clinical resolution, two were in follow-up as they were not in clinical resolution. These two patients had fragmentation and dislocation (Eichenholtz stage 1) and did not comply with the offloading protocol. The median duration for complete clinical resolution in patients with acute CN was 3 months [IQR 2.5–4.5 months]. Depending on the location of arthropathy, healing time in TCC varied. The median duration for clinical resolution for forefoot arthropathy was 2.5 months (IQR 2–3 months), and for midfoot and hindfoot arthropathies, it was 4 months (IQR 3–4.5 months) and 6 months, respectively. There was no statistically significant difference in the median duration of clinical resolution for Eichenholtz stage 0 and stage 1 [3 (IQR 2–4.5) months vs. 4 (IQR 3–6) months; $p = 0.25$] in all regions**.** On follow-up of ACN patients at the time of clinical resolution, there was a significant decrease in FPG, 2-h PPG, HbA1c, and inflammatory markers (ESR, CRP) from the baseline.

Follow-up of chronic CN patients

The median duration of follow-up in CCN patients was 6 months (IQR 4–8.7). Of the 13 patients with CCN, six had ulcers at the time of initial diagnosis. On follow-up with customized footwear and appropriate therapy, all the patients had healing of ulcers with a median duration of 1.5 months. For the seven patients who had no ulcers at the time of diagnosis with proper customized footwear, none of them developed new ulcers at the end of the study. At the end of the study, there was a significant decrease in FPG, 2-h PPG, and HbA1c, but inflammatory markers showed no significant difference from baseline.

Limitations of the study

The present study was a single-centre study, and the duration of the study was short. We have not matched duration of diabetes for selection of controls. Inflammatory cytokines like TNF-α, IL-6 have not been measured in our study. X-ray of foot was not done on follow-up of CN patients as none had progression of foot deformity. Bone turnover markers and bone mineral density were not assessed in the present study.

Discussion

In the present study, diabetic CN was found to be present in 0.49% of diabetic patients in the fifth to sixth decades of life with bilateral foot involvement in only 4% of patients. There was a wide variation in the prevalence of diabetic CN reported earlier, varying from 0.08 to 35% [13, 14]. These variations in prevalence of CN may be attributed to the lack of uniform criteria for the diagnosis of CN and inclusion of various high-risk groups of patients in those studies. There was also conflicting data on bilateral foot involvement in previous studies, varying from 9 to 75% [15–17].

Diabetic patients developing CN had higher BMI, poor glycemic control, longer duration of DM, and higher prevalence of neuropathy and retinopathy complications. Obesity was implicated as a risk factor for diabetic CN by increasing the biomechanical load on a deranged foot. In a study by Stuck et al., obesity alone increased the risk of CN by 59% [18]. The poor glycemic control and long duration of DM increase the risk of neuropathy and risk of repeated microtrauma, which go unnoticed. The present study also showed 42% of patients had history of trauma prior to CN. This corroborates to the neurotraumatic theory [19] in the pathogenesis of diabetic CN.

ACN and CCN were defined clinically by inflammatory signs and radiologically by Eichenholtz staging. As expected, the inflammatory signs were present in all cases of ACN and absent in all cases of CCN. However, clawing of toes, rocker bottom feet, anhidrosis, and callosities were more common in CCN patients. The development of these complications could be due to autonomic neuropathy and motor involvement in these patients.

Radiological diagnosis of CN is the cornerstone in the diagnosis and management of CN. The most common involvement among foot bones was midfoot (65%) (Sanders and Frykberg's patterns II and III) followed by forefoot (23%) (Sanders and Frykberg's pattern I), and the least common was the hindfoot (12%). The present study is in concordance with previous studies where midfoot was the commonest site of involvement [7, 20, 21]. Increased Meary's angle and decreased calcaneal pitch and decreased cuboid height were found in 68%, 50%, and 58% of CN patients, respectively, in the present study. The measurement of these angles helps in assessing the progression of disease. However, X-rays were non diagnostic in very early ACN (Eichenholtz stage 0), which were picked by MRI foot as seen by various other studies [7, 22].

MRI is useful as a diagnostic modality in most of the cases of CN. MRI could be able to detect four feet (3 patients, 25% of ACN), which were missed by X-rays. In these early cases (Eichenholtz stage 0), bone marrow oedema as identified by STIR images is a useful tool. MRI is also useful in assessing the progression of disease and identifying osteomyelitis. In the present study, two CCN patients had osteomyelitis, which were identified by MRI by the ghost sign.

The main stay of treatment in patients with ACN is immobilization with TCC. Understanding the duration of healing time is important in managing diabetic CN. The median duration of healing in ACN patients was 90 days (IQR 75–135 days). Depending on the location of arthropathy, healing time in TCC varied. This is useful while managing CN, affecting various regions of foot. Our finding is shorter than reports from studies in UK (median, 9 to 12 months) [23, 24] but is almost comparable to studies from USA (mean, 3 to 5 months) [25, 26] and other Asian countries (median, 5 months) [27]. This variation may be due to differing patient characteristics, patterns and staging of CN, definition used for Charcot resolution, type of offloading techniques used and adherence to offloading, experience in applying the TCC, protocols for monitoring Charcot progression, and study design [28]. The main concern in patients with CCN is to prevent progression of deformities, formation of new ulcers, and osteomyelitis. Only 2 patients had osteomyelitis at initial presentation, but none developed during follow-up.

Conclusions

The present study highlights that CN in DM patients are not uncommon complication. DM patients having poor glycemic control and longer duration of DM are at risk of developing CN. MRI is helpful in detecting early cases of CN, which may be missed on X-ray. Early diagnosis and appropriate offloading lead to clinical remission in majority of ACN and healing as well as prevention of foot ulceration in CCN.

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Data Availability Data is available through the corresponding author upon justified request.

Declarations

Ethics approval Ethical clearance was taken from the institutional ethics committee with registration no.783/ Chairman- IEC, M.K.C.G. Medical College, Brahmapur-4.

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

Conflicts of interest The authors have no conflicts of interest.

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ORIGINAL ARTICLE

A randomized, double-blind, active-controlled trial assessing the efficacy and safety of a fixed-dose combination (FDC) of MEtformin hydrochloride 1000 mg ER, SItagliptin phosphate 100 mg, and DApagliflozin propanediol 10 mg in Indian adults with type 2 diabetes: The MESIDA trial

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Abstract

Objective To assess the efficacy and safety of fixed-dose combinations (FDC) of triple-drug dapagliflozin, sitagliptin, and metformin (DSM) compared with FDC of two-drug sitagliptin and metformin (SM), in Indian adult patients with type 2 diabetes (T2D).

Methods A multicentric, randomized, double-blind, active-controlled, Phase 3 study (CTRI/2021/10/037461) was conducted on 274 Indian adult patients with T2D. Patients were randomized (1:1) to receive either an FDC of triple-drug ($n=137$) dapagliflozin propanediol 10 mg, sitagliptin phosphate 100 mg, and metformin hydrochloride 1000 mg extended-release (DSM) or FDC of two-drug ($n=137$) sitagliptin phosphate 100 mg and metformin hydrochloride 1000 mg sustained-release (SM), for 16 weeks. The primary endpoint was a change in HbA1c, while the secondary endpoints were changes in fasting plasma glucose (FPG), postprandial glucose (PPG), body weight, and safety.

Results Both DSM and SM FDCs reduced HbA1c significantly (-1.45% and -1.00%, respectively, both $p<0.0001$), however, HbA1c lowering was superior with DSM (Δ -0.45%; $p=0.0005$) compared to SM, at week 16. Similarly, both DSM and SM FDCs reduced FPG and PPG significantly, however, FPG (Δ -12.4 mg/dl; $p=0.003$) and PPG reduction (Δ -18.45 mg/dl; $p=0.01$) were significantly superior to DSM compared to SM, respectively. No significant reduction in body weight was observed between the two arms. Both FDCs were well tolerated.

Conclusion FDC of DSM was superior to SM in reducing HbA1c, FPG, and PPG in Indian adults with T2D. Both triple and dual FDCs had optimal safety profiles.

Keywords MESIDA study · Triple drug combination · Sitagliptin · Dapagliflozin · Metformin

Introduction

Two recent studies (VERIFY [NCT01528254] and GRADE [NCT01794143] having nearly similar (mean 5-year) follow-ups have now clearly suggested that the conventional approach of sequential addition of drugs in the treatment of type 2 diabetes (T2D) is an inferior strategy [1, 2]. Additionally, the VERIFY study showed initial combination therapy is superior to sequential addition therapy [1]. Intuitively, combination therapies that have different mechanisms of action and a. address various metabolic defects concerning the pathophysiology of T2D, b. having complementary and or synergistic actions, c. having the potential for a larger reduction of glycated hemoglobin (HbA1c) without potentiating hypoglycemia, d. can counter the undesirable effects

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produced by the individual agent, e. possessing cardio-renal protection, f. having good tolerability without any additional adverse effects, g. can reduce pill burden as a fixed-dose combinations (FDCs) allowing better compliance and h. administered through the oral route – are more likely to be a preferred modality for treating T2D [3]. To this end, triple-drug FDCs of metformin, dipeptidyl peptidase-4 inhibitors (DPP4i), and sodium-glucose co-transporter-2 inhibitors (SGLT2i) appear most promising. Indeed, combination therapy of metformin, DPP4i, and SGLT2i has the potential to correct seven pathophysiological defects of well-defined Ominous Octet, without provoking significant hypoglycemia [4–6]. A recent meta-analysis showed cardiovascular and renal benefits exerted by SGLT2i remain intact in association with DPP4i combination therapy with an expected additional HbA1c lowering [7]. Concerning international guidelines, the 2023 American Diabetes Association and European Association of Studies in Diabetes (ADA-EASD) have recommended using two drugs in T2D at diagnosis when HbA1c is ≥ 9% without osmotic symptoms and ≥ 8.5% in young adults with < 40 years of age [8], the 2022 American Association of Clinical Endocrinologists (AACE) guidelines [9] suggest using dual drug therapy when HbA1c > 1.5% above the desired target. The 2022 AACE guidelines recommend triple drug therapy as a first approach for treating asymptomatic patients with HbA1c levels > 9% (75 mmol/mol), while in patients with HbA1c levels ≤ 9% (75 mmol/mol), triple therapy is recommended if the patient has an inadequate response to monotherapy or dual therapy [9].

Several studies that have been conducted with FDCs of SGLT2i and DPP4i have shown superior HbA1c lowering compared with either agent, with or without background metformin therapy, without any notable increase in hypoglycemia or any other adverse events [10–17]. Indeed, the use of SGLT2i and DPP4i in the treatment of T2D has gained momentum, in light of the recent patent expiry of sitagliptin and dapagliflozin in some countries and the availability of several cheaper generic FDCs of these two drug combinations, especially in India. An expert consensus has recently highlighted the significant role of SGT2i and DPP4i FDCs in people with T2D in the Indian setting [18]. These findings supported studying the safety and efficacy of triple-drug FDC of metformin, sitagliptin, and dapagliflozin in the management of T2D in Indian settings.

Materials and methods

Study design

This study was a multicenter, randomized, double-blind, active-controlled, intention-to-treat, parallel-group, phase 3 trial (CTRI/2021/10/037461) that evaluated the efficacy and safety of triple-drug FDC in the treatment of T2D in Indian patients. This study was conducted following the ethical principles of the Helsinki Declaration, ICH-GCP (International Council for Harmonisation) E6 (R2) guidelines, local regulatory requirements for good clinical practice (GCP) for clinical research in India, and the national ethical guidelines of the Indian Council of Medical Research (ICMR) for biomedical and health research involving human participants [19–22]. The study enrolled 457 Indian patients with T2D who were inadequately controlled on metformin monotherapy (1000–1500 mg/day) for at least 6 weeks. The patients underwent a 2-week screening period before randomization. The eligibility criteria were as follows:

- Willing to provide voluntary written informed consent
- Male or female patients aged 18 to 65 years (both inclusive)
- Patients with HbA1c value between 8.0% and 10.0%

Patients with known hypersensitivity to metformin, sitagliptin, dapagliflozin, or the study product excipients, a body mass index (BMI) exceeding 40 kg/m^2, abnormal laboratory results (including eGFR < 60 mL/min by the CKD-EPI, hemoglobin < 10 g/dL, neutrophils < 2000/mm^3, platelets < 100,000/mm^3, total bilirubin > 1.5 X ULN (upper limit of normal), ALT/AST > 2.5 X ULN, serum amylase and/or lipase > 3 X ULN), type 1 diabetes, fasting plasma glucose (FPG) levels > 270 mg/dL, hypothyroidism, hyperthyroidism, hypotension, positive testing for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), genital mycotic or urinary tract infections, history of cardiovascular or respiratory diseases, and corticosteroid use for one week or more within 3 months before screening were excluded from the study.

Treatment regimen

A total of 274 eligible patients with T2D, recruited from 20 sites across India and meeting the study selection criteria, were randomized in a 1:1 ratio to either a fixed-dose combination (FDC) of three drugs (n = 137)—dapagliflozin propanediol 10 mg, sitagliptin phosphate 100 mg, and metformin hydrochloride 1000 mg extended-release (DSM), or an FDC of two drugs (n = 137)—sitagliptin phosphate 100 mg (SM) and metformin hydrochloride 1000 mg sustained-release. Out of the 274 patients, 253 completed the trial and were followed up at the end of 16 weeks having 126 patients in the DSM group and 127 patients in the SM group. The subject disposition of this trial is summarized in Fig. 1.

Study assessment and endpoints

The primary endpoint was to evaluate the change in HbA1c from baseline to week 16, analyzed using an analysis of covariance (ANCOVA) model with treatment as a factor and baseline as a covariate. Secondary endpoints were changes in FPG, postprandial glucose (PPG), and body weight from baseline to Week 16. A two-sample t-test was conducted to examine the mean difference between treatment groups. Additionally, the safety and tolerability of DSM were assessed.

Results

HbA1c change at week 16

Table 1 displays patient demographic and baseline characteristics for the two treatment groups, comparable in all the parameters. In all, 253 patients completed the trial (126 patients in the DSM group and 127 in the SM group). At the end of Week 16, the DSM group showed a significant decrease in HbA1c levels to 7.42 ± 0.973 (Δ -1.45%; p = 0.0005). In contrast, the SM group only reduced HbA1c levels to 7.87 ± 1.073 (Δ -1%) (Table 2). The difference between the two groups from baseline and Week 16 was statistically significant (-0.45% (-0.70, -0.20; p = 0.0005)) (Fig. 2).

FPG (Fasting Plasma Glucose) change at week 16

After week 16, a notable difference emerged in the mean change of FPG levels from baseline between the DSM group [from 153.79 mg/dL to 132.7 mg/dL (Δ -21.09)] and the SM group [from 162.70 mg/dL to 154.01 mg/dL (Δ -8.69)] (Table 2). The difference between the DSM and SM groups was Δ -12.4 mg/dL, with a statistical significance of P = 0.003 (Fig. 3).

PPG (Post-Prandial Glucose) change at week 16

By week 16, a substantial reduction in mean PPG levels from baseline was observed in the DSM group [from 228.90 mg/dL to 191.24 mg/dL] (Δ -37.66 mg/dL)], whereas the SM group demonstrated a lesser reduction [from 238.94 mg/dL to 219.73 (Δ -19.21 mg/dL)] (Table 2). This difference in PPG reduction was statistically significant, with the DSM group experiencing a more pronounced decrease (Δ -18.45 mg/dL; p = 0.01) as compared to the SM group (Fig. 4).

Body weight change at week 16

After 16 weeks, both groups experienced a significant reduction in body weight from baseline, with mean changes of -0.9 kg and -0.8 kg in the DSM and SM groups, respectively

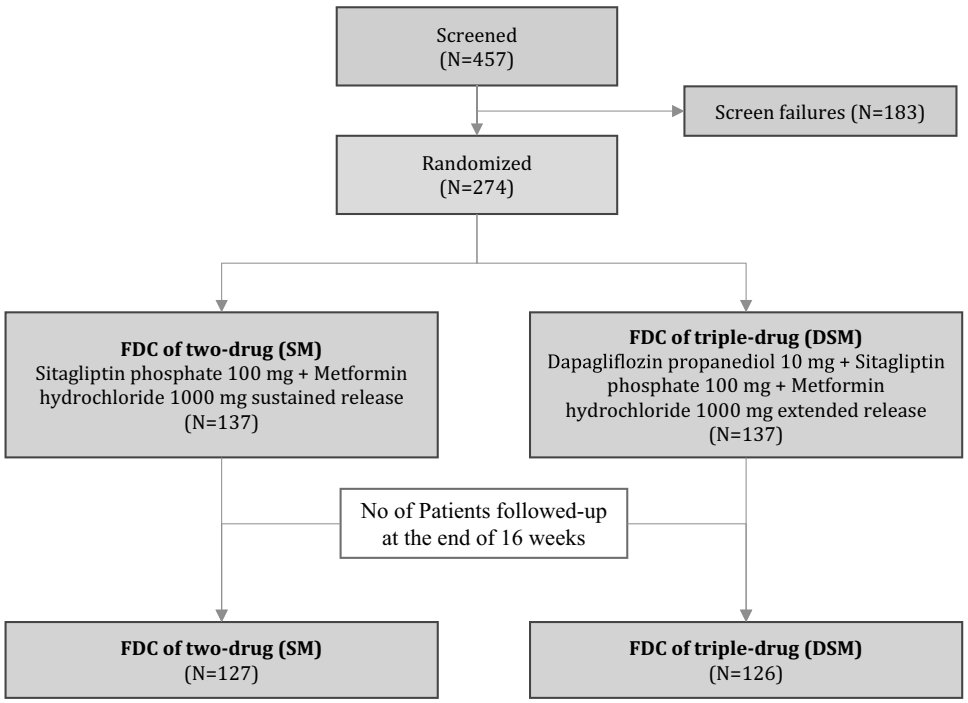

Fig. 1 Subject Disposition. Abbreviations: FDC: Fixed Dose Combination; SM: Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; DSM: Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release

Table 1 Demographic and Clinical Characteristics

Parameter	FDC of triple-drug (DSM) (*N* = 137)	FDC of two-drug (SM) (*N* = 137)	Overall (*N* = 274)	*p-value*
Age (years)				
Mean (SD)	50.7 (8.35)	49.3 (9.56)	50.0 (8.99)	0.2118
Height (cm)				
Mean (SD)	162.0 (6.82)	162.2 (8.36)	162.1 (7.61)	0.7781
Weight (Kgs)				
Mean (SD)	66.2 (10.78)	67.9 (10.75)	67.0 (10.78)	0.1898
Diabetes Duration (M)				
Mean (SD)	6.9 (11.75)	6.8 (6.08)	6.8 (9.29)	0.8867
HbA1c				
Mean (SD)	8.87 (0.54)	8.88 (0.57)	8.87 (0.55)	0.8640
FPG (mg/dl)				
Mean (SD)	153.79 (38.90)	162.70 (43.31)	158.24 (41.10)	0.0827
PPG (mg/dl)				
Mean (SD)	228.90 (61.54)	238.94 (71.76)	233.92 (66.65)	0.2280
Body Weight (kg)				
Mean (SD)	65.42 (10.07)	67.85 (10.80)	66.63 (10.43)	0.0621
Systolic Blood Pressure (mmHg)				
Mean (SD)	125.37 (7.44)	125.96 (7.75)	125.66 (7.59)	-
Diastolic Blood Pressure (mmHg)				
Mean (SD)	79.53 (4.69)	79.28 (5.54)	79.40 (5.11)	-

SD Standard Deviation; *M* Mean; *FDC* Fixed Dose Combination; *SM* Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; *DSM* Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release

($p < 0.0001$) (Table 2). However, there was no significant difference in body weight reduction between the DSM and SM groups (Table 2).

Safety and tolerability

Out of all 274 participants, 43 Treatment-Emergent Adverse Events (TEAEs) were reported in 33 subjects, with 16 subjects (11.7%) in the DSM group and 17 subjects (12.4%) in the SM group. None of these events were classified as serious treatment-emergent adverse events (SAEs). All reported events were categorized as mild (Grade 1), and no instances of moderate or severe events were observed. Eighteen of the reported events were considered related to the investigational product, 10 events in the DSM group and 8 in the SM group respectively (Table 3). In the DSM group, Gastritis (3 events), Dizziness (2 events), and Vomiting (1 event) were the most commonly reported related events. In the SM group, Headache (2 events), Cough (1 event), and Nasopharyngitis (1 event) were the most frequently reported related events (Table 3). All reported events resulted in complete recovery, with no unresolved events documented. These findings indicate that the investigational product was well-tolerated in both the DSM and SM groups, with no significant differences in the occurrence of TEAEs (Table 4).

Discussion

As β-cell activity gradually declines, oral antidiabetics may become less effective when used for extended periods. In such cases, switching from monotherapy to combination (dual or triple) treatments may be required [10]. Nearly half of the newly diagnosed T2D patients in the United Kingdom Prospective Diabetes Study (UKPDS) conducted half a century ago were found to have poor glycemic control with monotherapy after three years [23]. Surprisingly these findings were replicated in recent studies including VERIFY and GRADE studies [1, 2]. While poor treatment adherence may be linked to insufficient glycemic control, it can additionally be caused by other factors including polypharmacy, complicated treatment regimens, advanced age, obesity or hypoglycemia, lack of education, and occupation [24]. To this end, a triple-drug regimen that combines DPP4i, metformin, and SGLT2i can effectively treat different aspects of T2D, such as insulin resistance, β-cell dysfunction, and

Table 2 HbA1c, FPG, PPG, and Body Weight (kg) change at Week 16 from baseline

	DSM ($N=126$)	SM ($N=127$)
HbA1c		
Baseline	8.87 ± 0.541	8.88 ± 0.577
Visit 7/ Week 16 (Mean change from baseline)	7.42 ± 0.973 (-1.45)	7.87 ± 1.073 (-1.00)
p-value	< .0001	< .0001
FPG		
Baseline	153.79 ± 38.90	162.70 ± 43.31
Visit 7/ Week 16 (Mean change from baseline)	-21.09	-8.69
p-value*	< .0001	0.0389
PPG		
Baseline	228.90 ± 61.54	238.94 ± 71.76
Visit 7/ Week 16 (Mean change from baseline)	-37.66	-19.21
p-value*	< .0001	0.0028
Body Weight		
Baseline	65.42 ± 10.07	67.85 ± 10.80
Week 16 (Mean change from baseline)	-0.9	-0.8
p-value*	< .0001	< .0001

p-value* calculated using paired t-tests

HbA1c haemoglobin A1c; *FPG* Fasting Plasma Glucose; *PPG* Postprandial Glucose; *SM* Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; *DSM* Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release

glucose reabsorption. This combination is also likely to have a lesser risk of hypoglycemia and weight gain, which are common side effects of other antidiabetic drugs like sulfonylureas and insulin [12].

The only published Indian study that has shown the benefit of using a triple-drug combination of SGLT2i and DPP4i with metformin to control blood sugar levels is by Sahay et al. (2023). In phase 3 randomized 16-week study, that compared the safety and effectiveness of a triple-drug combination of dapagliflozin plus sitagliptin and metformin extended release (ER) and compared to both sitagliptin plus metformin sustained release (SR), and dapagliflozin plus metformin ER, showed a significant drop in HbA1c from baseline (-1.73%, -1.28%, and -1.33%, respectively; all $p < 0.001$). The triple drug combination of dapagliflozin, sitagliptin and metformin ER lowered HbA1c significantly better compared with dual therapy of sitagliptin plus metformin SR (Δ -0.46%; $P < 0.001$) and dapagliflozin plus metformin ER (Δ -0.4%; $p < 0.001$) [25]. These findings are concordant with our study that also finds superior HbA1c lowering of the triple drug combination of sitagliptin, dapagliflozin, and metformin ER compared with sitagliptin plus metformin SR (Δ -0.45%; $p = 0.0005$). Concerning global studies, in a 24-week, multicenter, randomized, double-blind study, involving 432 patients with T2D, Jabbour SA et al. (2014) showed adding dapagliflozin (10 mg/day) to sitagliptin (100 mg/day) with or without background metformin (≥ 1,500 mg/day) therapy led to more reductions in

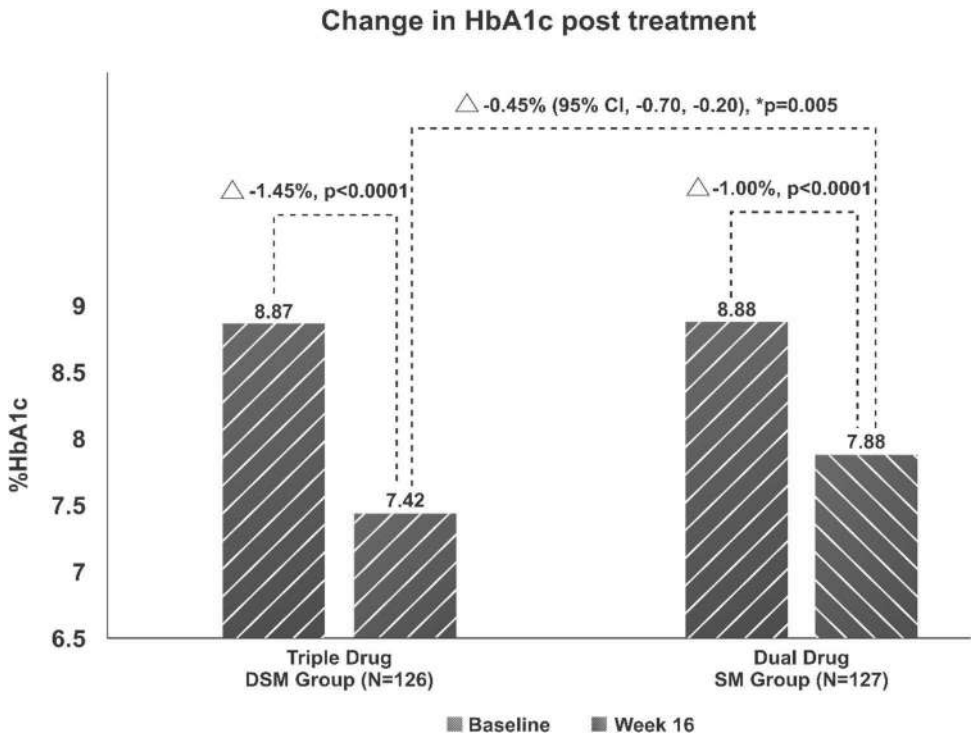

Fig. 2 HbA1c change at Week 16 from baseline. Abbreviations: HbA1c: haemoglobin A1c; SM: Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; DSM: Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release

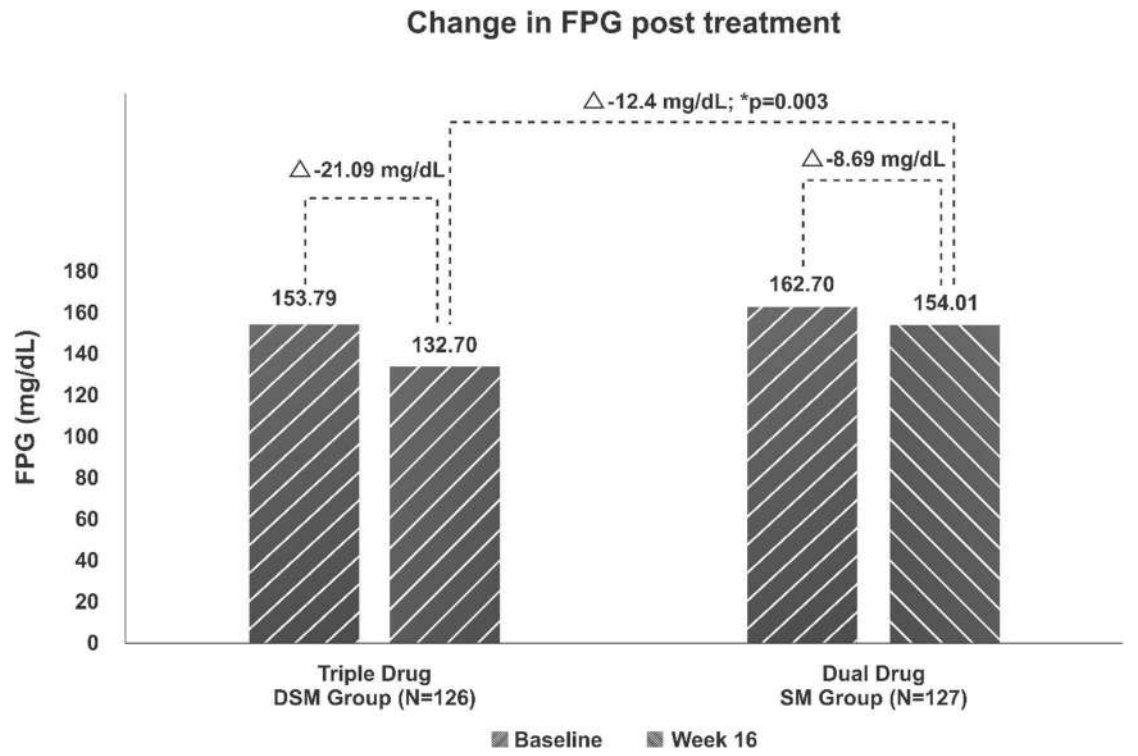

Fig. 3 FPG change at Week 16 from baseline. Abbreviations: FPG: Fasting Plasma Glucose; SM: Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; DSM: Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release; mg/dL: milligrams per deciliter

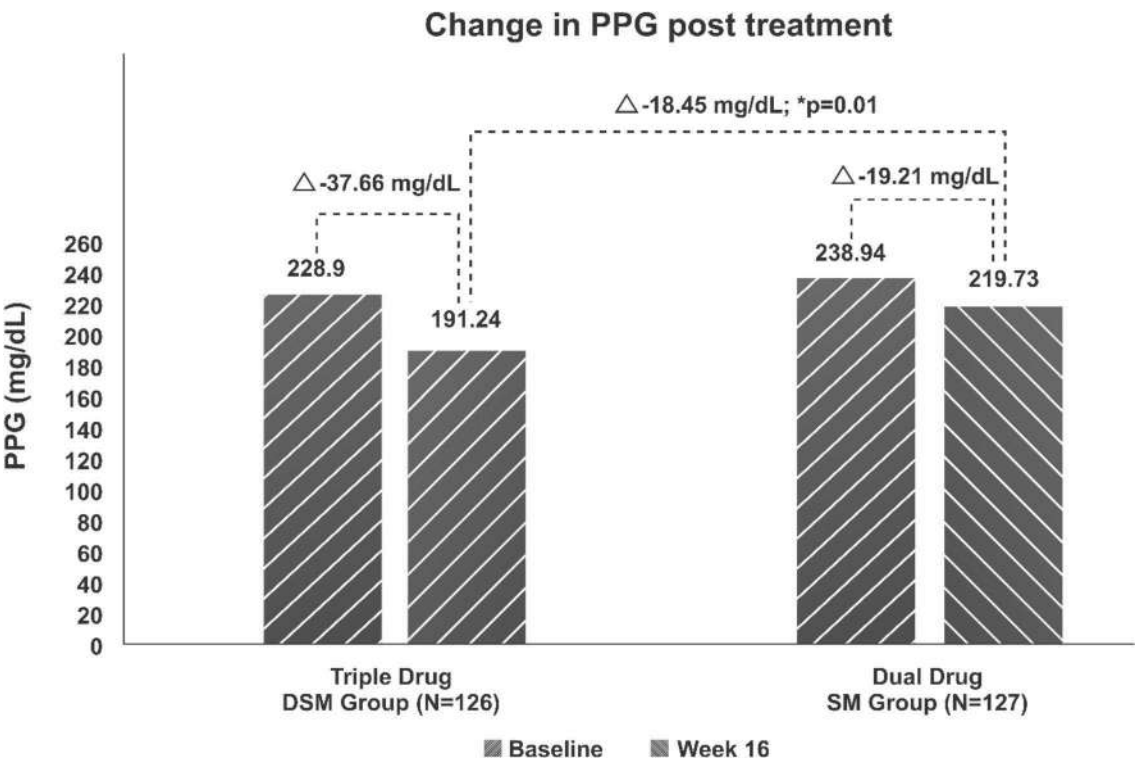

Fig. 4 PPG change at Week 16 from baseline. Abbreviations: PPG: Post-prandial Glucose; SM: Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg sustained release; DSM: Dapagliflozin propanediol 10 mg + Sitagliptin phosphate 100 mg + Metformin hydrochloride 1000 mg extended release; mg/dL: milligrams per deciliter

Table 3 Adverse Event-Overview

Categories	N [Events]
TEAE	33 [43]
SAE	0
Intensity	
Mild	33 [43]
Moderate	0
Relation of AE to Investigational Product	
Related	16 [18]
Unrelated	19 [25]
Outcome of AE	
Recovered	32 [42]
Ongoing	01 [01]
Not recovered	00 [00]

N Number of Events; *AE* Adverse Events; *TEAE* Treatment-Emergent Adverse Event; *SAE* Treatment-Emergent Serious Adverse Event

HbA1c levels (-0.5%), body weight (-2.1 kg), and FPG levels (-24.1 mg/dL) than placebo after 24 weeks, and these benefits were sustained until week 48 [10]. Mathieu et al. (2015) found dapagliflozin addition to saxagliptin plus metformin lowered HbA1c levels more than placebo (−0.82% *vs.* −0.10% respectively; $p < 0.0001$) at 24 weeks [13]. Similarly, Matthaei et al. (2015) showed saxagliptin along with dapagliflozin and metformin led to a greater reduction in mean A1C compared to placebo (−0.51% *vs.* −0.16%) at week 24 [12]. Moreover, in a 52-week study of 461 patients with T2D, Handelsman et al. (2019) found that the dapagliflozin and saxagliptin combination had a better HbA1c reduction than the sitagliptin and metformin combination at both 26 and 52 weeks, with similar safety and tolerability [26]. DeFronzo et al. (2015) assessed the safety and effectiveness of empagliflozin and linagliptin combinations as a second-line treatment for individuals with T2D who were not effectively managed on metformin. This study showed a sustained and superior HbA1c lowering with empagliflozin and linagliptin combination compared to either drug alone (linagliptin or empagliflozin) in a background metformin therapy, at 52 weeks. [15].

We acknowledge the strengths and weaknesses of our study. This is only a second randomized double-blind active comparator trial conducted in Indian people with T2D that compares the triple drug FDC of sitagliptin, dapagliflozin, and metformin combination therapy to dual therapy of sitagliptin and metformin combination. However, our study has some limitations. This study lasted only for 16 weeks, which may not be enough to see the long-term effects of the triple drug combination on glucose control. Also, the study had a small number of participants, which may limit the applicability of the results to other people with T2D in India. Notwithstanding these drawbacks, this study further strengthens our knowledge about the triple drug combination of SGLT2i, DPP4i, and metformin to be an effective agent in lowering HbA1c in Indian patients with T2D with an acceptable tolerability.

Table 4 TEAEs Related to Investigational and reference drug

SOC/PT/ Relationship, n (%)	Test (*N* = 137)	Reference (*N* = 137)	Overall (*N* = 274)
Gastrointestinal disorders	**4** [4]	1 [1]	**5** [5]
Gastritis	3 [3]	NA	3 [3]
Vomiting	1 [1]	1 [1]	2 [2]
General disorders and administration site conditions	**1** [1]	**NA**	**1** [1]
Fatigue	1 [1]	NA	1 [1]
Infections and infestations	**NA**	**1** [1]	**1** [1]
Nasopharyngitis	NA	1 [1]	1 [1]
Musculoskeletal and connective tissue disorders	**NA**	**1** [1]	**1** [1]
Pain in extremity	NA	1 [1]	1 [1]
Nervous system disorders	**3** [3]	**2** [2]	**5** [5]
Dizziness	2 [2]	NA	2 [2]
Headache	1 [1]	2 [2]	3 [3]
Respiratory, thoracic and mediastinal disorders	**NA**	**1** [1]	**1** [1]
Cough	NA	1 [1]	1 [1]
Investigations	**2** [2]	**2** [2]	**4** [4]
Creatinine renal clearance decreased	2 [2]	2 [2]	4 [4]

SOC System Organ Class; *PT* Preferred Term; *N* Number of Events; *NA* Not applicable

Conclusion

The triple combination (Dapagliflozin 10 mg, Sitagliptin 100 mg, and Metformin 1000 mg ER tablets) was superior to the combination of Metformin SR 1000 mg and Sitagliptin 100 mg in terms of HbA1c reduction, good control of FPG and PPG with favorable safety profile. This FDC may offer a promising treatment option to achieve optimal glucose for patients with T2D who are not controlled by mono or dual therapy.

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Author contribution The authors confirm contribution to the paper as follows: study conception and design: AS, MM, RP, AR, VS; study conduct: NG, ST, VK, IB, JS, MKS, VP, AD, MC, AN, AM, PG, SK, DK, YR, SS, OG, SK, DV, HC and DP; Data collection: VS; Analysis and interpretation of results: AKS, MM, RP, AR, VS; Draft manuscript preparation: AKS, RS, MP, NB, RA, AP, SG, SP, VK, MM. All authors reviewed the results and approved the final version of the manuscript.

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

Declarations

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

Conflict of interests Authors declare no conflict of interests.

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ORIGINAL ARTICLE

Role of sensory feedback in postural control of the patients with diabetic neuropathy

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Abstract

Background/Purpose Impaired balance is prevalent in patients with type II diabetes mellitus (T2DM). The aim of the current study was to evaluate the role of sensory information in these patients.

Methods Stabilogram-diffusion analysis was utilized to categorize the balance into local and central control modes based on the center of pressure (CoP) data acquired from quiet standing tests of 36 patients with T2DM and 20 healthy individuals. Local control was considered the efforts of muscles to stiffen the joints. Central control was the mode in which sensory information is used. Open- and closed-eye conditions were added to detect other sensory sources during the standing. Traditional linear measures of stability were also calculated for anterior–posterior and mediolateral directions.

Results Results showed that sway area, pathlength, and maximum velocity of the CoP are higher in the T2DM group ($p < 0.001$) in open-eye condition, but they did not change in eyes closed ($p > 0.158$). Both the local and central control mechanisms can cause postural instability in AP direction in patients with T2DM ($p < 0.017$). Contrarily, diabetes had no effect on the ML direction ($p > 0.051$). Patients with T2DM had a significant delay (about 225 ms greater than the controls) in the use of sensory information reflected in the AP direction outputs ($p < 0.009$).

Conclusion The patients with T2DM had poorer stability due to the delayed use of the sensory information. Diabetic postural stability was not fully provided in comparison with the healthy people even with using the sensory information. Elimination of the visual feedback led to reduced postural balance in these patients.

Keywords Diabetes mellitus · Peripheral neuropathy · Postural control · Sensory feedback · Stabilogram-diffusion analysis

Introduction

Type II diabetes mellitus (T2DM) causes several problems regarding the control of posture. About one-third of the older diabetic persons with peripheral neuropathy (PN) have at least one falling experience in the year [1]. The well-documented literature on postural stability acknowledges that T2DM impairs the visual and somatosensory systems [2, 3]. The T2DM, also, adversely affects the muscles' strength to exacerbate the standing instabilities [4]. Some researchers stated that the balance behavior of the patients with T2DM is compromised in comparison with their age-matched healthy individuals. However, these studies used different measurement methods such as body or center of mass sways [5], trunk inclinations [6], and the center of pressure (CoP)-related parameters of stability like sway range, velocity, area, variability, and time-to-boundary measures [7–12].

It was overlooked for a long time that the central processing of the feedback afferents may be impaired by the T2DM with peripheral neuropathy (PN) [13]. However, recent studies emphasized the effects of diabetes on the brain by indicating cerebral microvascular lesions [14], cortical atrophy [15, 16], and also the loss of neuroplasticity in animal models [17]. Because the normal postural control postulates motor and sensory coordination during standing, it is crucial to focus on the integration and use of sensory information for the motor responses. Meanwhile, several neuroimaging outcomes have shown decreased function of the motor and sensory cortices in patients with T2DM [18–21]. These findings complete the previous information about the motor behavior deficiency like posture instabilities and gait impairments in patients with

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T2DM [11, 12, 22, 23]; however, the correlation between the central and peripheral impairments has been rarely inspected using a single analytic method.

The maintenance of balance may have two modes of control: the local control, which is defined based on the efforts of muscles to stiffen the joints without using sensory information, and the central control, which is defined as the mode in which the sensory information is used. The discrimination between these two modes by means of routine postural measures like the CoP data has been suggested by Collins and Deluca (1993). They developed the stabilogram-diffusion analysis (SDA) based on the principles of statistical mechanics, which presumes the nature of random walk for the parameter of interest like the CoP. The SDA method calculates the squared-mean values for the desired parameter to correlate it to the time intervals by a linear constant called diffusion coefficient, representing the average stochastic activity of the posture analogous to a random walk movement [24]. Figure 1 graphically shows the details of this method. The first linear region in the SDA indicates the postural control without the use of sensory information, i.e., the local control mode, and the second linear behavior reveals the central mode of the control of posture by using sensory information [25, 26]. The SDA method has been widely used to determine the postural strategies among several pathological groups like diabetes [25], Parkinson's diseases [27], aging [28, 29], vertigo [30], etc. Toosizadeh et al. (2015) investigated the central versus local control of the posture in patients with diabetes and healthy controls using the SDA method applied on the center of mass (CoM) excursions [25].

The present study aimed to assess the role of type II diabetes mellitus with PN in central and local strategies during the control of posture in comparison with healthy age-matched individuals in terms of the CoP data. It was hypothesized that (i) the T2DM reduces the stability during standing; (ii) the central control of the posture in the patients with T2DM is affected more than the local control; (iii) patients with T2DM have more postural instabilities in the absence of visual information; and (iv) patients with T2DM have delay in use of sensory feedback.

Materials and methods

Participants

Thirty-six patients with type II diabetes mellitus (16 females, average age: 58.8 ± 7.3 years, BMI: 27.8 ± 4.9 kg/m2, duration of T2DM: 10.0 ± 3.9 years) and 20 healthy age-matched control subjects (6 females, average age: 52.1 ± 3.4 years, BMI: 27.3 ± 3.2 kg/m2) participated in this cross-sectional observational study. The participants entered the T2DM group if their age was between 40 and 75 years and had diabetes for 5 to 15 years, fasting glucose between 120 and 250 mg/dl, blood pressure between 100/60 and 140/90 mmHg, HbA1c between 6.5 and 9%, minimum visual acuity of 20/40, and should be able to

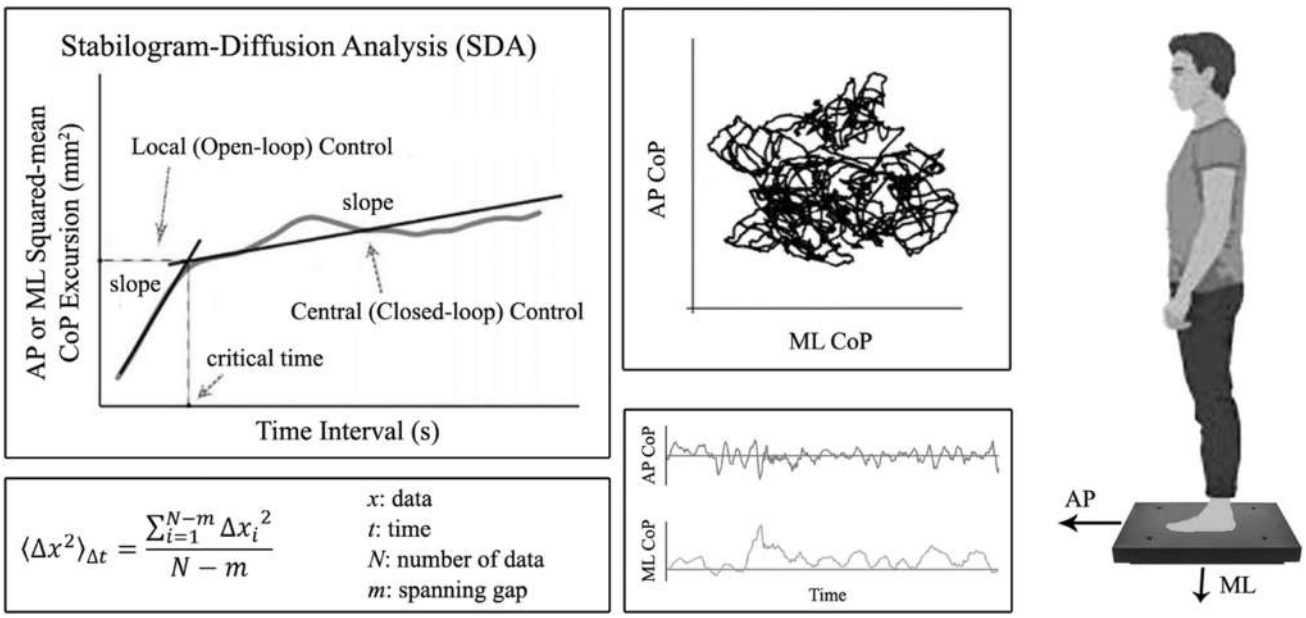

Fig. 1 Schematic representation of the test condition, raw CoP data in both AP and ML directions, data analysis to calculate critical time, local and central stability metrics in the SDA method

walk and stand without any aiding device. The PN of the patients has been evaluated by the Valk neuropathy score greater than 2 and also a recorded nerve conduction velocity less than 40 m/s for the peroneal tibial nerve. Exclusion criteria for these patients were suffering from cardiac disorders, vein thrombosis, CNS dysfunctions, vestibular dysfunctions, musculoskeletal disorders, foot wounds that prevent long-term standing, edema in the lower extremities, and any locomotive limitations. The participants in both groups received verbal and written explanations about the tests and signed the consent form. The ethical committee of the university approved this study.

Procedure

Both groups (T2DM and Ctrl) were asked to stand for 30 s on a force platform for 5 trials with a 2-min rest between the trials. They were barefoot with arms along the body with eyes open and closed using a blindfold.

Data acquisition and analysis

A force platform (Kistler, type 9286AA, Winterthur, Switzerland) measured the movement of the center of pressure during standing with a sampling rate of 100 Hz. Linear metrics of the postural stability were sway area (the area of an ellipse that encloses at least 95% of the CoP data), pathlength (the total length that the CoP travels during the task), maximum velocity, and the root mean square of the CoP data. The latter three metrics were calculated for both anterior–posterior (AP) and mediolateral (ML) directions. Nonlinear metrics of the postural stability were calculated based on the stabilogram-diffusion analysis including the critical time interval, local (open-loop) stability, and central (closed-loop) stability metrics (see Fig. 1). Details of this technique were presented in references [24, 26].

Statistical analysis

The normality of the data distribution was analyzed using the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was utilized to assess the roles of T2DM and vision on the linear conventional and the SDA metrics of postural stability. The significance level for all analyses was considered 5%.

Results

There was no significant different between the demographic data of age ($p = 0.611$) and BMI ($p = 0.798$). The distributions of all linear and SDA metrics were normal.

Table 1 shows the mean (SD) of the linear metrics for control and T2DM participants in open- and closed-eye conditions and the statistical measures. The sway area for patients with T2DM was significantly greater than the healthy controls ($F = 21.6$, $p < 0.001$). In the AP direction, the path length ($F = 65.4$, $p < 0.001$), maximum velocity ($F = 54.1$, $p < 0.001$), and RMS ($F = 83.2$, $p < 0.001$) of the CoP excursions were significantly increased in the participants with T2DM. In the ML direction, on the other hand, only the maximum velocity ($F = 23.0$, $p < 0.001$) and the RMS ($F = 25.1$, $p < 0.001$) were increased significantly in comparison to the healthy control subjects. The ML CoP path length was not changed between two groups ($F = 0.7$, $p = 0.408$).

The removal of visual feedback had no effect on all CoP-related linear metrics of stability ($p > 0.05$).

Table 2 also presents the mean (SD) values of SDA metrics for the test conditions. In AP direction, all SDA parameters, i.e., critical time interval ($F = 7.1$, $p = 0.009$), local ($F = 5.9$, $p = 0.017$), and center stability ($F = 15.7$, $p < 0.001$) were significantly higher in the patients with T2DM rather than the healthy ones. However, these SDA metrics in the ML direction were not different for the participants with T2DM ($F < 3.9$, $p > 0.051$).

Table 1 Linear variables of standing for healthy control (Ctrl) and type II diabetes mellitus (T2DM) participants and the related statistical results

	Ctrl		T2DM		Group *p*-value	Vision *p*-value
	EO	EC	EO	EC		
Sway area (cm^2)	1.3 (0.7)	1.5 (0.8)	4.3 (3.3)	5.4 (5.6)	**< 0.001**	0.293
AP direction						
Pathlength (cm)	15.8 (4.7)	16.6 (6.1)	27.1 (8.3)	30.3 (9.3)	**< 0.001**	0.219
Max velocity (cm/s)	2.8 (1.6)	2.5 (0.7)	4.6 (1.5)	5.3 (2.0)	**< 0.001**	0.392
RMS (cm)	0.3 (0.2)	0.4 (0.2)	1.6 (0.9)	1.9 (1.0)	**< 0.001**	0.332
ML direction						
Pathlength (cm)	16.3 (10.2)	17.8 (12.5)	15.8 (4.7)	16.6 (6.1)	0.408	0.158
Max velocity (cm/s)	2.5 (1.3)	2.5 (1.1)	3.3 (0.9)	3.9 (1.4)	**< 0.001**	0.175
RMS (cm)	0.6 (0.3)	0.7 (0.3)	0.4 (0.2)	0.4 (0.2)	**< 0.001**	0.751

The bold-faced values denote statistically-significant effects of the group and vision i.e. $p < 0.05$

Table 2 Nonlinear variables of standing for healthy control (Ctrl) and type II diabetes mellitus (T2DM) participants and the related statistical outputs whose main effects are presented in the last two columns but pairwise comparisons are developed by superscript letters noted below

	Ctrl		T2DM		Group *p*-value	Vision *p*-value
	EO	EC	EO	EC		
AP direction						
Critical time interval (s)	0.94 (0.40)	0.88 (0.39)	1.15 (0.43)	1.12 (0.47)	**0.009**	0.640
Local stability (cm^2/s)	0.41 (0.24)	0.40 (0.28)	0.49 (0.35) [a]	0.94 (0.57) [a]	**0.017**	**0.024**
Central stability (cm^2/s)	0.07 (0.06)	0.07 (0.07)	0.14 (0.10) [b]	0.27 (0.20) [b]	**< 0.001**	**0.017**
ML direction						
Critical time interval (s)	1.02 (0.48)	0.86 (0.46)	1.12 (0.47) [c]	0.82 (0.43) [c]	0.747	**0.005**
Local stability (cm^2/s)	0.52 (0.23) [d,e]	0.87 (0.50) [d]	0.82 (0.52) [e,f]	1.25 (0.67) [f]	0.051	**0.015**
Central stability (cm^2/s)	0.19 (0.28)	0.21 (0.24)	0.12 (0.11) [g]	0.49 (0.43) [g]	0.830	0.071

a: significantly diferrent ($F=6.7$, $p=0.012$); b: significantly diferrent ($F=7.3$, $p=0.009$); c: significantly diferrent ($F=8.0$, $p=0.006$); d: significantly diferrent ($F=7.7$, $p=0.008$); e: significantly diferrent ($F=5.6$, $p=0.021$); f: not significant ($F=3.2$, $p=0.077$); g: significantly diferrent ($F=5.9$, $p=0.018$)

The bold-faced values denote statistically-significant effects of the group and vision i.e. $p < 0.05$

The effect of vision was roughly significant for the SDA metrics. In particular, the local stability metric in the AP direction was significantly increased for the T2DM group ($F=6.7$, $p=0.012$), whereas not different for the healthy participants ($F=0.019$, $p=0.892$). The same results existed for the central stability metric, which is significantly increased for the T2DM group by elimination of visual feedback ($F=7.3$, $p=0.009$), and not changed for the healthy ones ($F=0.000$, $p=0.996$). The vision's main effects were significant for local and central stability metrics ($F=5.3$, $p=0.024$ and $F=0.9$, $p=0.017$, respectively). The critical time interval in the AP direction was not changed due to the closure of the eyes ($F=0.2$, $p=0.640$). In the ML direction, the critical time interval was significantly reduced during the closure of the eyes in the T2DM group ($F=8.0$, $p=0.006$), while not changed in the healthy group ($F=1.1$, $p=0.297$). The vision's main effect on the ML critical time was significant ($F=8.4$, $p=0.005$). The ML local stability metric was also increased significantly by the removal of visual feedback ($F=6.1$, $p=0.015$). The ML central stability was not changed due to closure of the eyes ($F=3.3$, $p=0.071$). But merely among the patients, the ML central stability was significantly higher in closed-eyes condition ($F=5.9$, $p=0.018$).

Discussion

The present study investigated the effects of type II diabetes mellitus on central and local control of the posture. For this purpose, stabilogram-diffusion analysis was utilized to discriminate two motor behaviors of the patients with T2DM with and without the sensory information. The main questions addressed in this study were (i) diabetes causes more postural instability in comparison with the healthy group, (ii) patients with T2DM have more instable central control than the local control, (iii) the removal of visual feedback has a destabilizing effect on balance, and (iv) patients with T2DM have delayed use of sensory information.

Effects of the diabetes

In terms of the linear measures of stability (Table 1), the patients with T2DM had considerably reduced stability during quiet standing for 30 s. Regardless of the visual condition, the healthy individuals had lower sway areas of the CoP, which indicates a higher stability level. The CoP pathlength that may reflect the amount of energy expenditure during the provision of stability was also lower in the healthy group. This outcome shows more optimal standing in comparison to the patients with T2DM. The maximum velocity was also two times greater in the patients than that of the healthy individuals. This parameter indicates the dexterity of postural control, which was impaired in the patients with T2DM. The higher velocity of the CoP may be originated by uncoordinated co-contractions of the muscles specifically acting on the ankle joint. Previous studies also developed similar results. For instance, sway area [31–35], path length [35, 36], and sway velocity [7] all showed greater values in the patients with diabetes in comparison with the healthy controls. The RMS of the antero-posterior CoP excursions was higher in the patients indicating farther CoP travels. This larger distance creates a larger moment arm for the ground reaction force, i.e., larger flexory/entensory moment about the ankle joint, which should be counterbalanced by the ankle muscles. Normally, the somatosensory afferent signaling would inform the CNS about the creation of such a large moment; nevertheless, the SDA time intervals of referring to the sensory information in patients with T2DM were

significantly greater in the AP direction (see Table 2). It was shown that the human and rodent models with diabetes have slower conduction velocity of upper motor neurons [37–39], maybe due to neuronal loss or demyelination [31, 40–42]. Abbruzzese et al. measured motor-evoked potentials in diabetic and healthy people and found that the central motor conduction times in upper and lower muscles are prolonged due to the diabetes seemingly independent from the PN [37]. The same results have been reported by Moglia et al. who investigated a larger population of the participants and found no correlation between the conduction time delays in the diabetic patients and their disease duration and also existence of the PN [38]. In the current study, the time delay in the use of sensory information may interfere with an effective feedback data processing in control of the posture.

In the T2DM patients, local (open-loop) instability is significantly increased, specifically in the AP direction when the vision was eliminated. Providing the local stability may be the result of the collaboration between different muscles of the lower extremity, which often uses a stiffening strategy while standing [25]. Decrease of muscle strength in the ankle [43], knee [4, 44], and the upper extremity [45, 46] due to diabetes (even without the PN) can lead to instabilities in the local control of posture among the patients with T2DM. The SDA local stability measure has been used as a discriminative index of human balance in many studies that employed the SDA method to differentiate between a variety of disorders, aging, or challenging conditions during standing. In most of the mentioned studies, the local stability indices were lower in the control group, which means a more stable condition. This local index has been generalized to the total stability in standing position. The researchers believed that if the short-term response is not stable enough, further endeavors cannot compensate this defect even after using the sensory information in the central response. But in two studies, the central stability (i.e., with the usage of sensory information) was unexpectedly greater in the control groups [25, 47]. This finding has been interpreted as a kind of adaptation to compensate for the unstable local-control mode.

It seems the interpretation of SDA results needs to consider study protocols. Collins et al. (49) applied the SDA method to the CoP data of young and elder people while Toosizadeh et al. (25) applied this method to the CoM of patients with neuropathic diabetes. The compensation in providing stability argued by Toosizadeh et al. (2015) might be originated from the somatosensory. In contrast, the literature confirmed that diabetes, specifically with the PN, has adversely affected the sensitivity and functionality of the muscle spindles in diabetic humans and mice [48–50]. Although the use of sensory information in the central control of the posture ameliorated the balance in both groups, the patients' central stability was still lower than the healthy individuals. Using a synchronized electromyographic analysis along the posturography during the standing task may elucidate the role of reduced muscle strength in diabetic patients [4, 43, 51, 52]. Furthermore, Toosizadeh et al. applied the SDA method to the CoM excursions, which have been considered non- or weakly chaotic behavior [53, 54]; hence, from a practical point of view, the assumption of the random walk motion for the CoM movements is not necessarily justifiable. The results of the current study did not confirm the outputs of the latter study, noting the application of the SDA to the CoP data here. The central stability of the patients with T2DM was significantly reduced in comparison with the healthy control individuals. It implies the deteriorative effect of diabetes on the anterior–posterior postural stability even during the existence of the sensory information. In fact, the CNS was faced to limitations in integration and processing of the the sensory information.

Effects of the vision

The linear measures of stability showed no significant effect of the vision on balance. Removal of the visual feedback whose role in the control of diabetic people's posture is highly emphasized by the literature [7] did not affect the linear measures of stability. But in contrast, the nonlinear SDA metrics showed a significant difference between open- and closed-eye conditions (see Table 2). The ML critical time interval, i.e., the parameter that quantifies the intervals of using the sensory data, was reduced by the elimination of visual feedback only in the diabetic patients. It implied that in the absence of vision, the CNS has relied on the other sources of information (here probably the vestibular system due to the PN) more frequently than when the eyes were open. Previous studies have shown that the stability in the ML direction is more dependent on the vestibular function [55]. Since the participants in this study had no symptomatic dysfunction in their vestibular systems, referring to the vestibular data as the only available and reliable feedback source in the ML direction was accelerated by the CNS. Individuals are often better able to maintain their balance in the ML than in the AP direction. The base of support is elongated in the ML direction between the feet so that the CoP has a wider area to travel far away from the margins of the base of support. In contrast, the base of support in the AP direction is narrower, and the probability of the CoP nearness to the base of support margin is higher.

The critical time interval of the healthy individuals changed neither in the AP nor in the ML direction by closure of the eyes. This highlighted the role of somatosensory feedback information in the healthy people and its defects in the patients with T2DM. The visual feedback also locally and centrally stabilized the posture in these patients especially in the AP direction.

The results of this study can shed light on the prospective treatments that would be designated for the improvement of posture in patients with T2DM. Since it was highlighted that the sensory information, which plays crucial roles in posture, is adversely affected by T2DM, exercise programs should consider proprioception. The goal of these exercises should be an improvement in proprioceptive acuity. For instance, Santos et al. (2016) proposed a set of circuit exercises with 13 stations to stimulate the proprioceptive sensation of women with diabetes [56]. From the outcomes of the present study, it could also be suggested as well that the physical therapies should comprise sensory stimulations of the feet soles, calf muscles, tibialis anterior/posterior, and peroneus longus/brevis.

Conclusion

In conclusion, the stabilogram-diffusion analysis could assess the balance in two segregated local and central modes of postural control in patients with T2DM. But it is necessary to note that the parameter of interest should vary like a random walk motion. The overall stability revealed in the linear metrics showed instable standings for these patients especially in the AP direction. These instabilities were more crucial in the central control of posture, i.e., with usage of the sensory information as the feedback, although the local, i.e., without sensory feedback control mode was instable too. Therefore, no compensation strategy was observed in the patients. The removal of visual feedback adversely affected the balance in the diabetic people. The patients with T2DM had a delayed use of the sensory information.

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Data Availability The data are available from the corresponding author.

Declarations

Conflict of interests The authors declare no competing interests.

Ethical clearance The Ethical Committee of Tabriz University of Medical Sciences approved the study (code: IR.TBZMED.REC.1397.655). All procedures were performed according to the Declaration of Helsinki. All participants signed informed consent forms before participating in the study.

Informed Consent was obtained from all participants included in the study.

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ORIGINAL ARTICLE

Changes of sweat gland function in type 2 diabetes mellitus patients with peripheral neuropathy

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Abstract

Background Millions of people suffer from diabetes mellitus. Most of them endure diabetes peripheral neuropathy (DPN) at the same time.

Objectives To examine the impairment of sweat gland function in type 2 diabetes mellitus patients with peripheral neuropathy (T2DM-DPN), and to explore the risk factors that may influence the impairment of autonomic function in T2DM-DPN patients by testing sweat gland function.

Methods Sweat gland function was tested with iodine and starch reagents on the sweat gland areas of the hands, feet and back of T2DM-DPN patients and healthy people. The ratio of the area in black spots to the whole tested area was calculated by ImageJ software. The participants' sweat gland function was also measured by SUDOSCAN conductance analyzer. The related hazard factors of the changes of sweat gland function was analyzed.

Results There was a significant difference in sweat gland function between the T2DM-DPN group and the healthy control group in hand area on the 1st and 2nd minute, in foot area on the 2nd minute ($p < 0.05$). The function of sweat glands in the hands, feet, and back of T2DM-DPN patients was weaker than that in healthy controls. Pearson correlation analysis showed that glucose (Glu) was correlated with sweat gland function significantly ($p < 0.05$).

Conclusion Sweat gland function was impaired in T2DM-DPN patients. The autonomic nerve may be injured in the T2DM-DPN patients at the same time. The injured degree of autonomic nerve in T2DM-DPN patients could be judged by sweat gland function test. Glucose may be one of the related hazard factors to the sweat gland function.

Keywords Type 2 diabetes mellitus (T2DM) · Diabetic Peripheral neuropathy (DPN) · Sweat glands · Autonomic nerve

Introduction

According to the International Diabetes Federation (IDF), approximately 537million adults aged 20 to 79 suffered from diabetes mellitus in 2021. This number is expected to rise to 643 million by 2030 [1]. Type 2 diabetes mellitus (T2DM) accounts for about 90% of all diabetes cases [1], which often leads to neuropathy and microvascular complications [2, 3].

Diabetes neuropathy includes diffuse neuropathy, mononeuropathy, radiculopathy, or plexus neuropathy [4]. Furthermore, diffuse neuropathy can be classed into distal symmetric polyneuropathy (DSPN) and autonomic neuropathy [5]. DSPN accounts for about 75% of diabetes neuropathy [6]. It is also commonly referred to as diabetes peripheral neuropathy (DPN) by scholars [7]. DPN includes small fiber, large fiber, and mixed fiber neuropathy, which is caused by diffuse and focal nervous system injury and occurs in up to half of diabetes patients [8, 9]. Following DPN, autonomic neuropathy takes the second place of diabetes neuropathy [8, 9]. Autonomic neuropathy includes cardiac autonomic neuropathy, gastrointestinal autonomic neuropathy, and urogenital autonomic neuropathy [10, 11]. Actually, one of the most simple and effective detection methods of autonomic neuropathy is the test of sweat gland function. However, it is often ignored in clinical practice. There are total about 4 million sweat glands in the body of human beings, which can be divided into two types: small sweat glands (accounting for about 90%) and apocrine glands [12]. Although the size of sweat glands varies among individuals, it is roughly proportional to the sweating rate [12]. The sweat gland function of T2DM patients is often impaired [13–15]. Previous studies have shown that

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axonal damage is the main feature of DPN [16, 17]. In the early stage, the unmyelinated small sympathetic fibers are destroyed, which can also make sweat gland function injured at the same time [18]. However, at present, there are few special studies on the damage of sweat gland function in T2DM-DPN patients. This study detected the changes of sweat gland function, analyzed the relevant indicators to the damage, and explored the impairment of autonomic nerve function in the T2DM-DPN patients, which will provide a new method for the diagnosis and treatment of autonomic neuropathy of T2DM-DPN patients.

Methods

Research design and methods

All the participants in the study were from the Affiliated Hospital of Putian University. Before the experiment, ensure that they did not take any drugs which would affect the diagnosis. People were divided into T2DM-DPN group (T2DM-DPN) and healthy control group (control). For the T2DM-DPN group, 17 patients first diagnosed with T2DM-DPN (other specific diseases excluded) were randomly selected, including 8 males and 9 females, aged from 53 to 70 years (62.23 ± 4.28). For the control group, a total of 18 healthy people aged from 51 to 66 years (57.13 ± 5.89) who underwent physical examination were randomly selected, including 9 males and 9 females, with diabetes history and other specific diseases excluded. The informed consents were signed by all the patients before test.

Diagnostic criteria of T2DM-DPN [19]: After excluding other reasons, the symptoms and / or signs of peripheral nerve dysfunction in patients with T2DM (the combinations of more than one examination below has more than 87% sensitivity in the detection of DPN: acupuncture, temperature, vibration perception with 128 Hz tuning fork, 10 g monofilament pressure sensation at the distal hallucination, and ankle reflex). The Guidelines for the Prevention and Treatment of T2DM in China (2020 edition) was used as the criterion of T2DM (a clear T2DM history of 9.38 ± 3.12 years) [20]. Patients enrolled in the study had no hematological diseases, type 1diabetes, autoimmune diseases, malignant tumors, gestational diabetes, renal insufficiency, and any other underlying diseases.

Collection of clinical data

General clinical data was collected. Items and methods were as follows: blood cell analysis (flow cytometry); HbA1c (high-performance liquid chromatography); FPG (hexokinase method); blood lipids (TG, LDL, HDL, ApoA, ApoB, etc., enzymatic method); electrolyte (electrode method); and another routine examination.

Test of sweat gland function

Iodine solution with a final concentration of 3.5% (iodine/75% alcohol) and starch solution with a final concentration of 10% (starch/castor oil), were used for the test of sweat gland function. Both of them were prepared fresh. In the two groups, the function of the developed sweat glands in the hands, feet, and back area was tested. The clean skin of each part (about $1cm^2$) was selected. A drop of iodine solution with a disposable straw was added, and then a drop of starch solution was added in the same area. Started timing immediately and took photos of this part on 30 s, 1 min, and 2 min separately. ImageJ analysis software was used to calculate the ratio of the black blot area to the measured area [21].

The sweat gland function of the participants' hand (bilateral) and foot (bilateral) was also measured by SUDOSCAN conductance analyzer (Impeto Medical, France) [22, 23]. The mean data of hand electrochemical conductivity (HEC) and foot electrochemical conductivity (FEC) were recorded. For both HEC and FEC, a value greater than 60 μs was defined as normal, and a value less than or equal to 60 μs was defined as abnormal [22, 23].

Analysis of data

SPSS (version 22.0) was used for statistical analysis. The normally distributed data was expressed in terms of mean and standard deviation. The two groups' comparison was performed using independent sample *t*-test. Correlation analysis was performed using Pearson's coefficient. Shapiro–Wilk test and Q-Q figure were used for ascertaining the normality of data. $p < 0.05$ was considered statistically significant.

Results

Analysis of sweat gland function

The analysis of sweat gland function with SUDOSCAN conductance analyzer is shown in Table 1. Test results of sweat gland function with starch iodine are shown in Fig. 1A–F (A, C, E: the pictures of the skin; B, D, F:

Table 1 The analysis of sweat gland function with SUDOSCAN conductance analyzer

	T2DM-DPN ($n=17$)	Control ($n=18$)	*t*	*p*
HEC (μs)	54.87 ± 7.93	72.49 ± 5.79	−7.472	< 0.0001
FEC (μs)	57.92 ± 7.00	75.48 ± 4.15	−8.967	< 0.0001

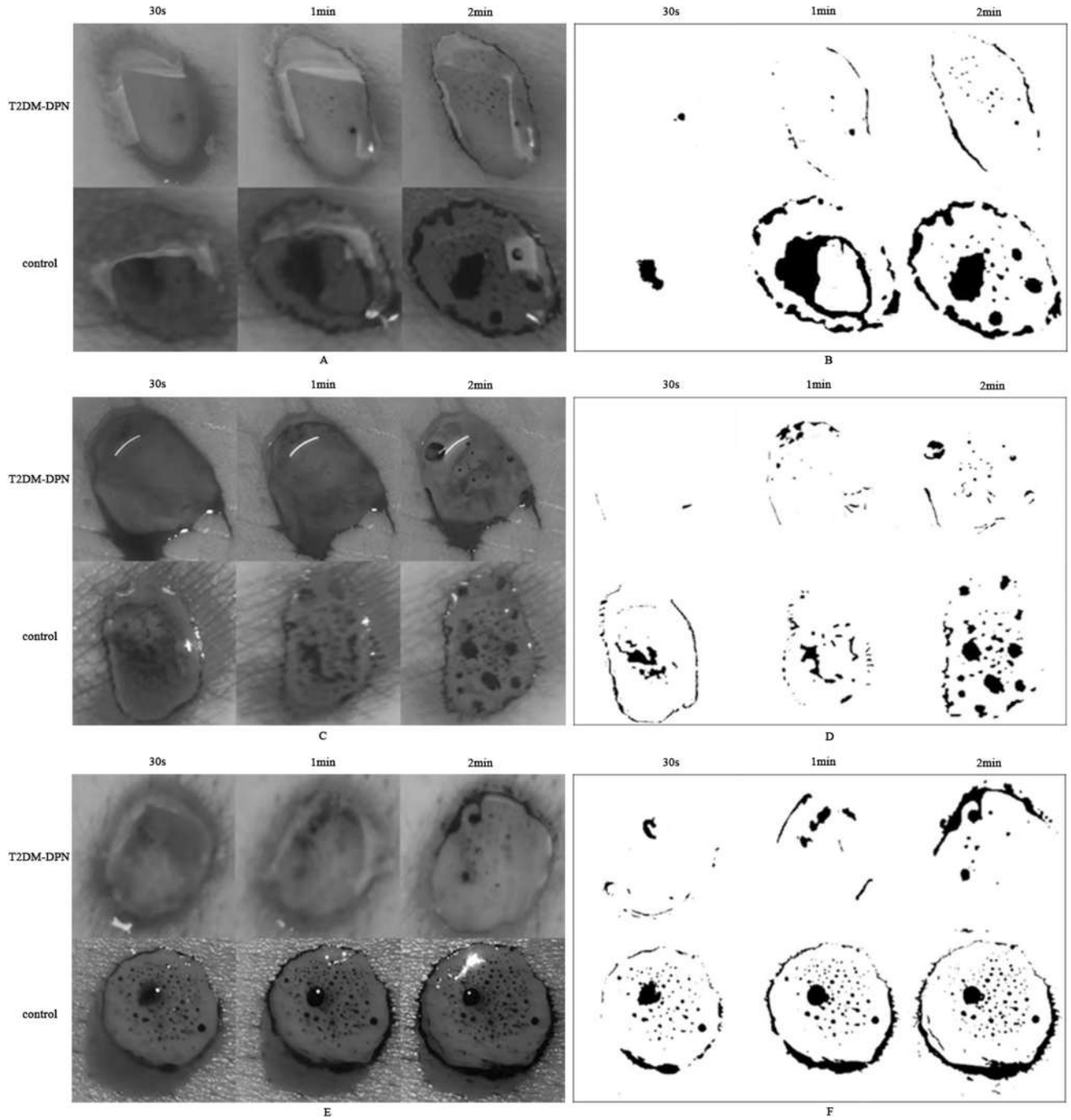

Fig. 1 Test results of sweat gland function. **A** Pictures of the hand skin; **B** analysis of sweat gland in hand; **C** pictures of the foot skin; **D** analysis of sweat gland in the foot; **E** pictures of the back skin; **F** analysis of sweat gland in the back

analysis with ImageJ software). There were significant differences between the two groups on the 1st and 2nd minute ($p = 0.044$, $p = 0.001$) in the hands area and on the 2nd minute ($p = 0.004$) in the foot area. Statistical analysis of sweat gland function is shown in Fig. 2.

Analysis of routine blood test

There was a significant difference of W-SCC and NLR between T2DM-DPN group and control group ($p = 0.009$, $p = 0.013$). The results are shown in Table 2.

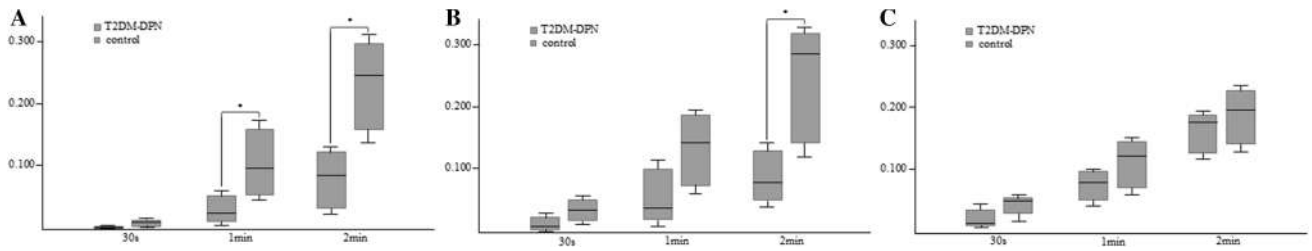

Fig. 2 Statistical analysis of sweat gland function. **A** Hand; **B** foot; **C** back (asterisk (*) $p < 0.05$)

Analysis of biochemical examination

The detailed results are shown in Table 3. There was a significant difference of Glu, TG, and ApoB between the two groups ($p = 0.009$, $p = 0.012$, $p = 0.015$).

Correlation analysis between clinical indexes and sweat gland function

Pearson correlation analysis was carried out between the quantified data of sweat gland function of the patient's hands on the 2nd minute and the 5 indicators which were statistically significant in the table above. The results are shown in Table 4. It was found that Glu was significantly correlated with sweat gland function ($p = 0.022$).

Discussion

In this study, the function of sweat gland was determined by the SUDOSCAN conductance analyzer and the principle of starch iodine test. The SUDOSCAN system is designed to evaluate the sweat gland function through reverse iontophoresis and chronoamperometry [23]. Professionals and specialized equipment were required during the test. While the method of starch iodine was economical, convenient, efficient, and accurate [21], the two kinds of assays yielded consistent conclusions. It can be found from the results that compared with the control group, the black sweat gland blots in T2DM-DPN group were irregular. The small marks gradually gathered together to a relatively large area. At the same time, a few small marks were isolated beside the large empty white area. This distribution form was in sharp contrast with the characteristics of the heathy control group, such as relatively uniform shape and color. From the graphs of sweat gland function test on 30 s, 1 min, and 2 min, it can be found that the T2DM-DPN group produced black blots slowly compared with the control group at the same time, and the black blots were not as obvious as that of the control group. In addition, the black spots on the back areas appeared earlier, but the increase was not as obvious as that on the hand and foot areas. In order to ensure the accuracy of quantitative data, the second minute data of hand area were selected for correlation analysis between clinical indicators and sweat gland function. It can be clearly seen from the figure that although the difference between the two groups is statistically significant only at the 1st and 2nd minutes of the hand or foot, the area and proportion of black marks produced by the sweat glands of the hands, feet, and back in the T2DM-DPN group were much smaller than those in the control group at any time point. It could be inferred that the sweat secretion was decreased and the sweat gland function was impaired in the T2DM-DPN patients.

In this experiment, areas of the hand, foot, and back with well-developed sweat glands were selected for sweat gland function testing [21, 24]. Through the comparison among the three parts, it can be found that the proportion of black marks on the back was larger in both of the two groups on the 30th second and 1st minute. Compared with the hand and foot at the distal end of the body, the sweat glands in the back of the trunk were more developed. In another words, the relatively small autonomic nerve at the distal end of the body may be damaged firstly in the T2DM-DPN patients. This result was consistent with the study of Liu et al. [25]. They reported the sweating nerve, a kind of slender and unmyelinated sympathetic C fiber, a small nerve fiber, could be damaged in the early stage of diabetes.

Table 2 Analysis of routine blood test

	T2DM-DPN ($n = 17$)	Control ($n = 18$)	t	p
WBC (10^9/L)	6.73 ± 0.95	6.39 ± 0.98	0.602	0.893
W-LCC (10^9/L)	4.45 ± 0.93	3.48 ± 0.63	1.116	0.218
W-SCC (10^9/L)	1.83 ± 0.42	2.57 ± 0.29	− 3.573	0.009
NLR (%)	2.57 ± 0.81	1.35 ± 0.17	3.62	0.013
W-MCC (10^9/L)	0.35 ± 0.13	0.34 ± 0.05	0.03	0.274
EC (10^9/L)	0.08 ± 0.04	0.06 ± 0.03	0.964	0.710
BC (10^9/L)	0.03 ± 0.02	0.04 ± 0.05	-0.499	0.206
RBC (10^{12}/L)	4.98 ± 0.54	4.75 ± 0.30	0.885	0.357
Hb (g/L)	144 ± 15.28	135.17 ± 6.05	1.316	0.179
PLT (10^9/L)	230.33 ± 32.98	216.50 ± 12.76	0.958	0.189

Table 3 Analysis of biochemical indexes

	T2DM-DPN ($n=17$)	Control ($n=18$)	t	p
TP (g/L)	67.88 ± 3.53	71.58 ± 4.93	− 0.793	0.77
ALB (g/L)	41.93 ± 2.52	40.97 ± 3.54	0.545	0.313
TBIL (μmol/L)	14.57 ± 6.61	10.72 ± 4.68	1.164	0.346
DBIL (μmol/L)	2.68 ± 1.23	2.88 ± 1.28	− 0.276	0.938
IBIL (μmol/L)	11.88 ± 5.44	7.97 ± 3.89	1.435	0.382
ALP (U/L)	80.83 ± 22.55	62.5 ± 13.88	1.696	0.064
K (mmol/L)	4.11 ± 0.35	4.29 ± 0.40	− 0.817	0.929
Na (mmol/L)	139.33 ± 3.78	141.5 ± 1.38	− 1.32	0.098
GLU (mmol/L)	17.84 ± 7.60	5.12 ± 0.50	4.088	0.009
UA (μmol/L)	276.33 ± 37.90	323.50 ± 60.69	− 1.615	0.616
Urea (mmol/L)	4.98 ± 1.06	5.01 ± 1.75	− 0.03	0.997
CRE (μmol/L)	50.33 ± 8.29	61.00 ± 10.13	− 1.866	0.730
CO_2 (mmol/L)	24.88 ± 4.76	25.38 ± 1.60	− 0.244	0.205
TG (mmol/L)	2.29 ± 1.10	1.03 ± 0.38	2.634	0.012
TC (mmol/L)	6.51 ± 1.21	4.63 ± 0.47	2.539	0.058
HDL-C (mmol/L)	1.34 ± 0.28	1.50 ± 0.33	− 0.935	0.611
LDL-C (mmol/L)	4.03 ± 0.72	2.34 ± 0.64	4.27	0.900
ApoA (g/L)	1.39 ± 0.21	1.41 ± 0.21	− 0.151	0.940
ApoB (g/L)	1.38 ± 0.29	0.78 ± 0.22	4.041	0.015
LDH (U/L)	173.33 ± 20.23	158.83 ± 17.29	1.335	0.461

DPN is one of the common but often neglected complications in diabetes patients [7–9]. Hyperglycemia is generally considered the main pathogenic factor of diabetes neuropathy [2, 26, 27]. The results of this study showed that the Glu was related to the function of sweat glands in the hand area of T2DM-DPN patients. In T2DM-DPN patients, long-term hyperglycemia promoted microvascular lesions, resulting in insufficient nutrition of sympathetic postganglionic fibers, which may eventually cause functional damage of sweat glands. At the same time, postganglionic sweating fibers also participated in peripheral neuropathy under the condition of hyperglycemic [28, 29]. Therefore, it is speculated that the autonomic neuropathy and peripheral neuropathy may exist at the same time in T2DM-DPN patients, and this damage may occur earlier at the distal end of the body. Moreover, this study found that there was a significant difference in TG content between T2DM-DPN group and control group. Although the further analysis showed that there was not a significant correlation between TG and sweat gland function, some studies have shown that the TG content in vivo might be related to diabetes peripheral neuropathy in some distance [30].

T2DM-DPN has been proven to be an inflammatory and immune system dysfunction disease [19]. Studies have shown that chronic inflammation could promote the occurrence and development of DPN in patients with T2DM [31–33]. Peroxidase and active oxygen are released during the increased activation of neutrophils, which may lead to enhanced oxidative stress and persistent inflammation. These cascades will eventually lead to an increase in neutrophils [34–36]. On the other hand, T2DM and its complications may be related to lymphopenia as well. NLR can precisely reflect the two main components of chronic inflammation: high neutrophil granulocytes and low lymphocytes [37, 38]. In this study, the difference of NLR between T2DM-DPN group and control group was statistically significant. Huang et al. [39, 40] also speculated that the increased NLR might predict the higher incidence of peripheral neuropathy in T2DM patients. NLR may not only accelerate the progress of DPN, but also serve as an auxiliary indicator for early diagnosis of DPN [41, 42].

Conclusion

In general, the onset of DPN is relatively insidious. Many patients usually have no obvious abnormal feeling in the early stage of the disease, and will not show obvious clinical symptoms until the disease progresses to the middle and

Table 4 Pearson correlation analysis between clinical indexes and sweat gland function

	Glu	TG	ApoB	RBC	NLR
r	0.651	0.472	0.164	0.149	0.171
p	0.022	0.121	0.611	0.644	0.594

r Pearson's correlation coefficient

late stages. Therefore, early screening and diagnosis is an effective method to treat T2DM-DPN. Although our result was obtained through a relatively small number of subjects, it may be helpful to track the progression of T2DM neuropathy. The accurate evaluation of sweat gland function damage can help clinicians strengthen the monitoring of autonomic neuropathy condition and effectively judge the occurrence, development, and severity of T2DM-DPN.

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Data availability Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, supporting data is not available.

Declarations

Ethical approval This study was approved by the Ethics Committee of Putian University, China.

Informed consent All the study subjects were informed of the significance of this study and signed informed consent forms.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Glycemic outcomes of people with diabetes mellitus in Brazilian primary health care

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Abstract

Background The capillary blood glucose monitoring program at home a challenge in primary health care. Therefore, it is fundamental to identify the glycemic control of people with diabetes mellitus through HBA1c and to analyze its associated factors.
Objective To identify the glycemic profile of people with Diabetes Mellitus (DM) through HbA1c and analyze factors associated.
Materials & methods Cross-sectional study developed in Ribeirão Preto, São Paulo, Brazil. Secondary data from the electronic health record of people registered in the Primary Health Care system were used. A sample of 3181 participants was obtained. People with HbA1c < 7.0% (53 mmol/mol) were considered to have adequate glycemic control. For people aged ≥ 55 years, a less stringent target, < 8.0% (64 mmol/mol), was also considered. The odds ratio was the measure of effect analyzed with their respective 95% Confidence Intervals (95% CI).
Results Adequate glycemic control with HbA1c < 7.0% (53 mmol/mol) was found in 44.8% of people and, when using the less rigid target, HbA1c < 8.0% (64 mmol/mol) for people aged ≥ 55 years-old, 70.6% had adequate glycemic control. Age and drug therapy were associated with adequate glycemic control ($p < 0.001$), which was more frequent among older people and those who used only metformin.
Conclusion The study shows that the achievement of adequate glycemic control is still a challenge, especially with regard to younger people and those who use insulin.

Keyword Diabetes mellitus · Glycemic control · Glycated hemoglobin A · Primary health care

Introduction

Chronic Non-Communicable Diseases (NCDs) are the leading cause of mortality worldwide and accounted for 71.0% of all deaths that occurred in 2016. While there has been a reduction in the overall rates of premature mortality (age 30–70 years) from chronic respiratory diseases, cardiovascular diseases and cancer, the diabetes mellitus (DM), in contrast, showed an increase of 5.0% between the years 2000 and 2016 [1, 2].

Estimates indicate that in 2019, every eight seconds a person aged 20–79 years died from DM, with almost half of the 4.2 million deaths occurring before the age of 60 years. The worldwide expenditure on DM complications is approaching U$760 billion. In addition, it is one of the diseases whose frequency is increasing in several countries around the world, with a projection of reaching 700 million people in 2045. Thus, DM imposes a high burden on society, in the form of high medical and hospital costs, loss of productivity, premature mortality, and compromised quality of life [2–5].

DM-related complications such as cardiovascular disease, retinopathy, nephropathy, neuropathy are related to hyperglycemia. After classical studies showed the correlation of hyperglycemia with the presence of complications of DM, glycated hemoglobin (HbA1c) has been consolidated as one of the main markers of glycemic control [6, 7]. In type 2

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DM, for each 1.0% reduction in HbA1c there is a 21.0% decrease in the risk of any outcome related to the disease, 21.0% for deaths, 14.0% for myocardial infarction and 37.0% for microvascular complications [8–10]. The goal of glycemic control through HbA1c recommended by the main guidelines is below 7.0% (53 mmol/mol) and may be more flexible depending on the clinical conditions of each person, life expectancy and episodes of hypoglycemia [9, 10].

Despite advances in therapy, adequate glycemic control of people with DM is a challenge in several regions of the world. A meta-analysis showed that globally, only 42.8% of people achieved optimal HbA1c targets, with Europe and North America showing the best results [11]. Recent studies found adequate glycemic control rates of 31.8% in Mexico, 53.4% in Colombia, 48.9% in Argentina, and 50.4% and 53.4%, respectively, in men and women in Korea [12–15]. In Brazil, a study in the southern region showed 30.2% of people enrolled in primary health care (PHC) with adequate glycemic control [16].

Recently, the new coronavirus pandemic has directed even more attention to people with NCDs, since the pre-existence of these comorbidities dramatically elevates mortality rates by COVID-19 [17, 18]. People with uncontrolled DM have impairment in innate immunity, the first line of defense against Sars-Cov-2, and other important alterations in the inflammatory response, which may lead to aggravation of COVID-19. On the other hand, infection by COVID-19 worsens dysglycemia, causing a vicious circle between DM and COVID-19 with unfavorable clinical outcomes. DM and hyperglycemia at hospital admission are associated with worse prognosis [18, 19].

Brazil is one of the countries with the highest prevalence of DM in the world. Recent studies have shown that most people who perform home self-monitoring experienced greater variability in blood glucose during the pandemic, in addition to many having postponed medical appointments and routine exams [20, 21]. In view of the recommendations for monitoring glycemic control, the Ministry of Health of Brazil instituted the evaluation of HbA1c in 2020 as an indicator of PHC performance in the country, and municipalities that meet the evaluation goals receive financial resources related to good performance in this indicator [10].

Few studies assessed glycemic control of Brazilian patients in PHC. Therefore, in order to provide subsidies for the planning of health care and actions, this study has the following objectives: to identify the glycemic profile of people with DM through HbA1c and analyze factors associated with glycemic control.

Methods

Study design and participants

Quantitative, observational, cross-sectional study developed in Ribeirão Preto, São Paulo, Brazil. Secondary data from the electronic health record of people registered in the PHC system were used. People aged ≥ 18 years, using oral antidiabetics (OAD) and/or injectables provided in the Brazilian PHC system (metformin, glibenclamide, gliclazide, regular insulin, and NPH insulin) and who had at least one record of HbA1c value in 2018 were included in the sample. The stratified random sampling method was applied, and people from the five health districts of Ribeirao Preto (North, South, East, West and Central) were proportionally selected. Therefore, a sample of 3181 participants was obtained. We adopted a significance level of 5.0%, relative error of 5.0% and prevalence of 27.0% of expected event [22].

Data collection

Data were extracted from electronic health records and recorded on a structured form containing sociodemographic variables: sex (male and female) and age (< 25 years, from 25 to 34, from 35 to 44, from 45 to 54, from 55 to 64, from 65 to 74 and ≥ 75 years of age); and clinical variables: drug therapy (metformin only, sulfonylurea only, insulin only, metformin + sulfonylurea and ADO + insulin) and HbA1c value.

People with HbA1c < 7.0% (53 mmol/mol) were considered to have adequate glycemic control. For people aged ≥ 55 years, a less stringent target, < 8.0% (64 mmol/mol), was also considered. For people with HbA1c values < 6.5%, the cut-off value for diabetes diagnosis, an investigation of the electronic health record was performed, searching for previous laboratory tests and/or medical records that would confirm or not the diagnosis of diabetes. This procedure was justified since some people with pre-diabetes also use ADO such as metformin, as shown in Fig. 1.

High-Efficiency Liquid Chromatography and Immunoturbidimetry were the laboratory analysis methods used in the reference laboratories of Ribeirao Preto's PHC.

Statistical analysis

Statistical Package for Social Sciences (SPSS) for windows version 20.0 was used for statistical analysis. Frequency calculations were performed by basic descriptive analysis. The relationship between glycemic control and sociodemographic and clinical variables was analyzed by means of Binary Logistic Regression, and the odds ratio (OR) was the measure of effect analyzed with their respective 95% Confidence Intervals (95% CI). The model was adjusted by selecting the independent variables that presented statistical significance in the univariate analysis, obtaining the adjusted odds ratio (ORaj).

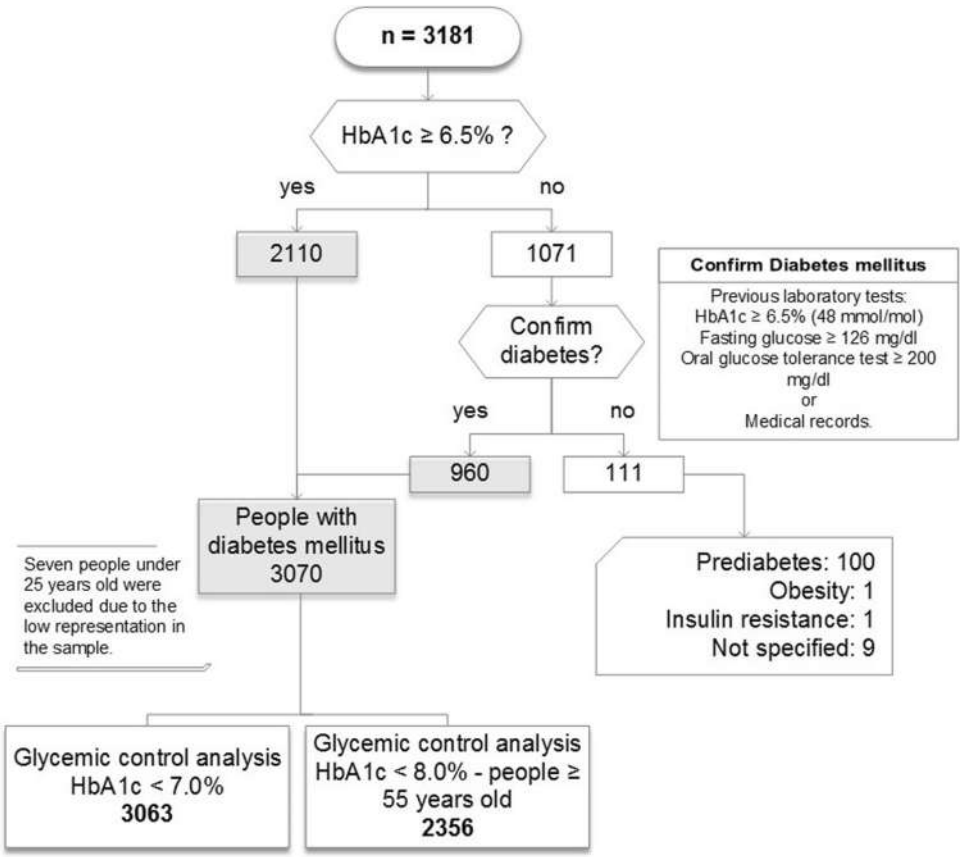

Fig. 1 Graphical representation of data collection and analysis, Ribeirão Preto, SP, Brazil

Ethical aspects

The project was approved by the Research Ethics Committeê of the School of Nursing of the University of Ribeirão Preto by opinion no. 3,340,774 on May 22, 2019.

Results

Among people being treated with ADOs in PHC, there was a predominance of females (60.5%) and adults and elderly, and most people (92.0%) were older than 45 years, according to Table 1. Metformin was the most used drug (60.8%), being used in isolation by 27.5% of people. Among the sulfonylurea drugs, gliclazide was used by 42.0% of people. Only 19.7% of people used sulfonylurea, while 21.1% used metformin + sulfonylurea. As for insulins, 13.4% used insulin alone and 18.3% associated with OAD.

We removed from the sample 111 people who, after obtaining data from medical records, did not confirm the diagnosis of DM. In addition, due to the low representation in the sample, it was decided to exclude people under 25 years of age. Thus, among people with confirmed DM diagnosis and age ≥ 25 years (n = 3063), HbA1c ranged from 3.3% (13 mmol/mol) to 18.1% (174 mmol/mol), with a mean of 7.6% (60 mmol/mol) [95% CI 7.5—7.6% (59—60 mmol/mol)]. Adequate glycemic control, HbA1c < 7.0% (53 mmol/mol), was found in 44.8% of participants with DM (95% CI 42.9—46.6%). Men and women had similar frequencies of adequate glycemic control**.**

According to Table 2, age and drug therapy were associated with glycemic control ($p < 0.001$). The frequency of adequate glycemic control increased progressively with age, and in people aged ≥ 75 years, the chance of achieving adequate glycemic control is 2.79 times higher than in people aged 25–34 years [ORaj = 2.79 (95% CI 1.35—5.76)]. People using insulin had the lowest frequencies of adequate glycemic control. The chance of achieving adequate glycemic control is 91.0% lower among people using insulin only [ORaj = 0.09 (95% CI 0.07—0.12)] and 93.0% lower among those using insulin + ADO [ORaj = 0.07 (95% CI 0.05—0.09)] when compared to those using metformin only.

Age is one of the factors to be considered in adjusting the HbA1c target, which may be less strict among older people. Thus, regarding the achievement of the less stringent glycemic target, HbA1c < 8.0% (64 mmol/mol), among people aged ≥ 55 years (n = 2356) it was observed that 70.6% (95% CI 68.8—72.6%) had adequate glycemic

Table 1 Sociodemographic and Clinical Characteristics (n = 3181)

Variable	Absolute frequency	Relative frequency (%)
Sex		
Female	1925	60.5
Male	1256	39.5
Age		
< 25 years old	10	0.3
25–34 years old	50	1.6
35–44 years old	194	6.1
45–54 years old	493	15.5
55–64 years old	968	30.4
65–74 years old	944	29.7
≥ 75 years old	522	16.4
Drugs used		
Metformin	1934	60.8
Glibenclamide	467	14.7
Gliclazide	1335	42.0
Insulin R	411	12.9
Insulin NPH	1003	31.5
Drug therapy		
Metformin only	874	27.5
Sulfonylurea only	626	19.7
Insulin only	425	13.4
Metformin + sulfonylurea	673	21.1
ADO[1] + insulin	583	18.3

[1]*ADO* oral antidiabetic

control. HbA1c ranged from 3.3% (13 mmol/mol) to 16.0% (151 mmol/mol), with a mean of 7.4% (57 mmol/mol) [95% CI 7.3—7.5% (56—58 mmol/mol)].

Even in cases of less stringent glycemic target, according to Table 3, age and drug therapy remained associated with glycemic control ($p < 0.001$). In people aged ≥ 75 years, the chance of finding HbA1c < 8.0% (64 mmol/mol) is 1.86 times higher than in people aged 25–34 years [ORaj = 1.86 (95% CI 1.42—2.43)]. Those using insulin had the lowest frequencies of adequate glycemic control. When compared to people using metformin only, the chance of finding adequate glycemic control for this goal is 93.0% lower in people using insulin only [ORaj = 0.07 (95% CI 0.04—0.10)] and 94.0% lower in people using insulin + ADO [ORaj = 0.06 (95% CI 0.04—0.10)].

Discussion

In our sample, 44.8% (95% CI 43.0—46.5%) showed adequate glycemic control with no differences between males and females. A study conducted in England among people with DM and aged ≥ 20 years, considering identical criteria to the present study to classify glycemic control, showed a frequency of adequate glycemic control of 49.7%, i.e., slightly higher than that found in the present investigation [23, 24]. Similar findings were recorded in countries such as Japan among people aged 20 to 69 years and also in China among those ≥ 18 years, in which 44.9% and 49.2%, respectively, had adequate glycemic control [25, 26].

On the other hand, in Portugal, 63.2% of people with DM and age between 25 and 74 years had adequate glycemic control. However, the criterion used in the study was having HbA1c ≤ 7.0% (53 mmol/mo) [27].

In our findings, age and drug therapy were associated with glycemic control. A municipality in the southern region of Brazil had similar findings, with elderly ≥ 70 years showing less elevation of HbA1c than people aged 50—69 years [16]. Similarly, in the United States, people with DM ≥ 75 years old had 40.0% more adequate glycemic control compared to those aged 40 to 49 years. According to the authors, the elderly were treated more intensively (use of insulin or two or more hypoglycemic medications) than young adults to achieve the goal of HbA1c < 7.0% (53 mmol/mol) [28].

Adherence to drug treatment is an important factor to be considered in achieving adequate glycemic control. Study in Sudan showed the relationship between low adherence and higher levels of HbA1c; and further, that people with low adherence were younger, which reiterates that the older the age the higher the frequency of adequate glycemic control, a fact that is presumed to have been strengthened by greater adherence to treatment by older people [29].

In Brazilian PHC, ADOs are prescribed on a large scale which was evidenced in the present investigation which recorded the use of metformin by 60.8% either alone or associated with sulfonylureas (gliclazide or glibenclamide). Before prescribing antidiabetics, professionals take into consideration the patient's general condition, the presence of comorbidities or obesity, the cost and the risk of hypoglycemia. Currently, metformin is the initial drug of choice recommended by the Brazilian Diabetes Society algorithm and, in the persistence of hyperglycemia, the combination with sulfonylureas becomes the option available at the Unified Health System [10]. In addition, the use of metformin has been related to the reduction of cardiovascular mortality, overall mortality, and cardiovascular events in people with coronary artery disease, besides being considered better than sulfonylureas in reducing the incidence of cardiovascular events [30]. It is worth noting that currently there are investigations that have shown encouraging results with the use of herbal medicines such as ginger and cinnamon in reducing the glycemic levels of ADO users [31, 32].

Table 2 Association between adequate glycemic control—HbA1c < 7.0% (53 mmol/mol)—and gender, age and drug therapy (n = 3063)

Variable	HbA1c < 7.0% (53 mmol/mol) n (%)	OR (95% CI)[1]	p-value	ORaj (95% CI)[2]	p-value
Sex			0.27		-
Female	842 (45.6)	Reference		-	
Male	531 (43.6)	0.92 (0.80–1.06)		-	
Age			< 0.001		< 0.001
25–34 years old	14 (30.4)	Reference		Reference	
35–44 years old	67 (35.4)	1.25 (0.63–2.52)		0.99 (0.46–2.15)	
45–54 years old	175 (37.1)	1.35 (0.70–2.59)		1.16 (0.56–2.40)	
55–64 years old	419 (45.1)	1.87 (0.99–3.56)		1.60 (0.78–3.26)	
65–74 years old	416 (45.2)	1.88 (0.99–3.58)		1.79 (0.88–3.65)	
≥ 75 years old	282 (55.8)	2.89 (1.51–5.55)		2.79 (1.35–5.76)	
Drug therapy			< 0.001		< 0.001
Metformin only	599 (78.4)	Reference		Reference	
Sulfonylurea only	281 (45.1)	0.23 (0.18–0.29)		0.21 (0.17–0.27)	
Insulin only	114 (27.1)	0.10 (0.08–0.13)		0.09 (0.07–0.12)	
Metformin + sulfonylureas	257 (38.2)	0.17 (0.13–0.21)		0.17 (0.13–0.21)	
ADO[3] + insulin	122 (20.9)	0.07 (0.06–0.09)		0.07 (0.05–0.09)	
Total	1373 (44.8)	-			

[1]OR (95% CI): Odds Ratio and 95% confidence interval

[2]*ORaj* Odds Ratio adjusted for age and drug therapy and 95% confidence interval

[3]*ADO* oral antidiabetic

With regard to insulin, the chance of finding adequate glycemic control was 91.0% lower among people using insulin alone. In our findings, insulin was used by 31.7% of people, being similar to the study by Mendes et al. [22] where 34.0% of patients with type 2 diabetes were on insulin treatment. In clinical practice, in the initial presentation of type 2 DM, modifications in lifestyle habits associated with the use of metformin are indicated. The frequency of insulin use becomes higher as

Table 3 Association between adequate glycemic control, according to the least stringent target—HbA1c < 8.0% (64 mmol/mol)—for people aged ≥ 55 years, according to the variables sex, age and drug therapy (n = 2356)

Variable	HbA1c < 8.0% (64 mmol/mol) n (%)	OR (IC 95%)[1]	Valor p	ORaj (IC 95%)[2]	Valor p
Sex			0.05		-
Female	1040 (72.1)	Reference		-	
Male	624 (68.3)	0.83 (0.69–1.00)		-	
Age			< 0.001		< 0.001
55–64 years old	633 (68.1)	Reference		Reference	
65–74 years old	636 (69.1)	1.05 (0.86–1.27)		1.16 (0.94–1.43)	
≥ 75 years old	395 (78.2)	1.68 (1.31–2.17)		1.86 (1.42–2.43)	
Drug therapy			< 0.001		< 0.001
Metformin only	559 (94.3)	Reference		Reference	
Sulfonylurea only	358 (74.3)	0.18 (0.12–0.26)		0.17 (0.11–0.25)	
Insulin only	176 (54.3)	0.07 (0.05–0.11)		0.07 (0.04–0.10)	
Metformin + sulfonylureas	342 (66.4)	0.12 (0.08–0.18)		0.12 (0.08–0.18)	
ADO[3] + insulin	229 (51.8)	0.06 (0.04–0.10)		0.06 (0.04–0.10)	
Total	1664 (70.6)				

[1]OR (95% CI): Odds Ratio and 95% confidence interval

[2]*ORaj* Odds Ratio adjusted for age and drug therapy and 95% confidence interval

[3]*ADO* oral antidiabetic

the duration of diabetes increases, given the natural progression of type 2 DM, which causes a gradual decrease in insulin production by the pancreas. Generally, after a decade of disease progression, it is necessary to associate the use of insulin with ADO. Thus, the Brazilian Diabetes Society stresses the importance of the timely initiation of insulin therapy, which often does not happen due to therapeutic inertia, fear of weight gain, fear of hypoglycemia, among other reasons [10].

However, even when setting less strict glycemic goals, people using insulin showed lower frequencies of adequate glycemic control. It is noteworthy that the less stringent glycemic target was established only considering the criterion of advanced age. Therefore, among people with DM and age ≥ 55 years, 70.6% (95% CI 68.8—72.6%) had HbA1c < 8.0% (64 mmol/mol). These findings are similar to others obtained by researchers who adopted the less rigid goal in the evaluation of people with DM. In this direction, we highlight the investigation developed in the southeastern United States, which identified adequate glycemic control in 63.5% of participants [33]. On the other hand, national data showed a slightly lower percentage, i.e., 60.0% with HbA1c < 8.0% (64 mmol/mol) [20].

It was evidenced in all analyses that the chance of finding adequate glycemic control is significantly lower in people using insulin than in those using metformin alone. Scholars pointed out that people who did not reach their HbA1c target were treated with substantially higher insulin doses than those who did. Therefore, it is noted that even by intensifying the dose of drug therapy, adequate glycemic control remains difficult to achieve [23]. The delay in intensifying drug treatment after the identification of elevated levels of HbA1c can reach, on average, more than one year, being even more significant in situations in which a larger number of antidiabetic drugs are used [34].

Furthermore, researchers draw attention to a worrying finding: 44.0% of people with HbA1c levels ≥ 9.0% (75 mmol/mol) did not have their drug therapy intensified [35]. It is also emphasized that therapeutic inertia may result from a combination of factors related to patients, health professionals and the health system, being a complex conduct that needs to be better investigated [36].

It is worth considering that adherence to insulin treatment is multifactorial, since it may be related to low socioeconomic status, fear of hypoglycemia, the fact that it is injectable and, in most cases, requires more than one daily application [37, 38]. In addition, the technique of insulin application requires specific knowledge and skills, and errors regarding self- application or application by others are not uncommon, especially due to decreased visual acuity which can compromise the achievement of adequate glycemic control [39–41].

The present study did not distinguish between the different types of diabetes and neither the time of diagnosis due to incomplete information in the electronic health record. However, it is recognized that both are important in the analysis of the frequency of insulin use. Another limitation of the study is the fact that the data refer to drugs dispensed by pharmacies in the primary health care network, not having computed those acquired by other means. In this regard, the study on access, use and promotion of rational use of medicines showed that 97.8% of people diagnosed with diabetes reported having access to prescribed antidiabetic drugs and 70.7% get them completely free of charge [42].

Although the glycemic control data found in this study approximate those for developed countries, it was found that just under half of people with DM achieved the more stringent goal of adequate glycemic control, even before the covid-19 pandemic. It is believed that the changes arising from this current health scenario contribute even more to inadequate glycemic control, both by lockdowns and by the limitation of care, and even by the fear of crowding and contagion. Therefore, in the current pandemic context, we emphasize the need for investment in strategies to overcome the new challenges imposed, such as the use of distance interaction technologies, teleconsultations, digital educational materials and guaranteed access to essential medicines and continuity of care [43].

Conclusion

The present study showed that less than half of the participants achieved adequate glycemic control and younger people with DM and on insulin treatment showed the lowest rates of adequate glycemic control. In addition, the study data showed that in the population studied, only 63.9% of people with DM had their HbA1c evaluated. As this is a component of the performance evaluation for PHC recently incorporated by the MS, there is an imminent need for further expansion of access and professional training so that HbA1c appears in the assessments of people with DM and, in this way, health teams guarantee the associated financial resources to this indicator.

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Declarations

Conflicts of interest Authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Association of albumin, globulin and albumin/globulin ratio with renal injury in type 2 diabetic nephropathy patients

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Abstract

Background Studies have shown that albumin/globulin ratio (AGR) can assess the extent of kidney damage in type 2 diabetic nephropathy (T2DN). However, there is a lack of similar clinical data to support this.

Objectives This study sought to inquire into the correlation of albumin (ALB), globulin (GLB) and albumin/globulin ratio (AGR) with renal injury in patients with type 2 diabetes mellitus (T2DM).

Methods A retrospective analysis was performed on the clinical data of 82 patients with T2DM (Control group) and 110 patients with type 2 diabetic nephropathy (T2DN) who were admitted to the First Affiliated Hospital of Wannan Medical College from October 2019 to April 2022. T2DN patients were classified into mild renal impairment group ($n=75$) and moderate renal impairment group ($n=35$) according to urinary albumin excretion rate (UAER). Then, the general data of all groups were compared. Furthermore, Pearson correlation was used to analyze the correlation of serum ALB, GLB and AGR with UAER in the three groups. A receiver operating characteristic curve (ROC) was utilized to evaluate the diagnostic value of ALB, GLB and AGR for moderate renal injury in T2DN patients.

Results There were significant differences in course of disease, history of hypertension, levels of fasting plasma glucose and glycosylated hemoglobin among the three groups. Besides, compared with the Control group, the levels of ALB and AGR were lower while GLB levels were higher in the mild and moderate renal impairment group. In particular, ALB and AGR levels were lower in the moderate renal impairment group relative to the mild renal impairment group, but the GLB levels exhibited no significant difference between the two groups. According to the results of Pearson correlation analysis, a negative correlation of ALB and AGR levels with UAER was revealed in T2DN patients. ROC curves displayed the area under the curve (AUC) of ALB (0.88) and AGR (0.71) predicting moderate renal injury in T2DM patients ($p<0.05$). However, GLB has no significant diagnostic value for moderate renal injury in patients with T2DN.

Conclusion The course of disease, hypertension and glycemic control may affect the occurrence and development of T2DN. ALB and AGR are of high value in predicting renal injury in patients with T2DN and can serve as the foundation for the clinical diagnosis of the condition.

Keywords Type 2 diabetic nephropathy (T2DN) · Albumin (ALB) · Albumin/globulin ratio (AGR) · Urinary albumin excretion rate (UAER) · Renal injury · Correlation

Abbreviations

ALB	Albumin
GLB	Globulin
AGR	Albumin/globulin ratio
T2DM	Type 2 diabetes mellitus
T2DN	Type 2 diabetic nephropathy
UAER	Urinary albumin excretion rate
BMI	Body mass index
ROC	Receiver operating characteristic
FPG	Fasting plasma glucose
SD	Standard deviation

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Introduction

Diabetic nephropathy (DN) is a chronic microvascular kidney disease induced by diabetes mellitus (DM) and is clinically characterized by microalbuminuria and/or decreased glomerular filtration rate [1]. It's reported that DN accounts for 16.3% of end-stage renal diseases in China [2]. Due to the insidious onset of DN, most patients are diagnosed with DN in the clinical stage. And renal injury is so difficult to reverse that it gradually develops into end-stage renal failure. Therefore, early detection, early diagnosis and effective control of DN seem to be of the utmost importance [3]. Data statistics in 2020 revealed that more than 90% of the diabetic population in China had type 2 diabetes mellitus (T2DM) [4]. Hence, the focus of this investigation was on determining the severity of renal injury in patients with type 2 diabetic nephropathy (T2DN).

Urinary albumin excretion rate (UAER) is usually applied to assess the degree of renal injury. However, UAER is susceptible to interference by some other factors because of the time-consuming urine collection and the significantly increased UAER under urinary tract infection and menstrual status [5]. For the assessment of renal impairment in patients with T2DN, it is therefore crucial to develop markers with simple sampling and stable outcomes. Albumin/globulin ratio (AGR) is a new predicting indicator that can comprehensively reflect the inflammation and nutrition in the body [6]. It can't be ignored that inflammatory response is crucial in the development of DN [7]. Recent studies have reported that, as sensitive indicators to assess the degree of renal injury in T2DN, albumin (ALB) and AGR have advantages like simplicity and speed [8]. Based on the above, we retrospectively analyzed the clinical data of 192 T2DM patients in our hospital. And our study aimed to assess the relationship between serum ALB, globulin (GLB) and AGR and renal impairment in patients with T2DN and provide new ideas for clinical diagnosis and treatment of this condition.

Materials and Methods

Study design

A retrospective analysis was conducted on 110 T2DN patients and 82 T2DM patients with normal kidneys (Control group) who visited the First Affiliated Hospital of Wannan Medical College from October 2019 to April 2022. Our study was approved by the Ethics Committee of the First Affiliated Hospital of Wannan Medical College.

Inclusion criteria were shown as follows. All enrolled patients (1) satisfied the diagnostic criteria of T2DM in the literature of *Guidelines for the prevention and treatment of Type 2 diabetes in China: 2017* [9]. The diagnostic criteria included (a) typical symptoms of diabetes mellitus (irritable thirst, polyuria, polyphagia, unexplained weight loss); (b) random blood glucose $\geq$ 11.1 mmol/L; (c) fasting blood glucose $\geq$ 7.0 mmol/L; (d) glucose load 2 h blood sugar $\geq$ 11.1 mmol/L. The (a) one is necessary, and (a) plus any one of the (b), (c) or (d) one will give the patient a diagnosis of diabetes. If there are no typical diabetic symptoms, the diagnosis should be confirmed by re-examination at a later date; (2) met the criteria of UAER $\geq$ 30 mg/24 h and with hyperglycemia as etiology [10]; (3) aged from 18 to 75 years; (4) had complete clinical data.

Patients were excluded if they suffered from (1) acute diabetic complications such as ketoacidosis, (2) cardiac insufficiency, an autoimmune disorder of connective tissue, infection and respiratory disorder, (3) lupus nephropathy, (4) kidney lesions caused by tumors, (5) type 1 diabetes, gestational diabetes, and other special types of diabetes; (6) severe heart, liver, lung and cerebrovascular diseases, (7) various stress states (e.g. infection, acute myocardial infarction, acute stroke, trauma, surgery, etc.); had (8) long-term application history of steroid hormone therapy, (9) history of previous neuropsychiatric disorders; received (10) angiotensin converting enzyme inhibitors or angiotensin II receptor antagonist antihypertensive drugs or had an uncontrolled hypertension, (11) dialytic treatment.

According to the grading criteria for renal impairment reported by Mogensen et al. [11], UAER of 30–300 mg/24 h indicated mild renal impairment and UAER > 300 mg/24 h represented moderate renal impairment. T2DN patients were divided into the mild renal impairment group ($n = 75$) and moderate renal impairment group ($n = 35$) depending on the level of UAER. Then, general information including gender, age, body mass index (BMI), smoking history, drinking history, fasting blood glucose, glycosylated hemoglobin (HbA1c), ALB, GLB, AGR and others were collected from the three groups.

Determination of serum and urine indicators

Venous blood samples were collected in the morning after fasting for 10 h in all the included patients. Next, an automatic chemistry analyzer (Hitachi 7600; Hitachi High-Technologies Corporation, Japan) was applied to detect fasting plasma glucose (FPG), ALB and GLB, followed by the calculation of AGR. Finally, an automatic HbA1c analyzer (ADAMS A1c HA-8180, ARKRAY Factory, Inc., Japan) was adopted for the detection of HbA1c level.

Subsequently, the 24-h urine was collected from all the included patients, and the total amount of ALB in urine was detected by immunoturbidimetric assay. UAER could be obtained after calculation of urinary excretion of ALB per unit time.

Methods for 24-h urine collection: the patients were instructed to prepare a clean plastic bucket (4000 ml of size and with a lid) by themselves. The patients should urinate once in the morning at a certain time (e.g., 7:00 a.m.), and this time the urine should not be poured into the bucket. From this discharge, all discharged urine within 24 h should be poured into the plastic bucket. The last urine discharge was at 7:00 am the next morning, and this time the discharged urine should also be introduced into the plastic bucket. When filling the first urine into the plastic bucket, the patients need to pour the antiseptic provided by the nurse into the plastic bucket and shake it well. Shaking of the bucket should be performed after each introduction of the discharged urine, followed by covering the bucket to prevent evaporation of urine. Twenty-four hours later, the bucket will be picked up by the specialized nursing staff. Notably, collection of samples from female patients during menstruation should be avoided; patients ate and moved normally during urine collection.

Statistical analysis

The data from this study were analyzed and processed using SPSS 22.0 statistical software. To be specific, measurement data conforming to normal distribution were expressed as mean ± standard deviation (SD). One-way ANOVA was used for comparison between multiple groups and then last significant difference (LSD) test was used for comparison between two groups. Enumeration data were expressed as n (%) and the chi-square test was used for comparison between multiple groups. Pearson correlation was used to analyze the correlation between UAER levels with serum ALB, GLB and AGR levels in the T2DM group. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value of ALB, GLB, and AGR for moderate renal injury in T2DN. $p < 0.05$ was considered statistically significant.

Results

Comparison of clinical baseline characteristics among the patients in the three groups

As shown in Table 1, there was no significant difference in gender, age, BMI, smoking history, and drinking history among the patients in the three groups ($p > 0.05$). However, the duration of T2DM, history of hypertension, FPG, and HbA1c in the mild renal impairment and moderate renal impairment groups showed significant difference with those in the Control group ($p < 0.05$). The comparisons among the three groups disclosed the longest duration of T2DM and the highest proportion of hypertension history, FPG level, and HbA1c level in the moderate renal impairment group.

Comparison of albumin, globulin and albumin/globulin ratio levels among the three groups

As displayed in Table 2, the ALB and AGR levels in the mild renal impairment group and moderate renal impairment group were lower while GLB levels were higher than those in the Control group, and the differences were statistically significant ($p < 0.05$). Besides, compared with the mild renal impairment group, the moderate renal impairment group presented lower ALB and AGR levels, but there was no significant difference in GLB level between the two groups ($p > 0.05$).

Correlation of albumin, globulin, and albumin/globulin ratio levels with urinary albumin excretion rate in patients with T2DN

Pearson correlation analysis revealed (Fig. 1A–C) that ALB and AGR levels had a significantly negative correlation with the UAER level in patients with T2DN ($p < 0.05$), while GLB levels had no obvious correlation with the UAER level ($p > 0.05$).

Table 1 Comparison of clinical baseline characteristics among the three groups

Grouping	Control group ($n = 82$)	Mild renal impairment group ($n = 75$)	Moderate renal impairment group ($n = 35$)	X^2/F	p
Gender (male/female)	46(56.1)/36(43.9)	40(53.3)/35(46.7)	18(51.4)/17(48.6)	0.250	0.883
Age (year)	63.05 ± 12.87	59.24 ± 14.44	63.57 ± 10.03	2.124	0.122
BMI (kg/m^2)	23.73 ± 2.41	24.19 ± 2.46	24.43 ± 2.55	1.250	0.289
Smoking history (%)	19(23.2)	15(20.0)	10(28.6)	0.998	0.607
Drinking history (%)	9(11.0)	11(14.7)	8(22.9)	2.781	0.249
Duration of T2DM (year)	7.87 ± 1.82	9.35 ± 1.61*	12.09 ± 1.90*#	71.126	< 0.001
History of hypertension (%)	24(29.3)	36(48.0)*	26(74.3)*#	20.615	< 0.001
FPG (mmol/L)	7.24 ± 0.87	7.62 ± 1.03*	8.80 ± 0.97*#	33.151	< 0.001
HbA1c (%)	8.04 ± 0.83	8.38 ± 0.85*	9.46 ± 0.84*#	35.111	< 0.001

Qualitative data were expressed as n (%), and measurement data were expressed as mean ± SD; *BMI*, body mass index; *T2DM*, type 2 diabetes mellitus; *FPG*, fasting plasma glucose; * indicated $p < 0.05$ vs. Control group; # indicated $p < 0.05$ vs. mild renal impairment group

Table 2 Comparison of albumin, globulin and albumin/globulin ratio levels among the three groups

Grouping	Control group ($n=82$)	Mild renal impairment group ($n=75$)	Moderate renal impairment group ($n=35$)	F	p
Albumin (mg/L)	4.13 ± 0.38	3.84 ± 0.43*	3.02 ± 0.64*#	72.521	< 0.001
Globulin (mg/L)	2.65 ± 1.14	3.38 ± 1.19*	3.73 ± 1.56*	11.707	< 0.001
albumin/globulin ratio	1.76 ± 0.55	1.32 ± 0.70*	0.94 ± 0.41*#	25.858	< 0.001

Measurement data were expressed as mean ± SD; * suggested $p < 0.05$ vs. Control group; # indicated $p < 0.05$ vs. mild renal impairment group

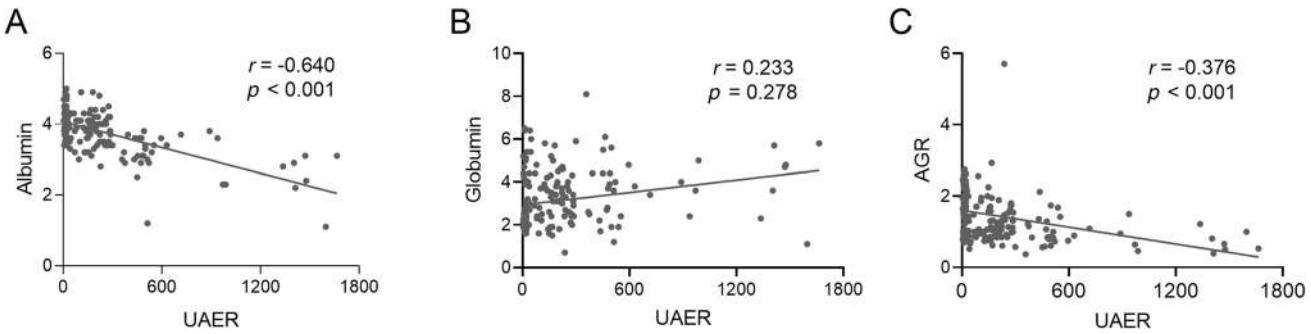

Fig. 1 Correlation analysis of albumin, globulin, and albumin/globulin ratio levels with urinary albumin excretion rate in patients with type 2 diabetes mellitus (T2DM). –**C**: scatter plots of albumin (ALB), globulin (GLB), albumin/globulin ratio (AGR) and urinary albumin excretion rate (UAER)

Diagnostic value of albumin, globulin, albumin/globulin ratio and urinary albumin excretion rate levels in moderate renal injury in patients with T2DN

ROC curves showed that the AUCs for ALB and AGR levels in predicting moderate renal injury in patients with T2DN were 0.880 (95% CI: 0.817–0.943) and 0.714 (95% CI: 0.608–0.820), respectively. The above findings indicated that ALB and AGR levels were of high value in assessing moderate renal injury in patients with T2DN ($p < 0.05$). As for GLB level, it had no significant diagnostic value for moderate renal injury in patients with T2DN ($p > 0.05$) (Fig. 2 and Table 3).

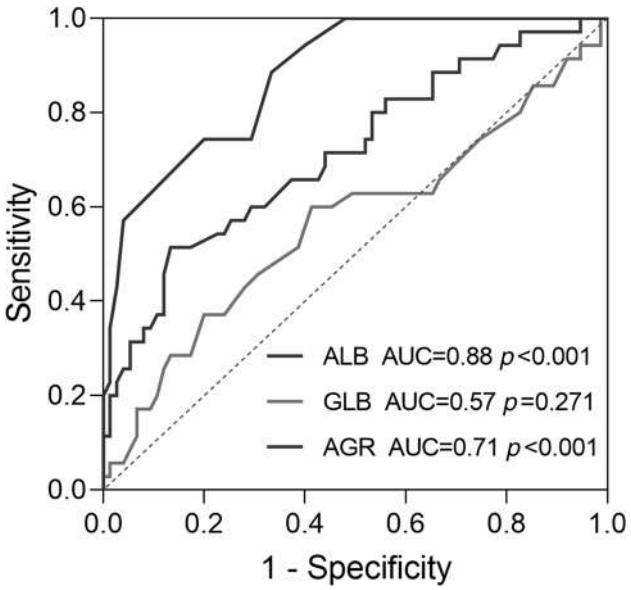

Fig. 2 ROC curves for the diagnostic value of albumin (ALB), globulin (GLB), and albumin/globulin ratio (AGR) in moderate renal injury in patients with type 2 diabetes mellitus (T2DM)

Discussion

As a disease with an insidious onset, DN leads to an aggressive renal deterioration that worsens with time, and even progresses to renal insufficiency and uremia [12]. Some studies have pointed out that DN can be induced by multiple factors. Among these factors, glucose metabolism disorders, changes in renal hemodynamics, a variety of cytokines and genetic background play crucial roles [13]. Hence, more novel predictors have also been applied for the assessment of renal impairment in patients with T2DN. This study was designed to explore the correlation of ALB, GLB and AGR with renal injury in T2DM patients.

In our study, there were significant differences in the duration of T2DM, incidence of hypertension, FPG and HbA1c levels among the three groups. Moreover, long T2DM duration, a history of hypertension and poor glycemic control may affect the occurrence and development of T2DN. It's reported

Table 3 Diagnostic value evaluation for albumin, globulin, and albumin/globulin ratio in moderate renal injury in patients with T2DN

	AUC	*95%CI*	*p*	Sensitivity (%)	Specificity (%)	Youden Index	Cut-off value
Albumin	0.880	0.817–0.943	< 0.001	88.6	66.7	0.552	3.650
Globulin	0.565	0.443–0.688	0.271	60.0	58.7	0.187	3.550
Albumin/globulin ratio	0.714	0.608–0.820	< 0.001	51.4	86.7	0.381	0.845

that long-term hyperglycemic infiltration is a major determinant in the development of diabetic microangiopathy, and diabetic patients with long disease duration and poor glycemic control are prone to microangiopathy like nephropathy. Briefly, the duration of disease and glycemic control have a great impact on renal injury in T2DM patients. Hypertension is also closely related to renal impairment. Diabetic patients with hypertension suffer from more severe insulin resistance and hyperinsulinemia and are prone to renal injury [14, 16]. The above findings are consistent with the results of our study.

Pearson correlation analysis in this study revealed a significantly negative correlation between the ALB and AGR levels and the UAER level in patients with T2DN. Patients with UAER > 30% suggest the presence of mild renal impairment [11], i.e., early renal disease. If patients suffer from massive albuminuria, the early renal disease will develop into clinical kidney disease. The clinical kidney disease will lead to thicker glomerular basement membranes than the early kidney disease, and even results in compensatory glomerular hypertrophy. The resulting symptoms will seriously impair the renal functions of patients. In this case, early diagnosis of kidney disease and timely treatment are crucial to improving the prognosis of the patients [17]. Low ALB, AGR level and high UAER level in patients with T2DN in our study suggested severe renal injury. Accordingly, the relationship between ALB, AGR and the degree of renal impairment in T2DN patients can be established by UAER.

After comparative analysis, ALB and AGR levels were much lower in the moderate renal impairment group than those in the mild renal impairment group and the Control group; the mild renal impairment group exhibited lower ALB and AGR levels than the Control group. In addition, GLB level was higher in the Control group than that in both the moderate renal impairment group and the mild renal impairment group; there was no significant difference in GLB level between the moderate renal impairment group and the mild renal impairment group. The above results demonstrated a certain sensitivity of ALB and AGR levels to the judgement of renal injury. Studies have reported that ALB is negatively correlated with C-reactive protein (CRP) [18], so the up-regulation of plasma ALB level has a positive effect on the improvement of chronic inflammatory diseases. Li Jie et al. [19] claimed that, on the basis of conventional treatment, increasing the intake of compound α-keto acids could effectively regulate the proteinuria in patients with chronic kidney disease (CKD), significantly increase serum ALB level, and finally delay CKD progression. ALB has antioxidant effects on carcinogens and can stabilize cell growth and DNA replication [20]. Hypoproteinemia may be a sign of malnutrition, which raises the risk of sepsis and can impair the immune system. Anyhow, hypoproteinemia is effective in predicting morbidity and mortality of patients with renal diseases [21]. GLB is a low molecular weight protein synthesized by immune organs, and its rate of synthesis and release is constant in healthy humans. Specifically, GLB is present in cells other than placental trophoblast cells and mature erythrocytes. The relatively small molecular mass of GLB allows it to freely traverse capillary walls, be excreted and decomposed by the kidneys, and be measured with ease [22, 23]. When glomerular filtration membrane is damaged, GLB level in urine is significantly increased; GLB can serve as a sensitive indicator to detect early renal damage in diabetic patients [24, 25]. However, several studies have stated that despite high sensitivity, GLB has poor specificity and is easily affected by many factors such as tumor, inflammation, and immunity [26]. Hence, GLB level alone do not accurately reflect the degree of renal impairment.

AGR is a comprehensive indicator reflecting all non-ALB proteins and ALB. In recent years, research has pointed out some benefits for AGR in predicting the prognosis of colon cancer, liver cancer, and lung cancer [6, 27, 28]. For instance, Tsai C C et al. reported [29] that AGR was superior to ALB in predicting mortality in peritoneal dialysis because AGR could comprehensively reflect the inflammation and nutritional status of the body. Compared with serum ALB, AGR combined with ALB and GLB is a relatively more stable and reliable indicator because it is less affected by inflammation, hepatic synthesis, catabolism, and changes in hydration status. Similar to the findings of previous studies, the results of ROC curve analysis in this study showed that ALB and AGR had high diagnostic value for moderate renal injury in patients with T2DM. All in all, ALB and AGR are valuable and practical biomarkers for assessing renal function impairment in patients with T2DN clinically.

There are still some limitations in this study. For example, the retroactive nature of our article and the small sample size could lead to outcome biases. In other words, the conclusions above require further verification through large-scale prospective studies.

Conclusion

Taken together, the expression level of ALB and AGR are decreased in T2DN patients. Moreover, ALB and AGR may be valuable in clinical application as a sensitive indicator for the diagnosis of T2DN.

Author contribution Dao-qin Liu and Xia Fu designed the study. Cheng-cheng Yang and Ru Zhou collected and analysed the data. Hong-jing Zhao, Ling-dan Zhuang and Qi-wen Wu wrote the manuscript. All authors have approved the submitted manuscript.

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

Declarations

Ethics approval and consent to participate Our study was approved by Ethics Committee of The First Affiliated Hospital of Wannan Medical College (2022–67).

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

The importance of active B12 (holotranscobalamin) measurement in the diagnosis of vitamin B12 deficiency in type 2 DM patients using metformin

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Abstract

Background B12 deficiency, which can be seen in type 2 DM patients using metformin, can cause peripheral nerve damage that can easily be confused with peripheral neuropathy, a complication of diabetes. Therefore, early diagnosis is important in patients.

Objective In our study, we aimed to investigate benefit of Active B12 measurement in diagnosing B12 deficiency, as it might be a better alternative in follow-up of B12 levels in type 2 DM patients using metformin.

Methods Patients were divided into three groups according to their Total B12 levels: Deficient, Borderline and Sufficient. Homocysteine and Active B12 (holotranscobalamin) levels were studied with Chemiluminescent Microparticle Immunoassay (CMIA) for quantitative determination. Duration of use, daily metformin dose, and other parameters were collected from records and interviews.

Results A statistically significant positive correlation was found between Total B12 and Active B12 ($r = 0.624$, $p = 0.000$). A statistically significant negative correlation was found between metformin dose and Active B12 and Total B12 levels ($r = -0.309$, $r = -0.212$, respectively; $p < 0.05$). While effects of metformin dose and duration of use groups were not found statistically significant in binary logistic regression analysis for Total B12 groups, the OR value for Active B12 was found to be 5.575 (95% CI 1.456–21.343, $p = 0.012$).

Conclusions It is important to closely follow up Type2 DM patients with high daily metformin dose in terms of B12 deficiency. Active B12 is a significant predictor of B12 deficiency compared to other parameters.

Keywords Type 2 DM · B12 deficiency · Metformin · Active B12 · Holotranscobalamin

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Introduction

Vitamin B12 (Vit B12) is an essential vitamin that is crucial for cell function, proliferation, and metabolism. Impaired absorption from gut, intrinsic factor (IF) deficiency or inactive IF synthesis, autoimmune diseases, metabolic diseases, inadequate nutritional intake, especially in vegetarians, certain drugs such as metformin cause vit B12 deficiency [1–3].

Total B12 (Cobalamin) measurement is the most commonly used routine diagnostic test for evaluating patients with suspected B12 deficiency. This test measures both "inactive" forms of cobalamin (holohaptocorrin) and "active" forms (holotranscobalamin) in serum. Total B12 test is a low-cost, widely used automated immune chemiluminescence method based on intrinsic factor binding of cobalamin [1, 3, 4]. In studies, it has been shown that Total B12 measurement is insufficient to diagnose B12 deficiency and that methylmalonic acid (MMA) and homocysteine increase are found together in B12 deficiency [5, 6]. The most critical disadvantage of homocysteine and MMA recommended in diagnosis of B12 deficiency is that they only increase after intracellular deficiency develops and give false positive values in renal dysfunction [7]. An immunoassay that can be used on automated clinical chemistry analyzers is available for measurement of Active B12 (Holotranscobalamin), 'active' fraction of plasma cobalamin. This assay has a smaller "gray zone" (uncertainty range) and better sensitivity and specificity than Total B12 assays [8].

Prevalence of type 2 diabetes mellitus (type 2 DM) is increasing worldwide, and metformin as oral antidiabetic drug is recommended as first-line therapy in most patients [9]. Studies are reporting that long-term metformin use is associated with B12 deficiency secondary to intestinal malabsorption [9–14]. Severe B12 deficiency can cause peripheral nerve damage that can easily be confused with peripheral neuropathy, a long-term complication of diabetes. This misdiagnosis can result in a reversible condition leading to permanent nerve loss. For this reason, it is recommended to screen metformin users for B12 deficiency [9, 12–15]. In our study, we aimed to determine Active B12 levels in patients with type 2 DM using metformin and to investigate its usefulness in diagnosing B12 deficiency.

Materials and methods

Population of study

Blood samples collected for routine clinical follow-up of Type 2 DM patients was used, which reaches Medical Biochemistry Laboratory and will be disposed of as medical waste after requested test results are determined. No extra blood was drawn from patients. The study was approved by Bezmialem Vakif University Noninterventional Research Ethics Committee. At least 153 subjects should be included in the study at a 95% confidence level, 90% power, 10% margin of error, and a 0.75 degree correlation coefficient between Active and Total B12 levels [16]. All study participants were selected based on predetermined inclusion criteria, and those who met one or more exclusion criteria were not included in the study. Patients were divided into three groups according to their serum Total B12 levels: Deficient (Total B12 < 250 pg/mL), Borderline (Total B12 250–350 pg/mL), and Sufficient (Total B12 > 350 pg/mL).

Inclusion criteria

Patients who had been diagnosed with Type 2 DM and had been using metformin for more than 6 months were selected.

Exclusion criteria

Patients diagnosed with Type 2 DM who have been using metformin for less than six months, patients with known B12 deficiency or who have taken vit B12 supplement during last six weeks, history of gastrectomy, alcoholism, ongoing pregnancy, kidney disease, malabsorption disorders; patients using proton pump inhibitors, vegetarians, and those who had a blood transfusion in the last three months were excluded.

Sample preparation

Total B12, folic acid, urea, creatinine, glucose, HbA1c, and hemogram tests are requested by clinicians within scope of indication from Type 2 DM patients using metformin. After 10–12 h of fasting, adult patient blood samples were taken into a yellow-capped vacuum tube and a purple-capped EDTA containing tube. After centrifugation at 2500 G for 10 min, the parameters requested by the clinicians were studied in the Medical Biochemistry Laboratory. Later on, samples classified according to serum Total B12 levels were aliquoted and stored at -80 °C for 1 year [17]. When target number of patients was reached, samples were thawed at room temperature, and Homocysteine and Active B12 measurements were performed.

Methods

Complete blood count was measured by laser reading method (Abbott CELL-DYN Ruby Hematology Analyzer). Creatinine and Urea Nitrogen levels were measured using

photometric kinetic alkaline picrate and urease method, respectively (Abbott creatinine/urea nitrogen analyte kit, Abbott ARCHITECT c8000). Glucose level was measured using photometric Hexokinase/G-6-PDH method (Abbott glucose analyte kit, Abbott ARCHITECT c8000). HbA1c (%) level was measured quantitatively immunoturbidimetrically from whole blood (Archem Diagnostics HbA1c kit, Abbott ARCHITECT c8000). For quantitative determination of Folate, Homocysteine, Total B12, and Active B12 (Holotranscobalamin) levels, Chemiluminescent Microparticle Immunoassay (CMIA) was studied (Abbott Architect i2000SR Immunoassay).

Statistical analysis

Analysis of obtained data was performed on IBM SPSS Statistics v.26.0 package program, and statistical significance level was accepted as 0.05. Conformity of quantitative variables to normal distribution was examined using Shapiro–Wilk test. Descriptive statistics of qualitative variables in study were presented as numbers and percentages, and descriptive statistics of quantitative variables were presented as median, interquartile range, minimum and maximum. Kruskal Wallis test was used for median comparison of groups with more than two categories, and Dunn's test was used as posthoc test. Relationships between quantitative variables were analyzed using Spearman correlation coefficient. To determine the best cut-off point for Active B12 value, receiver operator characteristic curve (ROC) analysis was performed, and Area under the curve (AUC) value was calculated. Cut-off point was determined by Youden index. Agreement between Active B12 and Total B12 was examined by Kappa coefficient. Linear regression analysis was performed for relationships of Active B12 and Total B12 values with other parameters. For Active B12 and Total B12 groups, binary logistic regression analysis was used to investigate relationship with other parameters. Enter method was used as variable selection method, and variables that were as significant as 0.25 in univariate analyzes were included in models.

Results

A total of 189 patients were included in our study, 121 of whom were men and 68 were women. Patients were divided into three groups according to their Total B12 level: Deficient, Borderline, and Sufficient. Table 1 shows statistical analysis results of demographic, clinical characteristics, and evaluated biochemical parameters of groups in the study.

Table 1 Demographic and Clinical Characteristics of the Deficient, Borderline and Sufficient Groups

Parameter		Deficient (n:59)	Borderline (n:61)	Sufficient (n:69)	*p* value
Gender	Female	37 (62.7%)	43 (70.5%)	41 (59.4%)	0.409
	Male	22 (37.3%)	18 (29.5%)	28 (40.6%)	
Age		58 (39–77)	57 (29–78)	56 (23–75)	0.918
Total B12 (ng/L)		212 (37)	290 (63)	464 (141)	$<0.001^{**}$
Folic Acid (µg/L)		7.5 (3.7)	6.5 (4)	8.3 (5.1)	0.056
Active B12 (pmol/L)		46.3 (31.1)	60.7 (30.4)	92.4 (75)	$<0.001^{**}$
Homocysteine(µmol/L)		11.03 (4.97)	10.58 (5.2)	10.0 (3.55)	0.028^{*}
MCV (fL)		87.63 (5.39)	87.99 (5.70)	87.58 (5.71)	0.390
Hb (g/dL)		13.12 (2.76)	13.625(1.83)	13.92(2.43)	0.045^{*}
Glucose (mg/dL)		121 (41)	122 (40)	120 (61)	0.976
HbA1c (%)		6.53 (1.29)	6.38 (1.20)	6.33(2.08)	0.940
Creatinine (mg/dL)		0.76 (0.19)	0.77 (0.16)	0.78 (0.12)	0.472
Urea (mg/dL)		27 (14)	28.5 (9)	29 (12)	0.850
eGFR		94 (17)	91 (18)	91 (18)	0.996
TSH (mIU/L)		1.66 (1.49)	1.62 (1.69)	1.76 (1.25)	0.925
Metformin Duration (year)		5 (1–30)	5 (0.6–25)	5 (0.5–25)	0.494
Metformin Dose (mg/day)		2000 (1000–4000)	2000 (500–2000)	2000 (850–2000)	0.148

In table gender *n* (%), age, duration of metformin use and metformin dose are shown as median (min–max), and values of other parameters are shown as median (IR)

MCV Mean Corpuscular Volume, *Hb* Hemoglobin, *eGFR* Glomerular Filtration Rate, *TSH* Thyroid Stimulating Hormone, *IR* Interquartile Range

$^{*}p<0.05$ $^{**}p<0.001$

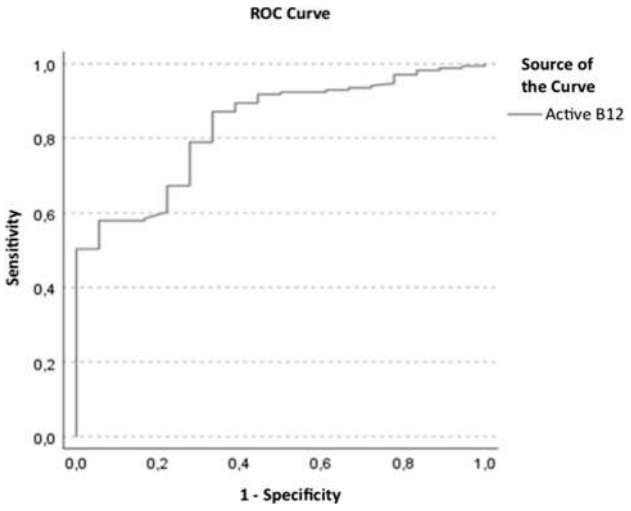

Fig. 1 ROC analysis for Active B12 in estimation of Total B12 deficiency

We found statistically significant differences between groups regarding medians of Total B12, Active B12, and Homocysteine ($p < 0.05$, Table 1). Figure 1 shows ROC analysis for Active B12 in estimating Total B12 deficiency. AUC value was 0.833 (95% CI 0.772–0.883) for Active B12. Cut-off value was determined as 35 pmol/L for Active B12, which predicted Total B12 deficiency with 89.47% sensitivity and 61.11% specificity (Youden index J = 0.5380, $p < 0.0001$). Rate of Active B12 level below 35 in those with a Total B12 level below 200 and Active B12 level above 35 in those with a Total B12 level above 200 was found to be significantly higher ($p < 0.001$). Agreement between Total B12 and Active B12 (using Kappa value) was found to be 38.5% and statistically significant ($p < 0.001$).

Deficient Group included 37 (62.7%) women and 22 (37.3%) men. Their age's median (min–max) value was 58 (39–77). Total B12 median (IR) value was 212 (37) pg/mL, while Active B12 median (IR) value was 46.3 (31.1) pmol/L. Homocysteine median (IR) value was 11.03 (4.97) µmol/L (Table 1). According to cut-off value of Active B12 level, 21 (36.6%) patients were deficient, and 38 (64.4%) patients were evaluated as sufficient. Homocysteine levels were elevated in 47.6% of patients with Active B12 deficiency, and homocysteine levels were found to be normal in 52.4%. Homocysteine levels were elevated in 21.1% of patients with sufficient Active B12, and homocysteine levels were found normal in 78.9%.

Borderline Group included 43 (70.5%) women and 18 (29.5%) men. Their age's median (min–max) value was 57 (29–78). Total B12 median (IR) value was 290 (63) pg/mL, while Active B12 median (IR) value was 60.7 (30.4) pmol/L. Homocysteine median (IR) value was 10.58 (5.2) µmol/L (Table 1). According to cut-off value of Active B12 level, 5 (8.2%) patients were deficient, and 56 (91.8%) patients were evaluated as sufficient. Homocysteine levels were elevated in 60.0% of patients with Active B12 deficiency, and homocysteine levels were found to be normal in 40.0%. Homocysteine levels were elevated in 30.4% of patients with sufficient Active B12, and homocysteine levels were found normal in 69.6%.

Sufficient Group included 41 (59.4%) women and 28 (40.6%) men. Their age's median (min–max) value was 56 (23–75). Total B12 median (IR) value was 464 (141) pg/mL, while Active B12 median (IR) value was 92.4 (75) pmol/L. Homocysteine median (IR) value was 10.0 (3.55) µmol/L (Table 1). According to cut-off value of Active B12 level, 2 (2.9%) patients were deficient, and 67 (97.1%) patients were evaluated as sufficient. Homocysteine levels were found to be normal in 100.0% of patients with Active B12 deficiency. Homocysteine levels were elevated in 23.9% of patients with Active B12 sufficient and homocysteine levels were found normal in 76.1%.

Spearman correlation analysis was performed to determine level of relationship between Total B12, Active B12, and Homocysteine. A statistically significant positive correlation was found between Total B12 and Active B12 (r = 0.624, $p = 0.000$, Fig. 2). Additionally, statistically significant negative relationships were found between Total B12 and Homocysteine (r = -0.226, $p < 0.05$) and between Active B12 and Homocysteine (r = -0.256, $p < 0.05$).

Median (min–max) values of metformin duration (years) and metformin dose (mg/day) were 5 (1–30) and 2000 (1000–4000) in deficient group, 5 (0.6–25) and 2000 (500–2000) in borderline group, 5 (0.5–25) and 2000 (850–2000) in sufficient group (Table 1). There was no statistically significant difference between groups regarding duration and dose of metformin use ($p = 0.494$, $p = 0.148$ respectively, Table 1).

Patients were divided into two groups in terms of duration of metformin use below and above five years and two groups in terms of metformin dose below and above 1000 mg/day. No statistically significant difference was observed between medians of Total B12 and Active B12 in those with metformin duration below and above five years ($p > 0.05$, Table 2). No statistically significant correlation was observed between duration of metformin use and Active B12 and Total B12. In terms of metformin dose, a statistically significant difference was observed between medians of Total B12 and Active B12 in those below and above 1000 mg/day ($p < 0.05$, Table 2). A statistically significant negative correlation was found between metformin dose and Active B12 and Total B12 (r = -0.309, r = -0.212, respectively; $p < 0.05$). After adjusting for more than one confounding factor using linear regression analysis, effects of metformin dose and duration of metformin use on quantitative variable of Total B12 were not found statistically

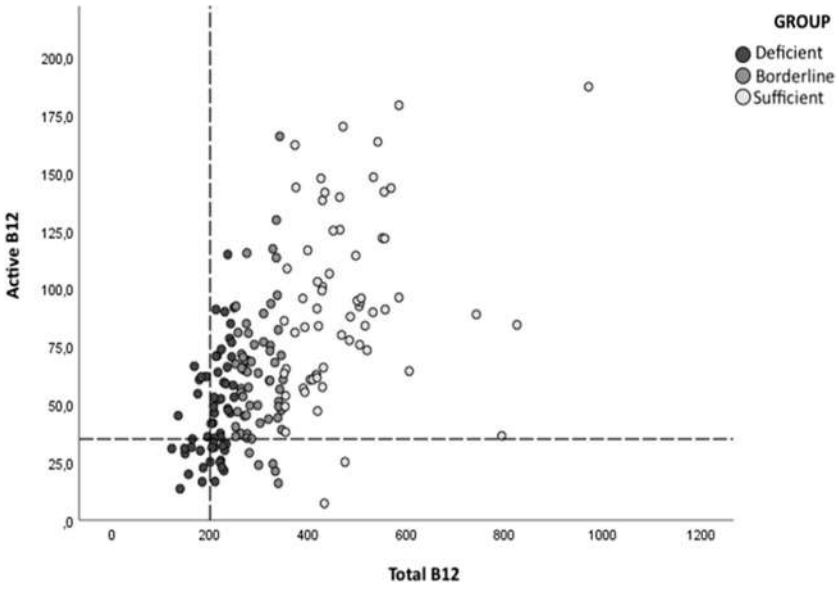

Fig. 2 Scatter plot showing the distribution of results in three groups according to cut-off values and the correlation between Active B12 and Total B12

Table 2 Comparison of medians of Total B12 and Active B12 in metformin duration and dose groups

	N	Total B12	Active B12	*p* value
Metformin Duration				
< 5 years	85	309(135–1820)	62.8 (13.5–892)	> 0.05
≥ 5 years	104	300.5(122–1476)	65.1(7.2–564.4)	
Metformin Dose				
≤ 1000 mg/day	22	360(175–1476)	84.5(24.2–564.4)	< 0.05*
> 1000 mg/day	84	300(122–1579)	60.5(7.2–492.2)	

Total B12 and Active B12 values are shown median (min–max) in table

*$p < 0.05$

Table 3 Binary logistic regression analysis result for Total B12 groups

		p value	OR	%95 CI
Step 1[a]	Metformin Duration Group	0.564	1.566	0.341–7.203
	Metformin Dose Group	0.577	0.532	0.058–4.878
	Homocysteine	0.789	0.973	0.796–1.190
	HbA1c	0.753	0.937	0.626–1.404
	Active B12	0.012*	5.575	1.456–21.343
	Constant	0.205	14.611	

*$p < 0.05$

significant; Active B12 effects were found to be statistically significant. A decrease of 1 pmol/L in Active B12 was associated with a decrease in Total B12 levels of 2.451 pg/mL (Beta:2.451**,** %95CI 2.155–2.746, $p < 0.001$). While effects of metformin dose and duration were not found statistically significant in binary logistic regression analysis for Total B12 groups, OR value for Active B12 was found to be 5.575 ($p = 0.012$, Table 3).

Discussion

Peripheral nerve damage due to B12 deficiency can easily be confused with peripheral neuropathy, one of the long-term complications of diabetes. Therefore, screening and early diagnosis of diabetes patients using metformin for vit B12 deficiency are important [9, 14, 15, 18]. In our study, we determined clinical conditions that would cause B12 deficiency other than metformin and affect our measurement methods as exclusion criteria. We investigated importance of Active B12 in early detection of deficiency by measuring Active B12 and homocysteine levels in samples that we divided into groups according to Total B12 levels diabetic patients using metformin.

Many studies have shown that using metformin in type 2 DM patients may cause B12 deficiency. They have focused on effect of metformin duration and dose on B12 deficiency. A large-scale study conducted in Korea found that use of metformin in type 2 DM patients caused B12 deficiency, and daily metformin dose and duration of treatment were important risk factors [18]. Our study evaluated relationship between duration and dose of metformin use and B12 deficiency in patients. There was no statistically significant difference between deficient, borderline, and sufficient groups when evaluated regarding median duration of metformin use and dosage. Then, evaluations were made by creating new groups based on these parameters.

In our study, it was found that duration of metformin use was not significantly associated with B12 deficiency, and there was no statistically significant difference between groups (< 5 years and ≥ 5 years) in terms of medians of Total B12 and Active B12. Some studies in the literature show no relationship between duration of metformin use and B12 deficiency. In study by Kim J et al., no association was found between B12 deficiency and duration of metformin use after adjusting for confounding factors [19]. Similarly, in study conducted by Beulens JW et al. including 550 type 2 DM patients, no correlation was found between duration of metformin use and concentrations of cobalamin and holotranscobalamin [20]. In other studies, it has been shown that there is a significant positive relationship between duration of metformin use and B12 deficiency. Krishnan et al. found in their study that long-term use of metformin (more than five years) was associated with a more than two-fold increased risk of B12 deficiency [21].

Our study found a negative correlation between metformin dose (mg/day) and Total B12 and Active B12. Total and Active B12 medians of group with metformin doses 1000 mg/day above were lower than those with 1000 mg/day and below. Type 2 DM patients using high-dose metformin should be followed closely for B12 deficiency since increase in the daily dose is associated with a decrease in Active and Total B12 concentrations. Our results are consistent with many studies examining relationship between metformin dose and B12 deficiency. In a study conducted in Qatar in 3124 type 2 DM patients, a high prevalence of B12 deficiency and a negative correlation between daily metformin dose and serum B12 were found [22]. In study conducted by Kim J et al., they found that B12 levels were negatively correlated with metformin dose and that B12 levels decreased significantly as metformin dose increased [19]. In another study involving 550 patients with type 2 DM, researchers found that higher and cumulative doses of the medication metformin were strongly associated with lower concentrations of HoloTC and cobalamin [20].

To prevent permanent neurological damage from B12 deficiency, it is important to determine true tissue deficiency and to diagnose patients at subclinical stage. Since there are many different methods for diagnosis of B12 deficiency, it is discussed which one would be ideal [23]. In routine practice, Total B12 (Cobalamin) is first measured from patient's serum. Major limitation of Total B12 is that 80% is haptocorrin bound, and this form is not suitable for cell uptake. It is also a method with low sensitivity and specificity in recognizing correct tissue deficiency [24]. In the early stages of deficiency, Total B12 level may be normal in patients. Therefore, it has been recommended to measure Total B12 together with metabolites such as homocysteine and MMA [25]. In B12 deficiency, these two metabolites, which are biochemical indicators of metabolic efficiency, increase. Homocysteine is not specific to cobalamin deficiency. It may also be elevated in some genetic polymorphisms in patients with folate deficiency, renal failure, and hypothyroidism. Since these clinical conditions will cause false homocysteine elevation, folate, creatinine, eGFR and TSH parameters were evaluated in patients and those with normal results were included in the study. In most laboratories and studies, the cut-off value of homocysteine in the diagnosis of B12 deficiency has been determined as 15 µmol/L. In line with these studies, we evaluated our patients by accepting the cut-off value as 15 µmol/L in our study [26]. A statistically significant negative correlation was found between serum homocysteine levels and both Total B12 and Active B12 levels. In addition, significant differences in medians were observed between B12 deficient, borderline, and sufficient groups, and median was higher in deficient group. In our study, homocysteine levels were elevated in 47.6% of the patients with both Total B12 and Active B12 deficiency. Observing high homocysteine levels in deficient patients makes us think of true tissue deficiency [1].

In our study, there was no difference between the groups in terms of MCV medians. Although MCV elevation is stimulating in B12 deficiency, it is not specific. It may increase due to myeloblastic syndrome, excessive alcohol consumption, drugs, etc. In early stage of deficiency, MCV value may be normal, but neurological damage process has begun in patients. Although there was no significant difference in Hb medians between the groups, it was almost normal in the patients. Since megaloblastic anemia is seen in the advanced stage of B12 deficiency, these results are expected to be like this [1, 27].

Active B12 (HoloTC) is active fraction of cobalamin, and this parameter can be measured today by immunoassays that can be run on automated analyzers. HoloTC has a shorter half-life than other B12 metabolites. Therefore, it is thought that the earliest change in B12 deficiency may be a low holoTC concentration [28, 29]. There are different values for Active B12 cut-off values in the studies. In a multicenter study, cut-off value of Active B12 for B12 deficiency screening was determined as 32 pmol/L [8]. In another study, it was found that a cut-off value of 40 pmol/L would be appropriate to evaluate B12 deficiency [30]. Therefore, it may be appropriate for each laboratory to determine a cut-off value. In our study, ROC analysis was performed to determine cut-off value of Active B12, and it was 35 pmol/L. When grouped and evaluated according to Total B12 and Active B12 cut-off values, it was found that Active B12 considered 61.1% of patients in Total B12 deficient group as deficient and 88.9% of the patients in sufficient group as sufficient. This result is in line with studies showing that Total B12 and Active B12 are similar in distinguishing between B12 deficient and sufficient individuals [30, 31]. Regression analysis was conducted to evaluate the effect of significant parameters on B12 deficiency in the study. While duration of metformin use, dosage, homocysteine, folic acid, and HbA1c were not

significant, Active B12 was found to be a significant predictor of deficiency (OR 5.575, 95% CI 1.456–21.343, Table 3). These findings reveal importance of Active B12 measurement in explaining B12 deficiency in patients.

The limitations of our study were absence of MMA comparison due to its high cost and inclusion of other anti-diabetic medications' effects**.**

Conclusion

We found a significant decrease in Total B12 and Active B12 concentrations at doses above 1000 mg/day, with a negative correlation between metformin dose and Total B12 and Active B12 levels. We also found that Active B12 was a significant predictor of B12 deficiency compared to other important parameters. Close follow-up of patients with Type 2 DM who have a high daily dose of metformin in terms of B12 deficiency is important. Total B12 and Active B12 can be examined together to detect deficiency at an early stage. The functional biomarkers homocysteine and MMA can be added to tests to detect true tissue deficiency.

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Data availability Based on a request, the data supporting the current work's findings is available from the corresponding author.

Declarations

Ethics approval This study was carried out with the permission no 07/128 (date: 22/ 05/2020) obtained from Bezmialem Vakif University Noninterventional Research Ethics Committee.

Author agreement All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

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

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ORIGINAL ARTICLE

Changes in HbA1c values of patients with type 2 diabetes mellitus during the pandemic period and their relationship to food literacy

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Abstract

Background Diabetes Mellitus is recognized as one of the most prevalent chronic diseases worldwide. The disruptions encountered in healthcare services during the pandemic period have significantly impacted the treatment and monitoring of these patients. While the concept of food literacy is relatively new, its potential impact on nutrition-related chronic diseases remains to be fully demonstrated.

Objective This study aimed to determine glycemic control of DM patients without physician supervision was affected by treatment adherence and food literacy during COVID-19.

Methods This was a cross-sectional study. A face-to-face survey was conducted among the patients who were admitted to the Internal Medicine outpatient clinic between April 2021 and October 2021 who had been ill with type 2 DM for more than one year, and whose medical follow-up was delayed by at least one year due to the pandemic.

Results A total of 154 patients 90 (58.4%) of whom were women, participated in our study. HbA1c in patients in 2020 ranged from 5.2 to 14.0 with a mean of 8.36 ± 2.00, whereas HbA1c in 2021 ranged from 5.6 to 14.3 with a mean of 8.38 ± 1.86. Minimal increase in HbA1c was observed in patients last year without a physician's check-ups, the difference was not significant. While the rate of change of HbA1c group in the good direction was higher in subjects with good food literacy ($p = 0.011$). The food literacy scores of patients whose HbA1c changed in the good direction (35.4 ± 4.3) were higher. Food literacy scores of patients with decreased HbA1c were significantly higher (33.0 ± 6.6).

Conclusion While the rate of change in the good direction in the HbA1c group was higher among participants with good food literacy, the rate of change in the poor direction in the HbA1c group was higher among participants with poor food literacy.

Keywords Diabetes mellitus · HbA1c · Food literacy · COVID-19

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia due to absolute insulin deficiency, insulin resistance, insulin secretion defect, and incretin hormone deficiency [1]. Diabetes is an important public health problem whose incidence has more than doubled in the last three decades [2]. According to the 2021 data, the number of people with diabetes, which is 536.6 million in the age group of 20–79 years, is expected to reach 783 million in 2045 [3]. Chronic hyperglycemia caused by diabetes is associated with damage to various organs, especially the eye, kidney, nervous system, and cardiovascular

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system, so that 5 million deaths worldwide can be associated with diabetes in 2017, while health care expenditures due to diabetes amount to $850 billion [1, 4].

Most diabetes cases are divided into two main groups in terms of etiology and pathology. These are type 1, which is caused by absolute insulin deficiency, and type 2 DM, which is characterized by insulin resistance, insulin secretion defect, and incretin hormone deficiency and accounts for 90% of diabetes cases [1]. Studies have linked the increase in the incidence of type 2 DM over the years to the increase in obesity, sedentary lifestyle, high-calorie and unhealthy diet, and aging population [5]. Studies show that type 2 DM can be prevented by maintaining a healthy body weight, eating a healthy diet, regular daily exercise, and abstaining from alcohol [6]. Experts agree that lifestyle modification, including dietary therapy, is the first-line treatment for type 2 DM [7]. It is said that nutrition is a very important tool to prevent complications in tertiary protection after the development of diabetes, and it is also important in the development of diabetes [8]. Food literacy is a broad definition that includes all concepts such as cultivation, storage, preparation, consumption, and information about the content of individuals or societies from production to consumption of a food, as well as how much of what type of food should be consumed for a healthy diet [9]. Therefore, the level of food literacy is an effective tool to measure whether diabetics have sufficient knowledge about nutrition [10].

Coronavirus 2 (SARS-CoV-2), which leads to acute respiratory syndrome from the coronavirus family, first appeared in Wuhan, China, in late 2019; affected millions of people; cost hundreds of thousands of lives; and its effects are still ongoing. The 2019 coronavirus disease (COVID-19), declared a public health emergency, has evolved into a pandemic [11]. As part of the global response to the COVID-19 pandemic, countries have implemented various measures such as quarantine, travel restrictions, working from home, and school vacations [12]. The measures led to social isolation, deterioration of diet, and lack of physical activity, and many DM patients had to cancel their routine clinical check-ups [13]. In addition to these measures to minimize mobility, prevent hospital contamination, and reduce the burden on healthcare facilities and staff, Turkey has opened the possibility for patients with chronic diseases to obtain their previously taken medications directly from the pharmacy without the need for a physician control to review them, with a decision by the Ministry of Health starting March 21, 2020 [14]. Although this situation is a solution to patients' problems in accessing drugs, it has resulted in DM patients taking drugs at the same dosage for a long time without being reviewed by a physician.

This study aimed to determine how glycemic control of DM patients without physician supervision was affected by treatment adherence and food literacy during COVID-19.

Material and methods

This was a cross-sectional study. Sample size was calculated using G*Power version 3.1.9.2. Assuming that the influence of the food literacy level on treatment adherence in type 2 DM patients is moderate, a two-way hypothesis with $\alpha = 0.05$ and $\beta = 0.95$ was established. The sample size was calculated, the aim was to reach at least 147 subjects, and it was determined that there were missing and incorrect answers in the questionnaire questions of 161 patients. Seven patients were excluded from the study, and the study was completed with 154 patients. A face-to-face survey was conducted among the patients who were admitted to the Internal Medicine outpatient clinic of Hatay Dörtyol State Hospital between April 2021 and October 2021, who had been ill with type 2 DM for more than 1 year, and whose medical follow-up was delayed by at least 1 year due to the pandemic. Drugs taken, hemoglobin A1c (HbA1c) value at the last medical check-up 1 year ago, and fasting blood glucose (FBG) and HbA1c value during the interview were recorded by reviewing the system. Patients' height and weight were measured with a standard precision scale while they kept their light clothing on, and their height was measured in the upright head and eye position by removing their shoes. Prior to the study, ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Hatay Mustafa Kemal University with decision dated 22 Apr 2021 and number 02.

The survey consists of 4 parts. The first part of the questionnaire consists of an information form containing questions, based on a literature review, including sociodemographic information, additional problems faced by patients due to diabetes, and life changes related to diabetes. In the second part, the 8-item treatment adherence scale, first used by Dr. Morisky [15] and whose Turkish validity and reliability were investigated by Sayıner [16], was used. In the third part of the study, a 12-question short form on food literacy was used, which was first developed by Krause [17] and whose Turkish validity and reliability were investigated by Durmuş et al. [18] and whose cut-off value was determined by Gökler et al. [19]. In the 4th part of the study, questions about the patient's HbA1c values at the last medical check-up 1 year ago, current HbA1c values, drugs taken, and fasting blood glucose levels are to be answered by the physician. Participants can score between 7 and 52 on the Food Literacy scale, with increasing scores indicating an increase in food literacy. The cut-off value is 31 points, with a score of 31 and above indicating good food literacy and a score below 31 indicating poor food literacy. Participants can score between 0 and 8 on the treatment adherence scale, with increasing scores indicating high treatment adherence and less than 6 points indicating low adherence, 6 and 7 points indicating moderate adherence, and 8 points indicating high adherence.

Table 1 Demographic characteristics of the patients

Factors	*n*	%
Gender		
Female	90	58.4
Male	64	41.6
Marital status		
Married	142	92.2
Single	12	7.8
Educational Status		
Primary school	81	52.6
Middle School	30	19.6
High school	23	14.9
University	19	12.3
Master's/Ph.D	1	0.6
Child education status		
No children	15	9.8
Pre-school	1	0.7
Primary school	11	7.1
Middle School	13	8.4
High school	48	31.2
University	59	38.3
Master's/Ph.D	7	4.5
Spouse education status		
No spouse	12	7.8
Primary school	76	49.4
Middle School	23	14.9
High school	31	20.1
University	10	6.5
Master's/Ph.D	2	1.3
Profession		
Not working	76	49.4
Retired	40	26.0
Employee	21	13.6
Officer	8	5.2
Other (tradesman, farmer, freelance)	10	5.8
Economic status		
Bad	31	20.1
Moderate	96	62.3
Good	27	17.5
BMI group		
Weak	1	0.7
Normal	13	8.4
Overweight	57	37.0
Obese	83	53.9
Chronic disease other than DM		
Present	96	62.4
Absent	58	37.6
Regular exercise walking		
Not doing	35	22.7
Daily	20	13.0
He/she does it a few times a week	57	37.0
He/she does it rather rarely	42	27.3
Food literacy		
Good	81	52.6
Poor	73	47.4
Total	154	100

Table 2 Diabetes-related characteristics of the patients

	n	%
Organ effect of DM		
Yes	84	54.5
No	70	45.5
Organ affected by DM		
Eye	44	28.6
Heart	26	16.9
Kidney	12	7.8
Leg vein	16	10.4
Sexual	19	12.3
Other	3	1.9
Time of DM diagnosis		
During a screening for another disease	28	18.2
After a visit to a physician when they suspected diabetes	79	51.3
During routine health check-ups	47	30.5
Diabetes in first-degree relative		
Present	116	75.3
Absent	38	24.7
Diabetes education at a hospital		
Attended	39	25.3
Not attended	115	74.7
Dietitian's nutritional assistance After DM diagnosis		
He/she applied to a dietitian and is still using the assistance	43	27.9
He/she applied to a dietitian, used the assistance and quit	36	23.4
He/she applied to a dietitian and did not use the assistance	14	9.1
No application for a dietitian's nutritional assistance	61	39.6
Annual DM physician checkups		
1 or 2 physician check-ups per year	85	55.2
More than 2 physician check-ups per year	69	44.8
Blood glucose measurement at home		
Unable to measure blood glucose at home	28	18.1
Daily measurements	44	28.6
Measure every other day	16	10.4
Take at least one measurement per week	28	18.2
Measure less frequently	38	24.7
Are you adhering to your diabetes treatment?		
I adhere to it	100	64.9
Sometimes I do not adhere it	49	31.8
I do not adhere it	5	3.3
Do you attend to your nutrition after being diagnosed with DM		
I do	116	75.3
I do not pay attention	38	24.7
History of hypoglycemia		
Present	28	18.2
Absent	126	81.8
History of hyperglycemia coma		
Present	15	9.7
Absent	139	90.3
History of emergency department admission due to DM		
Present	38	24.7
Absent	116	75.3
History of hospitalization due to DM		
Present	32	20.8
Absent	122	79.2
Treatment adherence for DM		
High adherence	41	26.6

Table 2 (continued)

	n	%
Moderate adherence	55	35.7
Low adherence	58	37.7
Treatment for DM		
Oral antidiabetic agents only	104	67.5
Oral antidiabetic agents + insulin	39	25.3
Insulin	11	7.2
Fasting blood sugar		
80–130 mg/dL	46	29.9
> 130 mg/dL	108	70.1
Total	154	100

According to the American Diabetes Association, glycemic and HbA1c therapeutic targets for individuals with type 2 diabetes mellitus should be individualized based on comorbidity and the needs of the patient. However, as a general guideline, the recommended therapeutic targets for type 2 diabetes mellitus are an HbA1c level of less than 7%, fasting blood glucose levels between 80 and 130 mg/dL, and levels of less than 180 mg/dL at the 2nd hour [20]. Based on WHO criteria, patients' body mass index (BMI) values were classified as underweight when BMI was < 18.5, 18.5 ≤ BMI < 25 as normal, 25 ≤ BMI < 30 as overweight, and ≤ 30 as obese [21].

Statistical analyses were performed using the SPSS 20 program for Windows. Frequency analysis, chi-square test, the Kolmogorov–Smirnov test for normal distribution, ANOVA analysis of variance, the post hoc Bonferroni analysis, Student's *t* test, and Wilcoxon's test were used for statistical analysis. Variables with normal distribution were reported as mean ± standard deviation, and variables that were not normally distributed were reported as median-minimum–maximum. The study's independent variables were demographic information, comorbidity and external chronic diseases due to diabetes, and hospitalizations due to diabetes. The dependent variables were the results of the Treatment Adherence scale and the Food Literacy scale.

Results

A total of 154 patients aged 22 to 77 years with a mean age of 53.4 ± 10.1 years, 90 (58.4%) of whom were women, participated in our study. While 81 (52.6%) patients had a primary school degree, the proportion of patients with a university or higher education degree was 12.9% ($n = 20$). Of the patients, 62.3% ($n = 96$) described their economic status as moderate. Patients' BMI values ranged from 17.6 to 51.2, with a median value of 30.3, and 53.9% ($n = 83$) of them were obese. The proportion of patients with a chronic disease other than diabetes was 62.4% ($n = 96$), and the most common concomitant disease was hypertension, with a rate of 40.2% ($n = 62$), followed by 24 patients (15.5%) with cardiac problems and 20 patients (12.9%) with hyperlipidemia. Thirty-five (22.7%) patients did not exercise regularly. Patients rated on the Food Literacy scale with a mean score of 32.0 ± 6.3, and the percentage of patients with good food literacy was 52.6% ($n = 81$). The demographic characteristics of the patients are given in Table 1.

The length of time DM patients spent varied from 1 to 26 years, with a median of 5.0 years. The percentage of patients whose organs were affected due to diabetes was 54.5% ($n = 84$). In 22 patients (14.2%), more than one organ was affected by DM, and the most commonly affected organ was the eye with 28.6% ($n = 44$). While 51.3% of patients ($n = 79$) reported that they were diagnosed with diabetes after a visit to a physician when they suspected diabetes, 47 (30.5%) patients were diagnosed with diabetes during routine health check-ups. Thirty-nine of the patients (25.3%) reported that they had previously attended diabetes education at a hospital. The proportion of patients who did not apply for a dietitian's nutritional assistance after diabetes diagnosis was 39.6% ($n = 61$), whereas the proportion of patients who were still using the dietitian's nutritional assistance was 27.9% ($n = 43$). In the period before COVID-19, the number of visits to the physician for check-ups ranged from 1 to 12 per year, with a median value of 2.0, and the number of patients who went to the physician for check-ups once or twice per year was 85 (55.2%). While 81.8% ($n = 126$) of patients were able to measure their blood glucose at home, 44 patients (28.6%) reported that they measured their blood glucose daily.

The median score of patients' ratings between 1 and 8 on the treatment adherence scale was 6.5 points, and the proportion of patients with high adherence was 26.6% ($n = 41$). The number of patients who used only oral antidiabetic agents for treatment was 104 (67.5%). Out of all the patients, 46 (29.9%) had fasting blood glucose values within the therapeutic limits of 80–130 mg/dL, while 108 (70.1%) had high glucose values above 130 mg/dL. Table 2 provides an overview of the diabetes-related characteristics of the patients.

In 2020, HbA1c values among patients ranged from 5.2 to 14.0, with a mean of 8.36 ± 2.00. In 2021, HbA1c values

Table 3 HbA1c change between April–October 2020 and April–October 2021

HbA1c	*n*	Mean ± standard deviation	Min	Max	25th	50th (median)	75th	*Z**	*p*
First	154	8.36 ± 2.00	5.20	14.0	6.73	8.00	10.00	−0.170	0.865
Second	154	8.38 ± 1.86	5.60	14.3	6.77	8.05	9.82		

*Wilcoxon test was used

Table 4 Change in HbA1c values of patients during the pandemic period

		Current HbA1c group			Test
	n (%)	< 7.0		7.0 <	*p*
Previous HbA1c group					
< 7.0	49 (%31.8)	34 (%69.4)		15 (%30.6)	Mc Nemar
7.0 <	105 (%68.2)	10 (%9.5)		95 (%90.5)	$p = 0.424$
Total	154 (%100)	44 (%28.6)		110 (%71.4)	
		HbA1c group change			Test
	n	The change in the good direction	Unchanged	The change in the poor direction	*p*
Food literacy					
Good	81	8—9.9%	67—82.7%	6—7.4%	$X^2 = 3.989$
Poor	73	2—2.8%	62—84.9%	9—12.3%	$p = 0.136$
Total	154	10—6.5%	129—83.8%	15—9.7%	
Treatment adherence for DM					
High adherence	41	1—2.4%	39—95.1%	1—2.5%	$X^2 = 5.785$
Moderate adherence	55	5—9.1%	44—80.0%	6—10.9%	$p = 0.216$
Low adherence	58	4—6.9%	46—79.3%	8—13.8%	
Treatment for DM					
Oral antidiabetic only	105	9—8.7%	83—79.8%	12—11.5%	$X^2 = 4.681$
Oral antidiabetic + insulin	39	1—2.6%	35—89.7%	3—7.7%	$p = 0.322$
Insulin	11	0—0.0%	11—100.0%	0—0.0%	
BMI group					
Normal and below	14	0—0.0%	14—100.0%	0—0.0%	$X^2 = 6.436$
Overweight	57	5—8.8%	43—75.4%	9—15.8%	$p = 0.169$
Obese	83	5—6.0%	72—86.7%	6—7.3%	
Gender					
Female	90	4—4.4%	77—85.6%	9—10.0%	$X^2 = 1.498$
Male	64	6—9.4%	52—81.2%	6—9.4%	$p = 0.473$
Spouse education					
Primary school	76	6—7.9%	62—81.6%	8—10.5%	$X^2 = 0.288$
Above primary school	66	4—6.1%	56—84.8%	6—9.1%	$p = 0.866$
Education					
Primary school	81	7—8.6%	63—77.8%	11—13.6%	$X^2 = 4.533$
Above primary school	73	3—4.1%	66—90.4%	4—5.5%	$p = 0.104$
DM in relative					
Present	116	8—6.9%	95—81.9%	13—11.2%	$X^2 = 1.352$
Absent	38	2—5.3%	34—89.4%	2—5.3%	$p = 0.509$
Diabetes education					
Attended	39	4—10.3%	31—79.4%	4—10.3%	$X^2 = 1.099$
Not attended	115	6—5.2%	98—85.2%	11—9.6%	$p = 0.577$
Dietician's advice					
He/she uses it actively	43	3—7.0%	38—88.3%	2—4.7%	$X^2 = 3.492$
He/she does not use it actively	50	4—8.0%	42—84.0%	4—8.0%	$p = 0.479$
No application to the dietician	61	3—4.9%	49—80.3%	9—14.8%	
Annual DM physician checkups					
1 or 2 physician check-ups	85	5—5.9%	72—84.7%	8—9.4%	$X^2 = 0.150$
More than 2 physician check-ups	69	5—7.2%	57—82.6%	7—10.2%	$p = 0.928$
Blood glucose measurement at home					
Be able to measure	126	9—7.2%	106—84.1%	11—8.7%	$X^2 = 1.187$
Not to be able to measure	28	1—3.6%	23—82.1%	4—14.3%	$p = 0.552$

Table 4 (continued)

Frequency of blood glucose measurement at home					
Daily	44	2—4.5%	40—91.0%	2—4.5%	$X^2=2.364$
Rare	82	7—8.5%	66—80.5%	9—11.0%	$p=0.307$
Do you attend to your nutrition after being diagnosed with DM					
I do	116	10—8.7%	99—85.3%	7—6.0%	$X^2=10.044$
I do not pay attention	38	0—0.0%	30—78.9%	8—21.1%	$p=0.007$
Inpatient treatment for DM					
Received	32	2—6.2%	27—84.4%	3—9.4%	$X^2=0.011$
I did not receive	122	8—6.6%	102—83.6%	12—9.8%	$p=0.995$

ranged from 5.60 to 14.30, with a mean of 8.38 ± 1.86. Although a minimal increase in HbA1c values was observed in patients who did not have physician check-ups in the last year, the difference was not statistically significant ($p=0.856$). Out of all the patients, 75 showed an increase in HbA1c values from the previous year, while 79 showed a decrease. Table 3 provides an overview of the HbA1c changes among patients.

Table 4 shows changes of HbA1c in pandemic. The study found that patients who reported paying attention to their diet after being diagnosed with diabetes showed a significantly higher rate of improvement compared to those who did not ($p=0.007$). Conversely, the rate of deterioration was significantly higher in patients who reported not paying attention to their diet.

HbA1c and food literacy relation shows in Table 5. The food literacy scores of patients with decreased HbA1c values were significantly higher (33.0 ± 6.6) than those of patients with increased HbA1c values (30.9 ± 5.8) ($t=2.051$—$p=0.042$).

Discussion

This study is the first study to investigate the extent to which glycemic control of DM patients during the COVID-19 period without a physician's check-ups is affected by treatment adherence and food literacy. According to this study's results, although there was an increase in blood glucose and HbA1c values before and after COVID-19, no statistically significant difference was found. However, it was also found that the food literacy scores were lower in patients with increased HbA1c values and higher in patients with decreased HbA1c values (Table 5).

As the COVID-19 pandemic has become a global health problem affecting the entire world, the measures taken and the fear of COVID-19 have led to a decrease in enrollment in health centers. A study that included five states in the USA showed that during the COVID-19 period, emergency department admissions decreased by 41.5 to 63.5% [22]. This situation increases the importance of treatment adherence and nutrition, which is recognized as the most important treatment modality in DM [23]. Indeed, individuals who paid attention to their nutrition showed better changes in HbA1c in the results of the study (Table 4).

At the onset of the pandemic, curfews were enforced as the primary measure to control its spread. Consequently, individuals who were compelled to stay home experienced a reduction in their physical activity levels. Given the well-established association between type 2 diabetes and obesity, patients who remained at home and engaged in less physical activity experienced a further increase in their obesity rates [24]. In our study group, only 13% of individuals engaged in daily physical activity before the pandemic. Moreover, as previously mentioned, only one-third of the patients had fasting blood glucose levels at

Table 5 The relationship between the change in HbA1c value and the food literacy scale score

	n	Food literacy scale score mean ± standard deviation	Test *p*
HbA1c group change			
The change in the good direction	10	35.4 ± 4.3	$F=1.972$ p = 0.143
Unchanged	129	31.9 ± 6.4	
The change in the poor direction	15	30.3 ± 6.3	
HbA1c value change			
Decreased HbA1c value	79	33.0 ± 6.6	$t=2.051$
Increased HbA1c value	75	30.9 ± 5.8	$p=0.042$

the desired level (Table 2). These findings suggest that patients were not adequately informed about the importance of physical activity, and the impact of the restrictions on going out may have had a minimal effect on their glycemic control.

Studies have shown that HbA1c increases in both type 1 and type 2 DM patients, although this is not statistically significant [25]. The change in HbA1c in the patient group where the research was conducted was minimal, with an average of 8.36 and 8.38 before and after the pandemic, respectively (Table 3). It can be said that overall, patients' blood sugar control was poor in both periods. However, when patients were grouped according to their HbA1c levels, 15 out of 49 patients (30.6%) with levels below 7 had values above 7 (Table 4). Given this information, it would not be incorrect to say that patients' glycemic control is moving in a poor direction. Although most countries have implemented distance learning and telemedicine applications to enable DM patients to self-care during the pandemic period, according to the results of a literature review, it has been shown that the controls of DM patients have moved in a poor direction during the pandemic period [26]. Our study group observed significant changes, particularly in patients who exclusively used oral antidiabetic drugs, which were predominantly negative. Although some diabetes patients may have paid more attention to their treatments due to the fear of contracting COVID-19 and being in the high-risk group, along with the facilitative policies that were implemented to improve medication access during the pandemic period, there was still a higher incidence of disrupted treatment adherence among patients. In fact, a study conducted in Germany indicated that the use of antidiabetic drugs among diabetes patients increased during the pandemic period [27].

It has been established that food literacy is one of the indicators of a healthy diet and is directly related to nutrition [28]. In our study, the level of food literacy was at a good level in slightly over half of the patients, and the most striking finding was that 9.9% of patients with good food literacy had improved HbA1c levels, while in patients with poor food literacy, the HbA1c levels changed in a negative direction with 12.3% (Table 4). It was also found that patients with decreased HbA1c values had higher food literacy scores (Table 5). Patients whose HbA1c values moved in a good direction had higher food literacy scores. It can be seen that 40% of patients do not receive support from a dietitian, and about 3 out of 4 patients do not receive diabetes education (Table 2). It is inevitable that the patients who are deprived of this training, which has a positive impact on improving food literacy, will adhere to the diet and naturally have poor glycemic control. Scheduling this training to improve food literacy levels instead of general health interventions will increase tertiary protection in DM patients [10]. In cases where restrictions occur, such as pandemics, and people must remain at home, it is important to take advantage of technology to ensure continuation and continuity of patient education. It has been shown that there is a strong association between cell phone applications and healthy lifestyle changes in patients with type 2 DM [29]. During the COVID-19 pandemic, there is evidence that a social media–focused education program helps to improve self-care in DM patients, increase their health literacy, and improve their knowledge, attitudes, and behaviors related to diabetes care [30].

Conclusion

It has been concluded that the glycemic control of patients with diabetes is irregular during the pandemic period and HbA1c increases more, especially in patients with low food literacy. To our knowledge, this study is the first to investigate the relationship between food literacy and glycemic control in DM patients, and its contribution to the literature is significant. The study also provides information on DM patients whose follow-up was delayed during the pandemic. However, because of the study's retrospective nature, one of its limitations may be that it is not possible to compare pre-pandemic levels of food literacy. For a widespread disease such as type 2 DM, more accurate results may be obtained by multicenter prospective studies with a larger number of patients. Nonetheless, this study will provide researchers with clues for future educational efforts to improve food literacy in patients with type 2 DM.

Author contribution Yavuzalp Solak: conception, design, data collection, analysis, writer, critical review.

Hasan Durmuş: conception, design, data collection, literature review, writer.

Gürgün Tuğçe Vural Solak: data collection, literature review, writer.

Seher Çetinkaya Altuntaş: data collection, literature review, writer.

Ahmet Gazi Mustan: data collection, literature review, writer.

Declarations

Statement of ethics This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved Mustafa Kemal University Faculty of Medicine Ethical Committee 22/04/2021, approval number: 02.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Diabetes-specific eating disorder and social exclusion in adolescents with type 1 diabetes

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Abstract

Background Adolescents with type 1 diabetes are at risk of developing eating disorders and social exclusion, and these disorders are associated with serious diabetes-related medical and social complications.

Objective The present study, which had a descriptive and correlational design, was conducted to determine the relationship between diabetes-specific eating disorders and social exclusion in adolescents with Type 1 Diabetes (T1DM) who were treated at a university hospital in Türkiye between November 2021 and April 2022.

Method The sampling of the study consisted of 124 adolescents who had T1DM between the ages of 14 and 18 who were followed up in the pediatric endocrinology clinic of a university hospital. The data of the study were collected with the Descriptive Characteristics Form, the Diabetes-Specific Eating Disorder Scale (DEPS-R), and the Adolescent Social Exclusion Scale (OES-A). The study was conducted with the permission of the institution and ethics committee, and written consent was obtained from the adolescents and parents. The descriptive statistics, Mann–Whitney U-Test, Kruskal–Wallis Analysis of Variance, and Simple Linear Regression Analysis were used in the evaluation of the data.

Results The mean scores of the DEPS-R and OES-A scales of the adolescents who had T1DM were 43.29 ± 17.15 and 34.51 ± 8.41, respectively. A relationship was detected between the OES-A mean scores of the adolescents and the DEPS-R mean scores, and the OES-A mean score explained 74.7% of the DEPS-R mean score ($R2 = 0.747$) ($p \leq 0.001$).

Conclusion It was determined in the present study that adolescents with T1DM had high levels of social exclusion and eating disorders. It was also found that as the level of social exclusion of adolescents with T1DM increased, the level of eating disorders increased.

Keywords Type 1 Diabetes · Social exclusion · Eating disorder · Adolescent

Introduction

Type 1 Diabetes Mellitus (T1DM), which is one of the most common endocrinological and metabolic disorders in childhood, makes up approximately 10% of the diabetes cases in the entire world [1]. It was reported in the 10th Diabetes Atlas (2021) of the International Diabetes Association (IDF) that the population of children aged 19 and under was 2.61 billion in the world, approximately 1.2 million of these children had T1DM, and there were approximately 150 thousand new children diagnosed with T1DM in the world every year. It was also stated in the same report that there were approximately 26 thousand children aged 19 and under with T1DM in Turkey [2].

T1DM causes compulsory medical practices (i.e. blood glucose measurement, insulin injection, etc.), lifestyle changes (diet, exercise, etc.), and social life (family, friend

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relations, etc.) in the daily life of adolescents [3], which cause both adolescents and their parents to face more problems and have adjustment problems. The reasons why the adolescents cannot adapt to these changes in life at the desired level can be listed as being dependent on parents because of T1DM, feeling different from their friends or peer groups, and the chronic illness negatively affecting the adolescents' perception of being normal [4].

Feeling different from friends or peers and the deterioration of the perception of being normal might cause adolescents to feel excluded from peers. Social exclusion, which can be defined as being excluded and ignored by others, is a common issue for many individuals. The exclusion or rejection of the individual from the group/society threatens the sense of belonging and may cause the individual faces painful experiences [5]. These experiences might manifest as anxiety, depression, eating disorders, and behavior problems in adolescents.

Adolescents who have T1DM have to regulate their eating habits and lifestyles to keep blood sugar under control throughout their lives. Because of the nature of the disease, factors such as diet lists to be followed, prohibited foods, and chronic disease cause anxiety in patients, leading to focus on food and weight control. Along with these, the exclusion of the adolescent by friends or peer groups also brings anxiety and causes the adolescent to not manage nutrition therapy appropriately. For all these reasons, adolescents who had T1DM experience deterioration in eating attitudes and behaviors. It was reported in previous studies that eating disorders are associated with diabetic ketoacidosis, acute or chronic complications of diabetes, electrolyte imbalances, cardiac/musculoskeletal complications, and increased mortality in adolescents who have T1DM [6]. The present study was planned to determine the relationship between diabetes-specific eating disorders and social exclusion in adolescents with T1DM.

Materials and methods

This study was planned as a descriptive and relational study to determine the relationship between diabetes-specific eating disorders and social exclusion in adolescents with type 1 diabetes who were treated at a university hospital in Türkiye between November 2021 and April 2022. The sampling of the study consisted of 124 adolescents who had T1DM between the ages of 14 and 18 who were followed up in the pediatric endocrinology clinic of a university hospital. The results of the post-power analysis performed in G-Power were; $\beta = 0.95$. ($n = 124$, $\alpha = 0.05$, $d = 0.33$). Ethics Committee (Decision No: 2021/697) and institutional permission were obtained for the study. Before the study started, the purpose of the study was explained to the adolescents and written and verbal consent was obtained from their parents and themselves.

The data of the study were collected with Adolescent Information Form, Diabetes-Specific Eating Disorder Scale (DEPS-R) and Ostracism Experience Scale for Adolescents (OES-A). Data collection process was carried out in the pediatric endocrinology outpatient clinic of the hospital. A questionnaire form was applied to the adolescents who came to the control via a tablet computer. The application period of the questionnaire took an average of 15 minutes.

Adolescent information form There are 14 questions about the introductory characteristics (age, gender, grade, number of siblings, income status, diagnosis time, type of insulin used, person administering insulin, using insulin regularly, nutrition model, number of blood glucose measurements, compliance with the exercise program, compliance with the nutrition plan, BMI-for-age) and diseases of adolescents with T1DM in this form.

Diabetes-specific eating disorder scale (DEPS-R) The scale was developed by Markowitz et al. [7] to evaluate diabetes-specific eating disorders and was adapted into Turkish by Atik Altınok et al. [8]. It is a 6-point Likert-type scale (0: Never, 5: Always) consisting of 16 items. The highest score that can be obtained from the scale is 80, and the lowest score is 0. High scores indicate a high level of diabetes specific eating disorder. The total Cronbach's Alpha was determined as = 0.86 in the scale adaptation study [8]. In the present study, the total Cronbach's alpha was found to be 0.93.

Ostracism experience scale for adolescents (OES-A) The Turkish validity and reliability of the scale, which was developed by Gilman et al. [9], was conducted by Akın et al. [10]. It is a 5-point Likert-type scale (1: Never, 5: Always) consisting of 11 items and two subscales (negligence and exclusion). There is no reverse-coded item on the scale. The highest score that can be obtained from the scale is 55, and the lowest score is 11. High scores indicate a high level of perception of social exclusion. The total Cronbach's Alpha was determined as 0.87 in the scale adaptation study for the Disregarding subscale of the scale [10]. In the present study, the total Cronbach's alpha was found to be = 0.86.

The data were evaluated by using the SPSS 22.0 (IBM Corp., Armonk, NY, USA) package program. Descriptive statistical methods are given as numbers (n), percent (%), mean ($\bar{x}$), standard deviation (SD), and min–max. The conformity of the data to the normal distribution was evaluated with the Shapiro–Wilk Test and it was determined that the data did not show normal distribution. The Mann–Whitney U Test was used to compare the pairwise independent groups that did not conform to the normal distribution, the

Kruskal–Wallis Analysis of Variance was used to compare three or more independent groups, and the relationship between scale scores was evaluated with Simple Linear Regression Analysis.

Results

It was found that the mean age of the adolescents who participated in the study was 15.60 ± 1.04, the mean BMI for age was -0.59 ± 1.13 in girls and 0.95 ± 1.65 in boys; 50.8% ($n = 63$) of the adolescents were female, 58.9% ($n = 73$) were in the 14–15 age group, 76.6% ($n = 95$) were studying at the 9-10th grades, 46.8% ($n = 58$) had two siblings, 72.6% ($n = 90$) perceived their income level as moderate, 43.5% ($n = 54$) were diagnosed between 1–3 years, 94.4% ($n = 117$) used an insulin pen, 88.7% ($n = 110$) self-administered the insulin, 93.5% ($n = 116$) used insulin regularly, 82.3% ($n = 102$) followed a fixed-meal (morning, noon, and evening) diet, 93.5% ($n = 116$) measured blood sugar three times a day or more, 64.5% ($n = 80$) did not follow an exercise program, 50.0% ($n = 62$) did not follow nutrition program, and 58.9% ($n = 73$) had a normal BMI for age (Table 1).

In the present study, the mean scores of the adolescents on the DEPS-R and OES-A scales were found to be 43.29 ± 17.15 and 34.51 ± 8.41, respectively (Table 2). A statistically significant positive correlation was found between the DEPS-R and OES-A scores of the adolescents with T1DM ($r = 0.865$; $p \leq 0.001$) (Table 3).

The mean DEPS-R scores were found to be higher in those who were male ($p \leq 0.001$), those who were educated in the 9-10th grades ($p = 0.001$), used an insulin pump ($p = 0.011$), administered insulin by their parents ($p = 0.046$), did not use insulin regularly ($p \leq 0.001$), applied carbohydrate counting as a nutrition model ($p \leq 0.001$), who did not measure blood sugar levels at all ($p = 0.037$), and who were obese according to the BMI classification for age ($p \leq 0.001$). It was also determined that the mean DEPS-R scores were not affected by the variables of the number of siblings, perceived income, duration of diagnosis, compliance with the exercise program, and compliance with the nutrition plan ($p > 0.05$) (Table 4).

It was determined that the mean OES-A scores were higher in adolescents who were male ($p \leq 0.001$), students who were educated in the 9-10th grades ($p = 0.001$), used an insulin pump ($p \leq 0.001$), administered insulin by their parents ($p = 0.002$), used carbohydrate counting as a nutrition model ($p \leq 0.001$), did not measure blood sugar levels at all ($p = 0.001$), and obese according to BMI classification for age ($p = 0.003$). It was also found that the mean OES-A scores were not affected by the variables of the number of siblings, perceived income levels, duration of diagnosis,

Table 1 Characteristics of adolescents with Type 1 Diabetes ($n = 124$)

Characteristics	Mean ± SD	
Age	15.60 ± 1.04	
Characteristics	*n*	%
Gender		
Female	63	50.8
Male	61	49.2
Age		
14–15 years	73	58.9
16–17 years	51	41.1
Grade		
9–10. grade	95	76.6
11–12. grade	29	23.4
Number of siblings		
One	17	13.7
Two	58	46.8
Three or more	49	39.5
Income Status		
Low	17	13.7
Middle	90	72.6
High	17	13.7
Diagnosis Time		
1–3 years	54	43.5
4–6 years	48	38.7
7 years and above	22	17.7
Type of Insulin Used		
Pen	117	94.4
Insulin pump	7	5.6
Person Administering Insulin		
Himself (adolescent)	110	88.7
Mom dad	14	11.3
Using Insulin Regularly		
Yes	116	93.5
No	8	6.5
Nutrition Model		
Carbohydrate count	22	17.7
Fixed meal (morning, noon, evening)	102	82.3
Number of Blood Glucose Measurements		
None	8	6.5
Three times or more	116	93.5
Compliance with the Exercise Program		
Yes	31	25.0
No	80	64.5
Partially	13	10.5
Compliance with the Nutrition Plan		
Yes	19	15.3
No	62	50.0
Partially	43	34.7
BMI-for-age*		
Malnutrition	19	15.3
Normal	73	58.9
Obese	32	25.8

*WHO growth reference data for 5–19 years

Table 2 DEPS-R and OES-A Mean Scores of Adolescents with Type 1 Diabetes ($n = 124$)

Scales	Med (Min–Max)	Mean ± SD
DEPS-R	46.50 (5–67)	43.29 ± 17.15
OES-A	36.00 (15–50)	34.51 ± 8.41

DEPS-R Diabetes-Specific Eating Disorder Scale, *OES-A* Ostracism Experience Scale for Adolescents

regular use of insulin, compliance with the exercise program, and compliance with the nutrition plan ($p > 0.05$) (Table 4).

Discussion

Intense emotional changes in adolescents with T1DM because of the nature of the period, the complex management of the disease, and the sense of being accepted or not different by their peers may predispose adolescents who have T1DM to some risky health behaviors. Negative emotional states such as stress and anxiety may appear in adolescents with T1DM who are socially excluded by their peers, which might then lead to eating disorders in adolescents with T1DM [11]. It was found in the present study that adolescents with T1DM had high levels of social exclusion and eating disorders (Table 2). Similar to the findings of the present studies, social exclusion in adolescents with T1DM [12–15] and eating disorders [16–21] were reported in previous studies.

It was determined that as the level of social exclusion increased in adolescents who had T1DM who participated in the study, eating disorders also increased ($p \leq 0.001$) (Table 3). No study was detected in the literature examining the relationship between social exclusion and eating disorders in adolescents with T1DM. In some studies, it was reported that eating disorders and social exclusion were detected in adolescents with T1DM who had high stress and depressive states [22, 23]. It can be argued that adolescents experience negative emotions such as stress, anxiety, and depression more intensely because of the characteristics of the period, and the addition of a disease with complex management such as Type 1 Diabetes increases the risk of social exclusion and eating disorders in adolescents with T1DM.

It was found that eating disorders and social exclusion were higher in males with T1DM who participated in the study

Table 3 Correlation (pearson correlation) between the mean scores of DEPS-R and OES-A of adolescents with Type 1 Diabetes ($n = 124$)

	DEPS-R	OES-A
DEPS-R	1.000	
OES-A	0.865*	1.000

* $p \leq 0.001$

Table 4 Distribution of DEPS-R and OES-A scale mean scores according to descriptive characteristics of adolescents with Type 1 Diabetes ($n = 124$)

Characteristics	DEPS-R *Mean Rank*	OES-A *Mean Rank*
Gender		
Female	48.44	48.29
Male	77.02	77.18
	$p \leq 0.001$	**$p \leq 0.001$**
Age		
14–15 years	66.42	61.62
16–17 years	56.88	63.76
	$p = 0.143$	$p = 0.742$
Grade		
9–10. grade	68.24	68.52
11–12. grade	43.69	42.79
	$p = 0.001$	**$p = 0.001$**
Income Status		
Low	51.94	78.82
Middle	63.31	58.20
High	68.76	68.94
	$p = 0.357$	$p = 0.067$
Diagnosis Time		
1–3 years	59.83	64.13
4–6 years	62.48	55.16
7 years and above	69.09	74.52
	$p = 0.591$	$p = 0.099$
Type of Insulin Used		
Pen	60.50	59.30
Insulin pump	96.00	116.0
	$p = 0.011$	**$p \leq 0.001$**
Person Administering Insulin		
Himself (adolescent)	60.32	58.98
Mom dad	79.64	90.18
	$p = 0.046$	**$p = 0.002$**
Using Insulin Regularly		
Yes	58.97	61.74
No	113.68	73.50
	$p \leq 0.001$	$p = 0.368$
Nutrition Model		
Carbohydrate count	70.50	92.86
Fixed meal (morning, noon, evening)	53.77	55.95
	$p \leq 0.001$	**$p \leq 0.001$**
Number of Blood Glucose Measurements		
None	88.00	103.00
Three times or more	60.74	59.71
	$p = 0.037$	**$p = 0.001$**
Compliance with the Exercise Program		
Yes	69.32	78.37
No	58.94	53.76
Partially	68.15	78.42
	$p = 0.323$	$p = 0.312$

Table 4 (continued)

Characteristics	DEPS-R *Mean Rank*	OES-A *Mean Rank*
Compliance with the Nutrition Plan		
Yes	49.68	64.45
No	65.24	57.61
Partially	64.21	68.69
	$p = 0.233$	$p = 0.286$
BMI-for-age*		
Malnutrition	31.32[a]	42.24[a]
Normal	57.70[b]	61.32[a,b]
Obese	91.97[c]	77.22[b]
	$p \leq 0.001$	**$p = 0.003$**

x^2: Kruskal-Wallis, *Z*: Mann-Whitney U

***The superscripts a, b, c show in-group differences in each group, and measurements with the same letters are similar

($p \leq 0.001$) (Table 4). Unlike the findings of the present study, some previous studies reported that the level of eating disorders was higher in girls with T1DM [20–22, 24]. Although female adolescents experience negative emotional states such as anxiety, stress, and depression more intensely, it is considered that this may be related to the fact that they support each other more and are less likely to be excluded than male adolescents [25]. The fact that males who had T1DM had higher exclusion levels in the study supports this situation.

In the present study, it was also found that eating disorders and social exclusion were higher in adolescents with T1DM who used carbohydrate counting as a nutrition model ($p = 0.011$, $p \leq 0.001$, respectively). Carbohydrate counting aims to improve glycemic control by providing flexibility in food choices. However, it can also be difficult for children and parents, and the training given must be adapted to the culture, preferences, capacities, and understandings [26]. Although there is no study conducted on children and adolescents who have T1DM, it was reported in a previous study conducted with adults with T1DM that carbohydrate counting is more difficult to implement in daily life because it fluctuates glucose levels and complicates diabetes management [27]. It was also reported that peer influences, social relationships, stress, and depression are among the factors preventing children and adolescents with T1DM from counting carbohydrates [28].

Eating disorders and social exclusion were found to be higher in those who used insulin pumps ($p = 0.022$, $p \leq 0.001$, respectively) in adolescents with T1DM who participated in the study. No study was detected in the literature examining the relationship between insulin pump use and eating disorders and social exclusion. However, in a previous study conducted with children who had T1DM, it was found that those using insulin pumps experienced more glycemic control and treatment-related problems and were more anxious [29]. Despite the increased use and defined benefits of the insulin pump, it is considered that it may also increase social exclusion in children because of constantly being reminded of the disease and making them feel different from their peers [30].

It was found that the levels of social exclusion and eating disorders were higher in obese adolescents who had T1DM who participated in the study ($p = 0.003$, $p \leq 0.001$, respectively) (Table 4). Previous studies support these findings [21, 22, 31–33]. Obesity causes negative conditions such as anxiety, stress, depression, inability to move around with peers, and exclusion from peers [34]. Both the negative emotions brought on by obesity and the complex disease management brought about by the disease may cause adolescents with T1DM to be exposed to more exclusion and to experience eating disorders.

Conclusion

It was found that the mean DEPS-R and OES-A scale scores of the adolescents were high. A high and positive significant relation was detected between DEPS-R and OES-A scores. It was also found that the variables of Body Mass Index according to gender, type of insulin used, the person administering the insulin, nutritional model, and age affected DEPS-R and OES-A scores.

Limitations

This study was conducted with 124 adolescents with type 1 diabetes. All data were limited with self-reports.

Author contributions All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work.

Data Availability Based on a request, the data supporting the current work's results is available from the corresponding author.

Declarations

Ethical Clearance Ethics Committee (Decision No: 2021/697) and institutional permission were obtained for the study. Before the study started, the purpose of the study was explained to the adolescents and written and verbal consent was obtained from their parents and themselves. This study was supported by Erciyes University Scientific Research Projects Department with the project code no: TSA-2022-11738.

Conflicts of ınterest The authors declare that there are no conflict of interests.

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ORIGINAL ARTICLE

Association between comorbid diabetes mellitus and mortality of patients with sepsis: A meta-analysis

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Abstract

Objective Although diabetes patients have a higher propensity to develop infection and sepsis, it is still controversial whether the mortality of sepsis patients is affected by diabetes (DM). We conducted a systematic review and meta-analysis to determine the relationship between diabetes and mortality in patients with sepsis.

Methods We comprehensively searched for relevant studies in PubMed, MEDLINE, EMBASE, and the Cochrane Library database from January 2000 to December 2021. Two reviewers independently selected studies, extracted data, and assessed quality. We used random-effects modeling to calculate the summary of risk ratios and confidence interval (CI) of mortality. Study quality was assessed using NOS score, and publication bias was assessed using Egger's statistic.

Results A total of 23 studies were included in the analyses, comprising 14,521,791 septic patients, including 2,866,429 DM patients. We stratified the in-hospital mortality data by duration for 30 days, 90 day, and mixed days. Meta-analysis of 23 studies showed slightly increased overall mortality among the patients with DM (RR, 1.12; 95% CI 1.00 – 1.25; I^2 96.1%; $p = 0.000$) by pooling of all data in the random effects model. Subgroup analysis did not demonstrate a statistically significant increase either in 30-day mortality (RR, 1.07; 95% CI 0.97–1.18; I^2 0.0%; p 0.963), 90-day mortality (RR, 1.00; 95% CI 0.95–1.07; I^2 0.0%; $p = 0.735$), or mixed-day mortality (RR, 1.16; CI 0.98–1.37; I^2 97.9%; $p = 0.000$). The quality of the included studies was good, and the median NOS score was 7.1 (range, 6–9).

Conclusions This systematic review and meta-analysis of studies suggests that DM does slightly increase sepsis overall mortality, however with statistical heterogeneity. Due to the limitations of the analysis, more well-designed clinical studies are still necessary in future.

Keywords Meta-analysis · Diabetes · Sepsis · Outcome · Mortality

Abbreviations

CI	Confidence interval
RR	Relative risk
DM	Diabetes mellitus

Introduction

Sepsis is a life-threatening organ dysfunction caused by over activation of inflammatory reaction and coagulation dysfunction response to severe systemic infection. It is a major medical problem worldwide and accounts for 20% of the global death [1]. Diabetes mellitus (DM) is a common and increasing comorbidity in sepsis patients. The incidence rate of DM is rising and has become a major public health problem worldwide [2], especially in low and middle-income countries. Sepsis is closely related to DM; in fact, Sepsis 2.0 used hyperglycemia (blood glucose > 7.7 mmol/L) in patients without a previous history of diabetes as one of the diagnostic criteria for sepsis, which shows the close relationship between sepsis and DM.

It is clear that DM patients are more prone to infection and sepsis, but the impact of diabetes on the outcome of sepsis is still uncertain. Two meta-analyses about this topic showed that presence of diabetes does not increase the risk of mortality in patients with sepsis [3, 4]. Neither of these two meta-analyses included Zoppini's study [5], a large-size observational study, which proved that diabetic patients had a twofold increased mortality for sepsis compared to non-diabetic patients. Due to the increase of relevant research in recent years, we searched studies January 2000 to December 2021 and conducted a systematic review and meta-analysis on this topic to determine the association between preexisting DM and mortality in humans with sepsis.

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Materials and methods

This study protocol was implemented following the Meta-analysis of Preferred Reporting Items for Systematic Reviews (PRISMA) [6].

Data sources and search strategy

We searched PubMed, MEDLINE, EMBASE, and the Cochrane Library database from January 2000 to December 2021. We use medical heading terms and cross search the following three categories for term search: (1) diabetes ("diabetes" or "diabetic"); (2) disease ("sepsis," "septic shock," "septic," or "septicemia"); and (3) others related ("outcome," "intensive care unit," "ICU," "critically ill patients," "death", "mortality," or "prognosis"). We limited the types of studies to "human" and "English" languages. Only studies that reported a comparison between diabetes patients and non-diabetes patients, whose ages were over 18 years of age, were included. All retrieved studies and recent bibliographies were screened to further expand the search scope.

Inclusion criteria

Two researchers independently read the titles and abstracts to determine eligible study. Studies were included if (1) the study population came from a well-established retrospective, prospective cohort, or case–control study, including a group of diabetic patients and a group of non-diabetic patients with sepsis; (2) the 28-day mortality, 90-day mortality, or hospitalization mortality was clearly reported on both group or provided sufficient data to calculate these parameters.

Data extraction and methodological quality

Two researchers (XY and QD) independently collected data from the included studies into a data standardized collection form. The following elements were extracted from the included studies: first author, year of publication, study design, study country, severity of sepsis, and number of diabetes patients and non-diabetic patients. The primary outcome was 28-, 30-, or 90-day mortality and mixed-day mortality. We equated 28-day mortality with 30-day mortality. The day of mortality not specified was assigned to mixed-day mortality. Newcastle–Ottawa Scale tool (available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) was used to evaluate the quality of the included studies.

Data synthesis and statistical methods

Stata Software (version 12.0 Stata Corporation, College Station, TX, USA) was used for statistical analysis. The dichotomous data of relative risk (RR) with 95% confidence intervals (CI) for in-hospital mortality in each study were pooled using the random-effects model; results were expressed by Forest plots.

In order to evaluate the effect of DM on the mortality of sepsis patients, we performed a subgroup analysis to evaluate the influence of DM. The first subgroup was sixteen studies that reported the day of mortality not specified. The second subgroup was studies that reported 30-day mortality, and the third subgroup was studies that reported 90-day mortality. Publication bias was assess by Egger's test [7]. A RR > 1 suggested that DM was associated with an increased risk of mortality.

We proposed to use Cochran's Q test and reported as I^2 to assess and calculate statistical heterogeneity between studies. Sensitivity analysis was used to determine the robustness of the data and the impact of individual research on the summary effect. In addition, p value < 0.05 was considered statistically significant.

Results

Search results

According to the initial search strategy, 5856 unique records were yielded, 1908 duplicates were removed, and 3796 records were eliminated by screening titles and abstracts. Full-text assessment was conducted in the last 156 articles. Of these articles, 23 studies satisfied the inclusion criteria. The study selection process was shown in Fig. 1.

Characteristics of included studies

All the studies were published from 2000 to 2021. There were a total of 14,521,791 septic patients, including 2,866,429 DM patients and 11,646,162 non-DM patients, ages ranging from 45 to 80 years and mostly older than 60 in these studies. Except for 5 prospective studies, the others are retrospective studies. The effect estimations of relative ratios (RRs) of mortality for diabetic patients were provided in each study. What should be mentioned is that four cohorts were included in Russell's study [8]. Four studies included six cohorts provided relative ratios (RRs) of 30-day mortality for diabetic patients. Four studies provided relative ratios (RRs) of 90-day mortality for diabetic patients. Of these studies, 8 studies enrolled patients with severe sepsis, septic shock patients, or ICU septic patients [8–15], 2 contained non-ICU patients [16, 17], and the left 14 studies enrolled sepsis patients with all stages. The sources of infection in the included studies were not limited to any specific systems or organs. The characteristics of each included study were presented in Table 1.

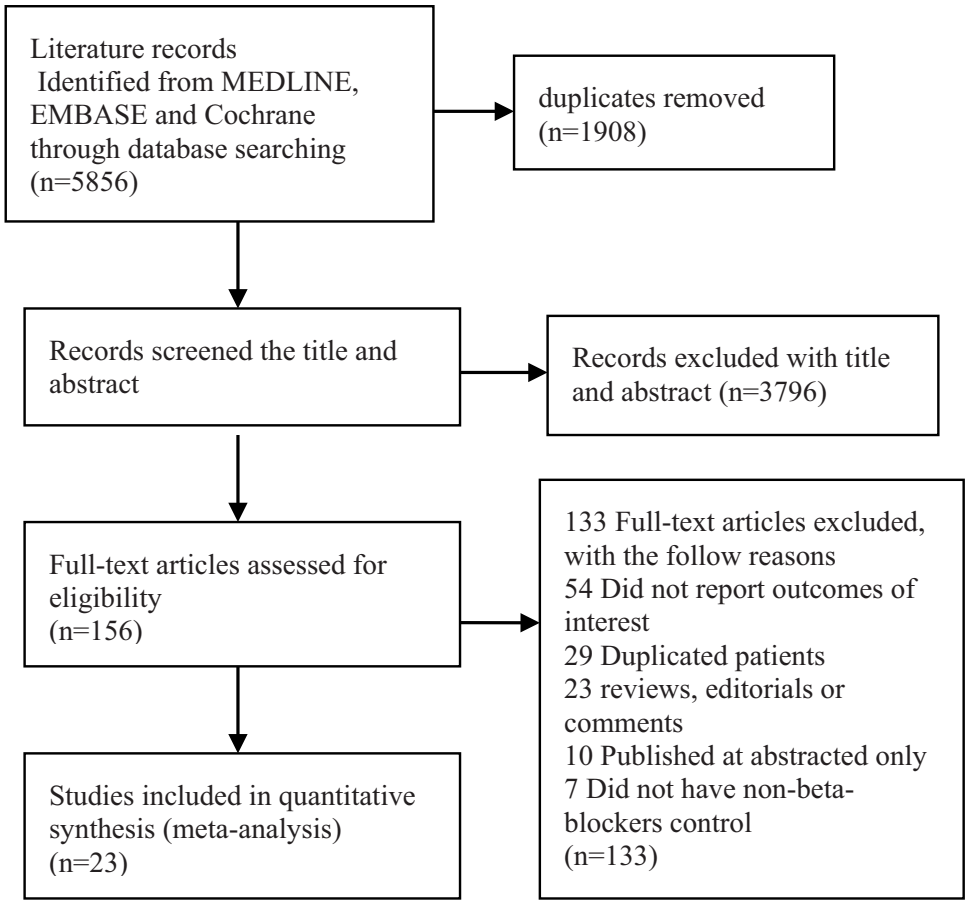

Fig. 1 Study flow diagram in this meta-analysis

The quality of each included study, assessed by the Newcastle–Ottawa Scale tool, was good. The NOSs was displayed in Table S1 (median score, 7.1; range from 6 to 9).

Quantitative data synthesis

The mortality RR was estimated using a random-effect model meta-analysis, and heterogeneity was evaluated by I^2. Meta-analysis of 23 studies showed that DM did slightly increase sepsis overall mortality (RR, 1.12; 95% CI 1.00–1.25; I^2 96.1%; $p = 0.000$) according to the random effects model, however with large heterogeneity. Subgroup analysis did not demonstrate a statistically significant increase either in 30-day mortality (RR, 1.07; 95% CI 0.97–1.18; I^2 0.0%; $p = 0.963$), 90-day mortality (RR, 1.00; 95% CI 0.95–1.07; I^2 0.0%; $p = 0.735$), or mixed-day mortality (RR, 1.16; 95% CI 0.98–1.37; I^2 97.9%; $p = 0.000$).

Inter-study variability

The pooled relative risk of DM related overall mortality in patients with sepsis was 1.12 (95% CI 1.00–1.25; $I^2 = 96.1\%$; $p = 0.000$). In subgroup analysis, no evidence of heterogeneity was observed in the analysis of 30-day mortality group ($I^2 = 0.0\%$; $p = 0.963$) and 90-day mortality group ($I^2 = 0.0\%$; $p = 0.735$), but a high degree of heterogeneity was observed among mixed-day mortality subgroup ($I^2 = 97.9\%$; $p = 0.000$) and among all the included studies (Fig. 2).

The leave-one-out sensitivity analyses by removing one study per time were used to test the replicability of the results. Two studies [14, 17] were identified as the source of heterogeneity (Fig. 3), and after the exclusion of these two studies, de Miguel-Yanes' [17] ($I^2 = 96.7\%$; $p = 0.000$) or Shah's study [14] ($I^2 = 96.0\%$; $p = 0.000$), only a little heterogeneity was removed in the mixed-day mortality subgroup. The omission of de Miguel-Yanes' [17] or Shah's study [14] seems to be not drastically changed in this analysis, and the RRs were in the range from 1.13 (95% CI 0.99–1.29) to 1.08 (95% CI 0.98–1.18). All the results were of marginal significance (Fig. 4).

Publication bias

We used Egger's regression asymmetry test to access the publication bias of included literatures, and no evidence of publication bias could be found ($t = 1.64$, $p = 0.113$) (Fig. 4).

Table 1 Characteristics of clinical studies investigating the association between diabetes and mortality for sepsis included in the meta-analysis

Authors	Year	Country	Study design	Participant	Mortality diabetes vs. no diabetes
Zohar [18]	2021	Israel	Retrospective cohort	Patients with community-onset sepsis 1527 (DM: 469; non-DM: 10,58)	Mort1ality (in-hospital): RR 1.21 (0.80–1.71) Mortality at 30 days: RR 1.10 (0.79–1.54) Mortality at 90 days: RR 1.13 (0.86–1.49)
Akinosoglou [19]	2021	Greece	Retrospective cohort study	The Hellenic Sepsis Study Group Registry non-ICU patients 812 (406 in each of the DM and non-DM groups)	Post-discharge: RR 1.04 (0.75–1.44)
Lin et al. [13]	2021	China	Retrospective analysis data	China mean age of 66.7 years; 51% males; Majority with bloodstream (44%) and urinary tract infection (21%) 5774 (2887 in each of the DM and non-DM groups)	Mortality (in-hospital): RR 0.73 (0.62– 0.87) Mortality at 28 days post-discharge: RR 0.86 (0.77–0.97)
Kushimoto et al. [20]	2020	Japan	Retrospective analysis of prospectively collective data	Mean age of 73 years; 60% males; majority with pulmonary (31%) and gastrointestinal infection (26%); 63% with septic shock 1127 (DM: 261; non-DM: 866)	In-hospital mortality: RR 1.32 (0.96– 1.81)
Russell [21]	2019	Canada		UK Biobank Cohort 1 Single Centre Cohort 2 Inflammation Mechanism Cohort 3 Lipid Mechanism Cohort 4 484,857 (DM: 25,430; non-DM: 459,407) 727 (DM: 37; non-DM: 690) 779 (DM: 165; non-DM: 614) 200 (DM: 9; non-DM:15)	Mortality at 28-days OR 1.18 (0.91– 1.54, $p=0.21$) Mortality at 28-days OR 1.35 (0.63–2.92, $p=0.452$) Mortality at 28-days OR 1.09 (0.76–1.55, $p=0.651$) Mortality at 90-days OR 1.59 (0.70–3.65, $p=0.28$)
Sathananthan [22]	2019	USA	Analysis of retrospective cohort data	Majority with age > 60 years; > 55% males; majority with severe sepsis (> 80%); 1698 (DM: 508; non-DM: 1190)	Mortality at 30 days *p*: RR 1.00 (0.81– 1.25)
Zoppini [5]	2018	Italy	Retrospective cohort study on a regional electronic archive	185,341 diabetic individuals	Increased risk of death from infection-related causes in diabetic people (especially in female and people aged between 30 and 64 years): RR for septicemia 1.91 (1.76–2.06)
Van Vught [16]	2017	Netherlands	Retrospective large national database review	41,492 ICU septic patients (8085 with diabetes)	Mortality (in-hospital): RR 1.14 (1.07–1.21) Mortality at 90-days post-discharge: RR 1.09 (0.72–1.66)
Van Vught [23]	2016	Netherlands	Prospective observational study	1104 ICU septic patients (241 with diabetes)	No association between diabetes and 90-day mortality: HR 0.90 (0.69–1.15) after correction for BMI, age, gender, hypertension, cardiovascular, and renal insufficiency HR 1.02 (0.81–1.29) after correction for APACHE IV score

Table 1 (continued)

Authors	Year	Country	Study design	Participant	Mortality diabetes vs. no diabetes
Venot [24]	2015	France	Case–control study based on a multicenter database	10,911 patients (3728 with severe sepsis or septic shock; among them, 451 with diabetes)	No difference in mortality between diabetic and non-diabetic septic patients (19.8% vs. 15% in the matched case–control analysis; $p=0.08$) Mortality (in-hospital): RR 1.32 (91.00–1.74)
De Miguel-Yanes [8]	2015	Spain	Retrospective cohort study	Mean age of 72 years; > 55% males; majority one or more organ failure 217,280 All sepsis (DM: 50,611; non-DM: 166,669)	In-hospital mortality 0.97 (0.96–0.98)
Schuetz [11]	2012	USA	Prospective cohorts study	Mean age of 60 years; around 48% were females; majority with pneumonia (22%) or skin/soft tissue infection (27%) or urinary tract infections (11%) 1849 (DM: 539; non-DM: 1310)	In-hospital mortality 0.95 (0.48–1.90)
Chang C et al. [25]	2012	Taiwan	Nationwide population-based retrospective cohort study	Mean age of 67 years; > 50% males; majority with pneumonia (43%) or gastrointestinal infection (34%) or urinary tract infections (26%); majority with severe sepsis/septic shock 16,497 (DM: 4573; non-DM: 11,924)	90-day mortality (in-hospital): RR 1.00 (0.94–1.07)
Schuetz [9]	2011	USA	Prospective cohorts study	Patients admitted to the hospital from the ED with suspected infection; mean age of 59 years; around 49% were males; around one-third (37%) had severe sepsis/septic shock 7754 (DM: 1844; non-DM: 5910)	0.85 (0.71–1.01)
Yang et al. [10]	2011	Singapore	Retrospective large database review	Mean age of 60 years; around 50% were males; majority with respiratory, urinary tract or gastrointestinal infections 9221 (DM: 2943; non-DM: 6278)	Mortality (in-hospital): RR 0.96 (0.88–1.05)
Stegenga [26]	2010	Multicentric study	Retrospective analysis of a previously published RCT	ICU patients with septic shock (188 with diabetes) 830 (DM: 188; non-DM: 642)	Mortality at 28 days: RR 1.03 (0.81–1.31) Mortality at 90 days: RR 1.00 (0.71–1.41)
Vincent [27]	2010	Belgium	Prospective study	3147 ICU septic patients (226 with insulin-treated diabetes) 3147	No difference in ICU and hospital mortality between diabetic and non-diabetic septic patients
Chen Y [28]	2009			121 Severe sepsis and septic shock, 34 with diabetes	Hospital mortality 0.97 (0.59–1.59)
Esper [14]	2009		Retrospective large national registry review	12,500,459 septic patients (2,070,459 with diabetes)	Lower hospital mortality in diabetic vs. non-diabetic patients (18.5% vs. 20.6%, $p<0.05$) Mortality 0.90 (0.81–1.0)

Table 1 (continued)

Authors	Year	Country	Study design	Participant	Mortality diabetes vs. no diabetes
Moutzouri [29]	2008	Greece	A prospective cohort study	64 severe sepsis or septic shock; mean age of around 60 years; around 50% were females; majority with urinary tract infections; most had severe sepsis/septic shock 64 (DM: 24; non-DM: 40)	In-hospital mortality1.30 (0.56–3.03)
Shah [12]	2003	Canada	Retrospective cohort study on population-based administrative data	513,749 diabetic individuals (matched to an equal number of non-diabetics)	Higher global infection-related mortality in diabetic patients (including home and hospital) risk ratio up to 1.92 (1.79–2.05) No significant difference in term of infection-related hospital mortality risk ratio up to 0.94 (0.87–1.01)
Bertoniet [30]	2001	USA	Retrospective cohort study on a national registry	9208 individuals (533 with diabetes)	Higher infection-related mortality in diabetic patients with cardiovascular disease RR 3.0 (1.8–5.0)
Moss [31]	2000	USA	A prospective cohort study	113 septic shock	0.67 (0.36–1.23)

Discussion

DM is the main comorbidity of sepsis because of its high prevalence; about 10–30% of septic patients have diabetes. However, the effect of diabetes on outcome of sepsis is not completely clear. There are two meta-analyses about this topic: one showed that the mortality rate of septic patients with DM was slightly lower than that of non-diabetic patients [30]; the other (included four loosely defined sepsis studies) demonstrated that there were no significant differences in the risk of mortality [6]. In a recent meta-analysis, it was reported that DM was associated with mortality, severe COVID-19, ARDS, and disease progression in patients with COVID-19 [30].

In these 23 included studies, the results by Zoppini et al. [7] and Bertoni [18] found increased mortality rate related to sepsis in diabetic compared to the general population, whereas others [9–13, 16, 19–29] failed to demonstrate such association, and four studies [8, 14, 17, 31] reported decreased mortality rates among DM patients during sepsis. The following factors have been proposed to explain this heterogeneity in mortality: different study populations (including different the duration, severity of diabetes, lack of stratification into type 1 and type 2 diabetes, different adjustments for comorbidities, sepsis etiology, stages, and severity) [32], anti-diabetic medication to control blood glucose, degree of glycemic control of during hospital, medical treatment, and nursing. The main finding of our meta-analysis is that pre-existing DM slightly significantly increased overall mortality in sepsis patients, but not 30-day mortality, 90-day, or mixed-day mortality in sepsis patients. From this meta-analysis, it is certain that presence of DM is not associated with reduced risk of mortality in sepsis patients.

To clarify the risk of DM in sepsis mortality, we need to clarify blood glucose level control and the risk of sepsis mortality. As an important cellular energy, blood glucose must be controlled at a specific level and kept relatively stable. Whether it is low or high, it is not conducive to cell survival. It has been demonstrated that hyperglycemia, irrespective of the DM status, is a major independent risk factor for in-hospital sepsis mortality [33], while hypoglycemia is associated with an increased risk of mortality too [15]. Dose–response analysis showed that the effect of blood glucose on mortality may differ in patients with DM versus without [34]. Critically ill patients undergoing intensive glucose control showed significantly reduced all-cause mortality, length of ICU stay, and incidence of acquired infection and sepsis compared to the same parameters in patients treated with the usual care strategy, while the intensive glucose control strategy was associated with higher occurrence of severe hypoglycemic events [35]. Septic patients with higher acute glycemic variability had significantly increased mortality risk compared to those with lower acute glycemic variability; higher acute glycemic variability may

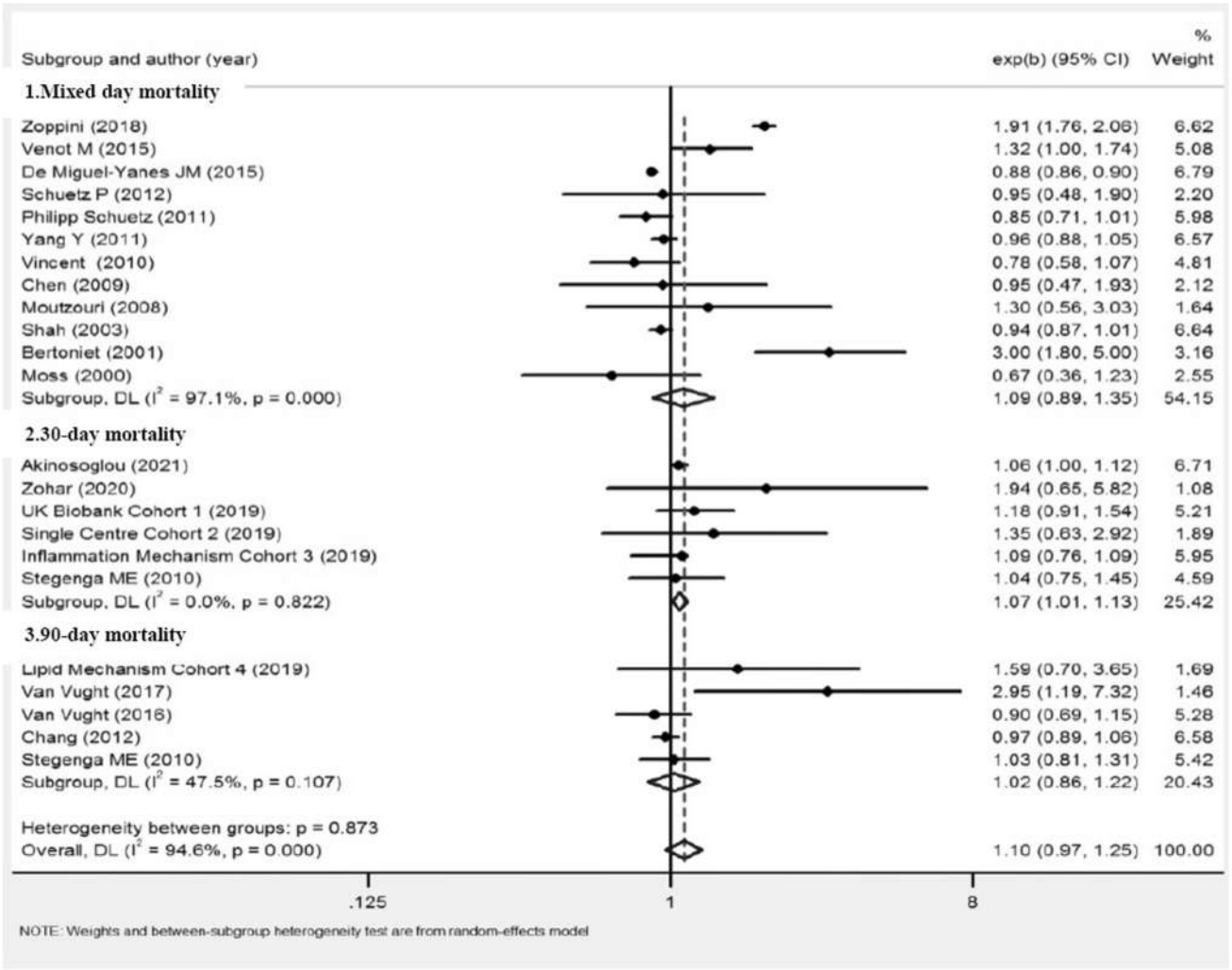

Fig. 2 Meta-analysis of the overall pooled odds ratios (ORs) of studies investigating the mortality of patients with diabetes mellitus in sepsis. Forest plot showing lightly increased risk of sepsis-related overall mortality according to the random effects model

be a predictor of mortality risk in patients with sepsis [36]. From these studies, we can draw conclusion that DM should impair the outcome of patients with sepsis; at least, it will not improve the prognosis of sepsis.

In this meta-analysis, the included studies showed a low-risk publication bias. Therefore, the heterogeneity was not considered statistically. The heterogeneity may be derived from methodological and clinical causes, such as the sample sizes, ethnically diverse, anti-diabetic medications, different DM type, different glucose control level, different adjustments for comorbidities, sepsis etiology, and disease severity. The relation between DM and risk mortality is weak across all three subgroups. Due to the weak nature of the association between DM and mortality, drawing conclusions about the practical significance of this relationship should be treated with caution.

This meta-analysis has several strengths. First, the risk publication bias assessment by using Egger's test showed a low risk of bias among the included studies. All the studies fulfilled the diagnostic criterion proposed by sepsis, and most of the included studies were of high NOS score, which demonstrated the relatively high quality of the included studies. Second, we pooled data for the primary outcome by the random effects model, which allows for more accurate representation of data that arise from complicated multilevel study designs. Finally, the outcome of the sensitivity analysis showed that this result slightly varies.

Study limitations

There are also several limitations in our study which are similar to other meta-analysis. First, there is a marked heterogeneity noted in study design, size, duration, the mean ages, severity, and DM type of the patients among the included studies.

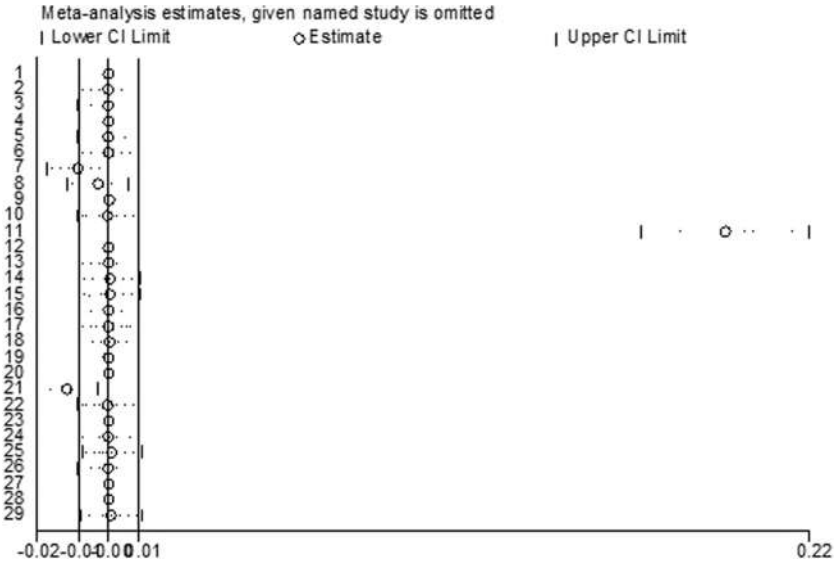

Fig. 3 Sensitivity analysis of the meta-analysis of the association between diabetes mellitus and mortality risk in sepsis patients. The meta-analysis is dominated by De Miguel-Yanes' study and Shah's study

Furthermore, most used a retrospective design, and the effect estimate was adjusted for different level confounders. For example, the diagnosis of diabetes in most of the studies depended on the medical history record and did not provide severity, duration, and anti-diabetic medication of diabetics. These heterogeneity might have an effect on the outcome. Second, our analysis only includes the articles published in full text and in English, so the publication bias is unavoidable. Finally, all these limitations of the available data make it hard to reach definitive conclusions of the effect of DM on mortality of sepsis.

Conclusions

Despite diabetes does not increase risk of 28-day mortality or 90-day mortality, it slightly does increase risk of sepsis-related overall mortality. Diabetes is not associated with beneficial survival outcomes in patients with sepsis. Considering the limitations of the meta-analysis, more high-quality original designed studies are required to confirm the association. Future research should aim to gain a deeper understanding of the relationship between DM and mortality using more reliable measures and accurate prospective research to elicit the truth.

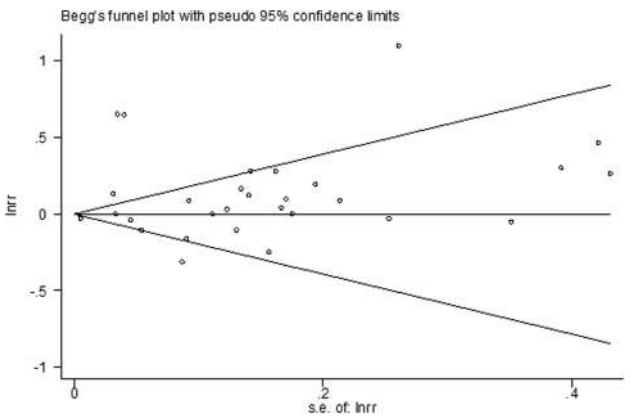

Fig. 4 Egger's funnel plots test with pseudo 95% confidence limits for studies reporting diabetes mellitus and mortality in sepsis patients. There is no evidence of bias in the test or the formal plot ($t=1.64$, $p=0.113$)

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01225-0.

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Data Availability The data that support the findings of this study are openly available within the article or its supplementary materials.

Declaration

Conflict of interest The authors declare no conflict of interest related to this work.

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ORIGINAL ARTICLE

Insulin glargine use and cancer risk among patients with type 2 diabetes mellitus: a real-world study in Shanghai, China

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Abstract

Objective The association of insulin glargine with overall and site-specific cancer risk remains uncertain. This study aims to provide relevant evidence from mainland China.

Methods Based on the Shanghai Link Healthcare Database, patients newly treated with insulin glargine or neutral protamine Hagedorn (NPH) insulin between January 1, 2013 and December 31, 2020 were identified and followed up until December 31, 2021. In addition to the original cohort, we created a 1:1 propensity score-matched cohort with balanced baseline characteristics. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for incident cancers, comparing insulin glargine with NPH insulin.

Results A total of 26,810 insulin glargine users and 47,765 NPH insulin users were enrolled in the final analysis. During a median follow-up of 4.18 years, 3903 patients developed cancer. Including a 1-year lag period, insulin glargine use was not associated with increased cancer risk compared to NPH insulin use in both the original cohort (HR 1.02, 95% CI 0.95–1.10) and the propensity score-matched cohort (HR 1.01, 95% CI 0.94–1.09). In stratified analyses by sex, age, and cancer site, as well as in sensitivity analyses at different lag periods, the estimated cancer risk, although fluctuating, remained uncorrelated without any substantial change.

Conclusion Insulin glargine is not associated with the risk of overall and site-specific cancers compared to NPH insulin among patients with type 2 diabetes mellitus.

Keywords Insulin glargine · NPH insulin · Cancer · Type 2 diabetes mellitus · Real-world study

Introduction

Diabetes is widely recognized to be associated with an increased risk of some site-specific cancers [1]. Our previous real-world study, which included 410,191 Chinese patients with type 2 diabetes mellitus (T2DM), also observed a substantially higher risk for 11 cancer sites in males and 13 cancer sites in females [2]. However, the exact mechanism of this association is not fully understood. A growing body of evidence suggests that antidiabetic drugs may also have an essential impact on cancer risk.

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Insulin glargine, a long-acting insulin analog, is commonly used in the treatment of diabetes. Four observational studies on the association between insulin glargine and cancer risk were published simultaneously in 2009, some of which showed a positive association, raising concerns about its potential carcinogenic effects [3–6]. Since then, this topic has been intensively studied, but inconsistent results have been obtained, with some studies reporting an increased risk, some studies indicating no association, and one study observing a decreased risk [7–28]. Notably, most studies have been criticized for methodological flaws, including the small number of cancer cases, the insufficient duration of follow-up, the inclusion of prevalent users, and time-related biases [29, 30]. Some studies even used inappropriate comparators, such as non-use [15] and other insulin [4–8, 11, 16, 22]. If the duration and severity of the disease are not adequately considered and adjusted, such a comparison design may lead to indication bias, thus limiting the interpretation of its findings. At present, except for one study from Taiwan, China [9], all other relevant studies are from Western countries [3–8, 10–28], with gaps still existing in mainland China. Therefore, we conducted this study to investigate whether insulin glargine affects the risk of overall and site-specific cancers compared to neutral protamine Hagedorn (NPH) insulin among patients with T2DM in Shanghai, China.

Methods

Data source

The data for this study were gathered from the Shanghai Link Healthcare Database (SLHD), which is developed and operated by an administrative agency of the Shanghai Municipal People's Government—the Shanghai Hospital Development Center. In China, hospitals are divided into three tiers, of which tertiary hospitals are the best and have stronger capabilities in medical care, medical education, and medical research. Therefore, most patients will go directly to the best tertiary hospitals when they feel unwell. The SLHD contains general medical practice data from 35 tertiary hospitals, covering more than 99% of Shanghai residents. After review and approval, researchers can access the encrypted data for academic research (since 2013).

This study was approved by the Ethics Committee of Ruijin Hospital (2020-226Y). Diseases were identified according to the *International Classification of Diseases (10th Revision)* and related diagnoses. The common cancers evaluated in this study were cancers of the stomach (C16), colorectum (C18–C21), pancreas (C25), lung (C33–C34), breast (C50), prostate (C61), kidney (C64–C66, C68), and thyroid (C73).

Study population

Figure 1 shows the selection process of the study cohorts. First, we identified a base cohort that included all patients aged 20–99 years with at least one prescription of insulin glargine or NPH insulin between January 1, 2013 and December 31, 2020. Cohort entry was defined as the date of the first prescription of insulin glargine or NPH insulin. To establish a cohort of new insulin users, patients without any medical record of T2DM or prescription of other antidiabetic drugs before cohort entry were excluded. We then excluded patients initiating treatment with insulin glargine and NPH insulin on the same date and patients with a history of cancer, secondary or unspecified cancer (only the first primary cancer was considered). All patients were followed up until the diagnosis of cancer, switch of exposure group, death, or December 31, 2021, whichever came first. To reduce protopathic and detection bias, we included a 1-year lag period to exclude patients with less than 1 year of follow-up [31, 32].

Results were adjusted for known or suspected risk factors to avoid biased estimates, including sex, age, hypertension, dyslipidemia, diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, ischemic heart disease, peripheral vascular disease, cerebrovascular diseases, chronic lung disease, metformin, sulfonylurea, thiazolidinedione, other insulin, other antidiabetic drugs, statin, aspirin, and non-steroidal anti-inflammatory drug (NSAID). Comorbidities and medications were assessed using relevant medical records before cohort entry.

To reduce potential confounding effects and treatment selection bias, we created a 1:1 propensity score (PS)-matched cohort with balanced baseline characteristics from the original cohort. PS is the conditional probability of having a particular exposure (insulin glargine or NPH insulin), derived from the baseline characteristics shown in Table 1 through multivariable logistic regression. Propensity score matching was conducted to select a 1:1 PS-matched cohort of insulin glargine users and NPH insulin users, using a caliper of 0.20 standard deviation of the logit of the estimated PS. Standardized mean differences (SMDs) were calculated for all baseline characteristics between the two groups, and values less than 10% may indicate relative balance [33].

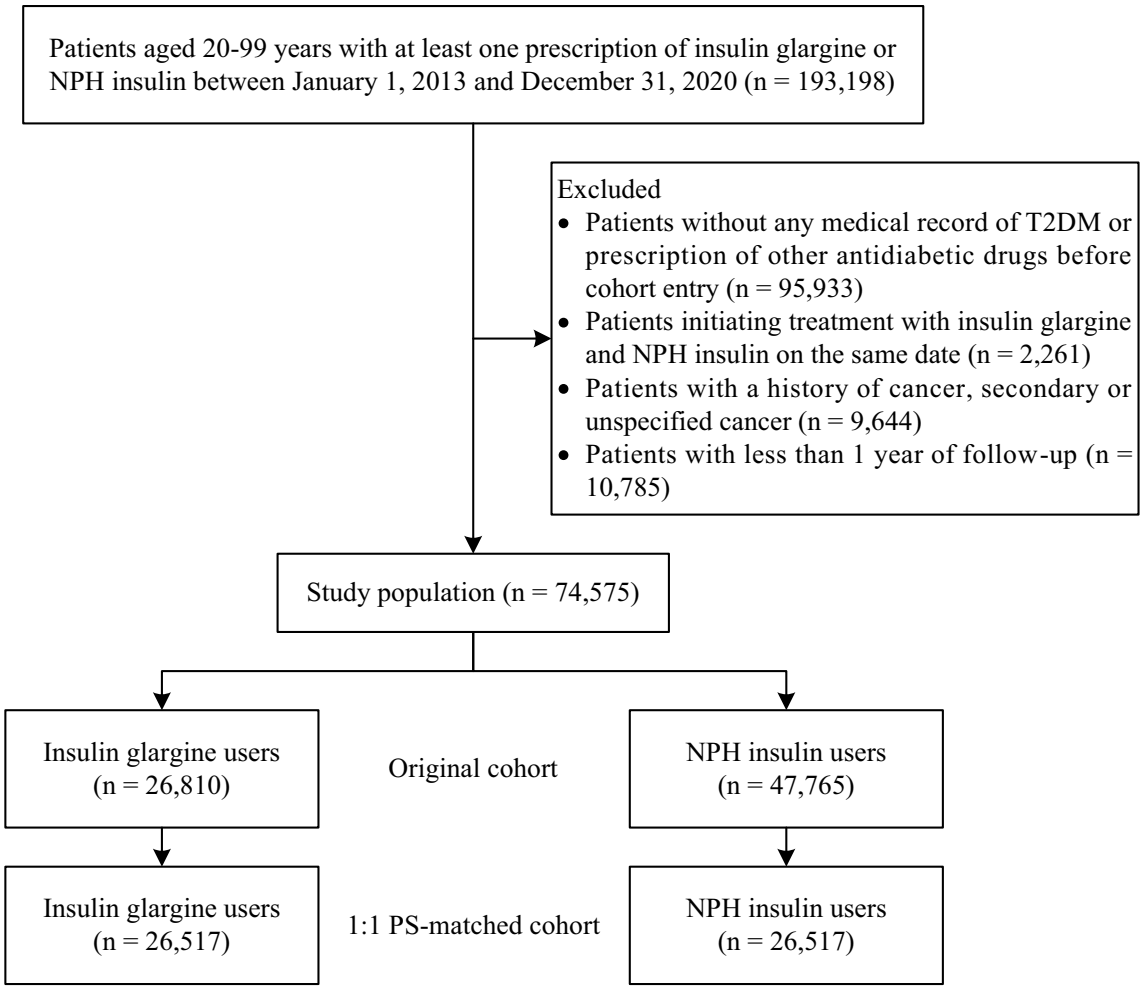

Fig. 1 Flowchart showing the selection process of the study cohorts. Note: Cohort entry was defined as the date of the first prescription of insulin glargine or NPH insulin. Abbreviations: NPH insulin, neutral protamine Hagedorn insulin; T2DM, type 2 diabetes mellitus; PS, propensity score

Statistical analysis

Baseline characteristics between insulin glargine users and NPH insulin users were compared using the Student's *t* test for age and the chi-square test for other variables. Baseline characteristics of patients are presented as means with standard deviations (SDs) for age and frequencies with percentages for other variables. The crude incidence rate of cancer was calculated by dividing the number of cancer cases by the number of person-years. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for incident cancers, comparing insulin glargine with NPH insulin. Stratified analyses were conducted according to sex (male or female), age (< 50 years, 50–64 years, or ≥ 65 years), and cancer site (stomach, colorectum, pancreas, lung, kidney, thyroid, breast, or prostate). Sensitivity analyses were carried out by setting different lag periods: 1-month lag period, 6-month lag period, 2-year lag period, or 3-year lag period. Statistical analyses were performed using R language software, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Table 1 presents the baseline characteristics of insulin glargine users and NPH insulin users in the original cohort and the PS-matched cohort. A total of 74,575 patients with T2DM were enrolled in the final analysis, including 26,810 insulin glargine users and 47,765 NPH insulin users. Among

Table 1 Baseline characteristics of insulin glargine users and NPH insulin users

	Original cohort			PS-matched cohort		
	Glargine ($n=26,810$)	NPH ($n=47,765$)	SMD	Glargine ($n=26,517$)	NPH ($n=26,517$)	SMD (p)
Male, *N* (%)	14,771 (55.10)	25,392 (53.16)	0.039	14,546 (54.86)	14,551 (54.87)	< 0.001
Age, mean (SD)	62.54 (13.41)	64.20 (13.01)	0.126	62.66 (13.38)	62.72 (13.06)	0.005
Age group						
< 50	4105 (15.31)	5525 (11.57)		3982 (15.02)	3661 (13.81)	
50–64	10,694 (39.89)	18,824 (39.41)		10,561 (39.83)	11,058 (41.70)	
≥ 65	12,011 (44.80)	23,416 (49.02)		11,974 (45.16)	11,798 (44.49)	
Comorbidities, *N* (%)						
Hypertension	10,770 (40.17)	20,552 (43.03)	0.058	10,699 (40.35)	10,693 (40.33)	< 0.001
Dyslipidemia	4144 (15.46)	6008 (12.58)	0.083	4010 (15.12)	4007 (15.11)	< 0.001
Diabetic nephropathy	1676 (6.25)	3580 (7.50)	0.049	1671 (6.30)	1672 (6.31)	< 0.001
Diabetic retinopathy	1716 (6.40)	3034 (6.35)	0.002	1694 (6.39)	1643 (6.20)	0.008
Diabetic neuropathy	1839 (6.86)	2559 (5.36)	0.063	1772 (6.68)	1829 (6.90)	0.009
Ischemic heart disease	5104 (19.04)	11,369 (23.80)	0.116	5103 (19.24)	5125 (19.33)	0.002
Peripheral vascular isease	738 (2.75)	1292 (2.70)	0.003	724 (2.73)	701 (2.64)	0.005
Cerebrovascular diseases	4807 (17.93)	8682 (18.18)	0.006	4773 (18.00)	4837 (18.24)	0.006
Chronic lung disease	2673 (9.97)	4344 (9.09)	0.030	2634 (9.93)	2580 (9.73)	0.007
Medications, *N* (%)						
Metformin	15,328 (57.17)	22,050 (46.16)	0.222	15,035 (56.70)	14,943 (56.35)	0.007
Sulfonylurea	12,290 (45.84)	16,792 (35.16)	0.219	12,005 (45.27)	11,977 (45.17)	0.002
Thiazolidinedione	4521 (16.86)	6348 (13.29)	0.100	4396 (16.58)	4395 (16.57)	< 0.001
Other insulin	13,628 (50.83)	24,130 (50.52)	0.006	13,478 (50.83)	13,829 (52.15)	0.026
Other antidiabetic drugs	19,262 (71.85)	29,207 (61.15)	0.228	18,972 (71.55)	19,109 (72.06)	0.011
Statin	11,932 (44.51)	21,919 (45.89)	0.028	11,826 (44.60)	11,804 (44.51)	0.002
Aspirin	9078 (33.86)	17,278 (36.17)	0.048	9025 (34.03)	9200 (34.69)	0.014
NSAID	8495 (31.69)	14,932 (31.26)	0.009	8403 (31.69)	8454 (31.88)	0.004

PS, propensity score; *NPH insulin*, neutral protamine Hagedorn insulin; *SMD*, standardized mean difference; *N*, number; *SD*, standard deviation; *NSAID*, non-steroidal anti-inflammatory drug

insulin glargine users and NPH insulin users, the percentage of males was 55.10% and 53.16%, and the mean age was 62.54 years (SD 13.41 years) and 64.20 years (SD 13.01 years), respectively. After propensity score matching, 26,517 pairs of insulin glargine users and NPH insulin users were identified, with all baseline characteristics well balanced (SMD much less than 10%).

During a median follow-up of 4.18 years (interquartile range 2.53–6.49 years), 3903 patients developed cancer, with a crude incidence rate of 1150.26 per 100,000 person-years. Table 2 shows the HRs (95% CIs) for overall and site-specific cancers. The results were consistent across the original cohort and the PS-matched cohort. Including a 1-year lag period, insulin glargine use was not associated with increased cancer risk compared to NPH insulin use in both the original cohort (1109.36 versus 1169.64 per 100,000 person-years, HR 1.02, 95% CI 0.95–1.10) and the PS-matched cohort (1117.99 versus 1149.04 per 100,000 person-years, HR 1.01, 95% CI 0.94–1.09). In stratified analyses by sex and cancer site, none of the HR values were statistically significant, although they fluctuated between 0.69 and 1.31. The results were also robust in stratified analyses by sex and age, as well as in sensitivity analyses at different lag periods (Supplementary Table 1 and Table 2).

Discussion

This is the first real-world study from mainland China to investigate the effect of insulin glargine on cancer risk. We found that insulin glargine is not associated with the risk of overall and site-specific cancers compared to NPH insulin among patients with T2DM. In stratified analyses by sex, age, and cancer site, as well as in sensitivity analyses at different lag periods, the estimated cancer risk, although fluctuating, remained uncorrelated without any substantial change.

Our findings are consistent with most [5–9, 13–20, 24–27], but not all, of the observational studies. Some

Table 2 HRs (95% CIs) for overall and site-specific cancers, comparing insulin glargine with NPH insulin

	Original cohort					PS-matched cohort				
	Glargine		NPH		Adjusted HR (95% CI)	Glargine		NPH		HR (95% CI)
	Events	Incidence rate[a]	Events	Incidence rate[a]		Events	Incidence rate[a]	Events	Incidence rate[a]	
Any sites										
Total	1210	1109.36	2693	1169.64	1.02 (0.95–1.10)	1206	1117.99	1447	1149.04	1.01 (0.94–1.09)
Male	666	1124.98	1488	1246.05	0.99 (0.91–1.09)	661	1138.64	811	1196.50	0.99 (0.89–1.10)
Female	544	1090.81	1205	1087.31	1.07 (0.96–1.18)	541	1088.12	634	1090.23	1.04 (0.93–1.17)
Stomach										
Total	79	71.04	221	93.91	0.81 (0.62–1.05)	79	71.82	121	94.06	0.79 (0.59–1.05)
Male	52	86.25	130	106.60	0.86 (0.62–1.20)	52	87.94	69	99.72	0.92 (0.64–1.31)
Female	27	53.03	91	80.25	0.73 (0.47–1.13)	27	53.20	48	80.68	0.69 (0.43–1.11)
Colorectum										
Total	170	153.17	347	147.65	1.14 (0.94–1.37)	169	153.93	186	144.76	1.10 (0.89–1.35)
Male	98	162.91	200	164.25	1.11 (0.87–1.43)	97	164.39	113	163.58	1.06 (0.80–1.39)
Female	72	141.64	147	129.80	1.17 (0.87–1.56)	72	142.08	84	141.40	1.02 (0.74–1.39)
Pancreas										
Total	66	59.31	132	56.03	1.14 (0.84–1.54)	66	59.96	74	57.46	1.05 (0.75–1.47)
Male	39	64.65	70	57.33	1.22 (0.81–1.81)	39	65.91	37	53.42	1.27 (0.81–1.99)
Female	27	52.99	62	54.63	1.05 (0.66–1.67)	27	53.16	37	62.15	0.86 (0.52–1.42)
Lung										
Total	224	201.96	527	224.39	0.96 (0.82–1.13)	223	203.26	291	226.68	0.93 (0.78–1.11)
Male	131	217.82	302	248.00	0.95 (0.77–1.17)	130	220.38	176	254.83	0.90 (0.72–1.13)
Female	93	183.16	225	198.97	0.98 (0.77–1.26)	91	179.77	122	205.70	0.91 (0.70–1.20)
Kidney										
Total	46	41.35	88	37.37	1.12 (0.78–1.61)	46	41.80	52	40.39	1.07 (0.72–1.59)
Male	34	56.38	56	45.89	1.23 (0.80–1.91)	34	57.48	31	44.78	1.31 (0.81–2.14)
Female	12	23.55	32	28.20	0.86 (0.44–1.70)	12	23.63	18	30.24	0.81 (0.39–1.70)
Thyroid										
Total	79	71.07	144	61.20	1.08 (0.81–1.43)	79	71.85	85	66.09	1.10 (0.81–1.50)
Male	23	38.13	47	38.51	0.85 (0.51–1.41)	23	38.88	30	43.33	0.90 (0.52–1.55)
Female	56	110.16	97	85.65	1.20 (0.86–1.68)	56	110.51	59	99.32	1.13 (0.78–1.63)
Breast	113	222.76	235	208.17	1.09 (0.86–1.37)	113	223.46	114	192.40	1.20 (0.92–1.55)
Prostate	96	159.53	191	156.84	1.17 (0.91–1.51)	96	162.65	100	144.71	1.16 (0.88–1.54)

[a]Per 100,000 person-years

PS, propensity score; *NPH insulin*, neutral protamine Hagedorn insulin; *HR*, hazard ratio; *CI*, confidence interval

studies have reported that insulin glargine use is associated with an increased risk of overall cancer [3, 10, 28] and breast cancer (the most studied cancer) [4, 11, 12, 21–23]. A study from the Netherlands even observed a decreased risk of overall and colon cancers, no difference in the risk of bladder, respiratory tract, and prostate cancers, but an increased risk of breast cancer among insulin glargine users [12]. However, the authors suggested that these associations may be attributable to residual confounding, lack of adherence, or competing risks. Although increased or decreased risk for site-specific cancers can be observed under specific analytical conditions, most previous studies have concluded that insulin glargine is not associated with cancer risk [5–9, 13–20, 24–27]. Furthermore, all randomized controlled trials, including the most influential ORIGIN study, also showed no effect of insulin glargine on cancer risk [34–37]. Based on the results of previous studies and our study, it appears that insulin glargine does not alter cancer risk among patients with type 2 diabetes.

T2DM is an extremely complex chronic metabolic disease, often accompanied by multiple comorbidities and medications, making it challenging to analyze the specific role of a particular factor. In fact, there is no direct, let alone definitive, biological evidence linking insulin glargine to cancer risk. Among the potential mechanisms, hyperinsulinemia is the most prominent and controversial. Hyperinsulinemia is a recognized risk factor for cancer, but whether insulin glargine exacerbates this cancer-promoting effect remains debatable [38]. Even if exogenous hyperinsulinemia has a cancer-promoting effect, it may be exaggerated by the tissue's prior long-term exposure to endogenous hyperinsulinemia [39]. Most importantly, the higher receptor affinity and mitogenic potency of insulin glargine observed in vitro may not reflect the situation in vivo, as after injection, insulin glargine is metabolized to various derivatives with different mitogenic activities [38, 40, 41]. An in vivo study demonstrated that neither insulin glargine nor non-metabolizable insulin glargine promoted cancer cell growth in a mouse model of T2DM [42]. Therefore, the effect of insulin glargine on cancer risk observed under specific conditions requires further study.

Due to the low incidence rate and long latency period of cancer, even if insulin glargine has a carcinogenic effect, it may only be observed in studies with large population sizes and long follow-up periods. The SLHD is the largest medical database in mainland China. Therefore, we were able to conduct this large-scale population-based study in which a sufficient number of cancer cases were observed during long-term follow-up. The statistical efficacy of studying overall and site-specific cancers was greatly improved. In addition, we used a new-user design combined with an active-comparator design (i.e., new insulin glargine users vs. new NPH insulin users) to minimize critical biases in observational studies, such as confounding by indication and time-related biases [43]. To assess the robustness of our results, we established the original cohort and the PS-matched cohort with balanced baseline characteristics, and the results were always consistent. Consequently, this study could provide a more credible assessment of the association between insulin glargine and cancer risk, contributing to the accumulation of relevant evidence from mainland China.

This study has several limitations. First, the database lacks complete information on the cumulative duration and dose of insulin, which may affect the further interpretation of our results. Second, as with all observational studies, this study was subject to unmeasured and residual confounding. However, we minimized confounding by selecting cohorts with similar indications and disease stages through a combined study design of new-user and active-comparator, as well as propensity score matching. Finally, the induction period of many carcinogens may last for years or even decades. Although the follow-up period in this study exceeded 5 years, studies with longer insulin exposure and follow-up are still needed to draw firm conclusions.

Conclusion

This study suggested that insulin glargine is not associated with the risk of overall and site-specific cancers compared to NPH insulin among patients with T2DM. As insulin glargine is widely used in clinical practice, further studies with longer follow-up periods are needed to confirm this null association.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01230-3.

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Author contribution Jiying Qi: formal analysis, investigation, writing—original draft. Ping He: data curation, resources. Huayan Yao: data curation, software. Wen Sun: data curation, software. Zizheng Zhang: formal analysis. Bin Cui: conceptualization, funding acquisition, methodology, writing—original draft, writing—review and editing. Guang Ning: conceptualization, project administration, supervision, writing—review and editing.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was approved by the Ethics Committee of Ruijin Hospital (2020-226Y).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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SHORT ARTICLE

A heterozygous mutation at promoter region of insulin gene (*INS*) accounts for early-onset diabetes: A case report and review of the literature

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Abstract
Insulin gene (*INS*) mutations are associated with a rare form of maturity-onset diabetes of the young (MODY). Here, we describe a proband with early-onset diabetes resulting from a heterozygous mutation in the promoter region of *INS* and summarize the clinical features of *INS* mutations caused by MODY (INS-MODY) reported in previous studies. The proband presented with proteinuria, mild hyperglycemia, and hypertension at the age of 39 years old; he was negative for the glutamic acid decarboxylase (GAD) antibody and had a family history of diabetes, including his father and aunt. The proband underwent whole exome sequencing, and the mutation in the proband and his father was verified by Sanger sequencing. A literature review was performed to examine all reported cases of INS-MODY to evaluate the clinical characteristics of the probands. A heterozygous *INS* mutation (c.-332C > G) was detected in the proband and his father, and their phenotypes had unique characteristics. Previous reports have described a total of 26 probands with 16 pathogenic mutations of the *INS* gene, with clinical features that exhibit great inter- and intrafamilial variability, and onset ages ranging from 2 years, 10 months to 62 years; 88% of patients were diagnosed before 40 years of age. Heterozygous mutations in the promoter region affecting the transcriptional activity of the *INS* gene may increase the risk of early-onset diabetes in adults, with patients presenting phenotypes that are very similar to type 2 diabetes, and genetic testing is needed to identify these individuals.

Keywords Diabetes · Insulin gene · INS · MODY10

Introduction

The insulin (*INS*) gene encodes preproinsulin, which comprises the signal peptide, insulin B-chain, C domains, and insulin A-chain. In addition to affecting the transcription and translation of insulin, mutations in the *INS* gene can also affect all steps of insulin biosynthesis in pancreatic β-cells [1], including endoplasmic reticulum (ER) targeting and translocation of preproinsulin, folding of proinsulin in the ER, trafficking and processing of proinsulin, and the binding of insulin to its receptor. In addition, misfolded proinsulin deposition in the ER can lead to ER stress and pancreatic β-cell apoptosis [2, 3], further promoting the occurrence of diabetes.

Pathogenic mutations of the *INS* gene are associated with a broad spectrum of clinical manifestations, ranging from severe neonatal onset diabetes to mild adult-onset hyperglycemia, suggesting that the products of different mutant *INS* alleles behave differently and cause diabetes via different mechanisms [1]. The clinical severity of *INS* mutations is related to the nature of the specific mutations and the steps of insulin biosynthesis affected by these mutations [4, 5]. In addition to autosomal recessive *INS* gene mutations that can cause permanent neonatal diabetes mellitus (PNDM), dominant mutations also contribute to the causation of PNDM [6], dominant and recessive *INS* gene mutations account for approximately 12% of diagnosed PNDM cases [7], and some maturity-onset diabetes of the young (MODY)-type diabetes cases are caused by heterozygous *INS* gene mutations

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(INS-MODY or MODY10). A series of *cis*-sequence elements and their homologous DNA-binding factors in the *INS* promoter region together ensure cell specificity and the rate of INS transcription [8]. Herein, we report the case of an early-onset diabetes patient carrying a heterozygous variant in the promoter region of the *INS* gene.

Materials and methods

Case report

The proband was a 49 year-old male who had hyperglycemia and hypertension for 10 years. Details of the laboratory tests were defined as previously described [9]. In 2012, he presented at our hospital with proteinuria and hematuria, with a fasting glucose level of 5.86 mmol/L and HbA1c reading of 6.2% (44.26 mmol/mol). For the next six years, his fasting blood glucose level ranged from 5.18–7.09 mmol/L. In 2018, he was referred to the Department of Endocrinology and Metabolism of Peking University People's Hospital for elevated fasting glucose levels. No diabetic ketoacidosis was observed. His body mass index (BMI) was 27.8 kg/m^2, and the following indices were recorded: fasting glucose 7.3 mmol/L, fasting insulin (Fins) 19.3uU/ml, fasting C-peptide (FCP) 4.21 ng/ml, and HbA1c 7.2% (55.19 mmol/mol). His glutamic acid decarboxylase (GAD) antibody test result was negative. The proband had good glycemia control for several years by diet, and his fasting insulin levels were not low, so even he had only measured the GAD antibody, the proband was diagnosed with type 2 diabetes and started taking SGLT2 inhibitors for proteinuria and diabetes in 2020. His laboratory data for the last decade are detailed in Table 1. The patient's father and paternal aunt were diagnosed with type 2 diabetes; however, his aunt had died without genetic testing.

The study protocol was approved by the Ethics Committee of Peking University People's Hospital (China). Written informed consent was obtained from the proband and his father.

Genetic screening

Genetic screening was performed at MyGenostics Inc. (Beijing). All DNA samples were extracted from peripheral blood samples. The GenCap Human Exon V4 capture chip (MyGenostics, China) was used for whole exome sequencing of the proband sample, which was performed using the Illumina HiSeq2500 system. The rare variant of *INS* was validated by Sanger sequencing in both the proband and his father.

Literatures review

A literature search was performed in the following databases: PubMed, ClinVar, and the Human Gene Mutation Database for mutant *INS* gene-induced MODY (as of January 2022). The search terms were "INS gene," "Insulin gene," "Maturity-onset diabetes of the young 10" and "MODY 10." All articles published in English reporting patients with the following criteria were included: (1) *INS* mutations classified as pathogenic or likely pathogenic according to the guidelines recommended by the American College of Medical Genetics and Genomics (ACMG); (2)

Table 1 The laboratory test of the proband in the last decade

	2012–2	2012–7	2014–1	2015–6	2016–8	2017–11	2018–5	2020-11[a]	2021–3	2021–6	2021–12	2022–3
FPG, mmol/l	5.86	5.4	6.52	6.59	6.43	7.09	7.3	7.03	5.9	6.64	6.4	6.37
Fins, uU/ml	/	/	/	/	/	/	19.3	18.11	/	/	11.78	/
HbA1c, %	6.2	5.9	/	/	/	/	7.2	6.6	5.9	6	6.2	6.4
HbA1c, mmol/mol	44.26	40.98	/	/	/	/	55.19	48.63	40.98	42.08	44.26	46.45
UA, umol/l	418	481	473	478	474	425	394	389	328	402	353	391
TG, mmol/l	1.64	2.2	1.94	1.9	2.07	2.66	2.44	2	1.27	1.44	1.28	1.51
TCHO, mmol/l	4.17	4.1	4.09	4.48	4.83	4.31	4.9	4.58	4.56	4.42	4.55	4.82
LDL-c, mmol/l	2.62	2.6	2.53	2.98	3.34	2.71	3.21	2.85	2.86	1.76	2.82	3.19
HDL-c, mmol/l	0.77	0.87	0.82	1	0.99	0.94	1.04	1.03	1.06	1.14	1.14	1.11
UACR, mg/g	/	/	/	/	/	/	/	322.62	100.67	71.94	92.15	63.78
24-h proteinuria, g/day	0.86	0.77	0.36	0.54	0.71	0.55	/	0.44	/	0.18	/	/
eGFR, ml/min/1.73m^2	95.09	90.06	87.28	96.22	84.01	79.96	90.99	75.23	93.03	68.78	94.25	80.2

FPG, fasting plasma glucose; *HbA1c*, hemoglobin A1c; *Fins*, fasting serum insulin; *TG*, triglyceride, *TCHO*, total cholesterol, *LDL-c*, low-density lipoprotein cholesterol; *HDL-c*, high-density lipoprotein cholesterol; *UACR*, urinary albumin/creatinine ratio, *eGFR*, estimate glomerular filtration rate

[a]The proband began taking a SGLT2 inhibitor after this follow-up

non-autoimmune diabetes and non-neonatal diabetes; (3) no treatment dependent on insulin and/or measurable C-peptide at least one year after diagnosis of diabetes.

Results

A heterozygous mutation (c.-332C > G) in the promoter region of *INS* was detected in the proband and his father (Fig. 1). Whole exome sequencing revealed no other mutation of known monogenic diabetes. According to the ACMG guidelines, this mutation can be classified as likely pathogenic (PS3[8], PM2).

INS-MODY is a relatively rare type of MODY. The clinical features of the proband found in this study were similar to those of type 2 diabetes, and it is difficult to distinguish him from type 2 diabetes without genetic testing. Therefore, we reviewed the previous literatures to find the clinical characteristics of patients with INS-MODY. A total of 26 probands with 16 pathogenic mutations of *INS* gene of INS-MODY have been reported in the literature, including one with intronic mutations, two with nonsense mutations, two with frameshift mutations, and 21 with heterozygous mutations (Fig. 2). According to the provided clinical data for probands with pathogenic *INS* mutations, 92% (24/26) were diagnosed with diabetes, 88% (21/24) of which were diagnosed before 40 years of age, 71% (17/24) of which were diagnosed before 25 years of age, and 63% (15/24) of which were treated with insulin. All of the probands carried heterozygous mutations and the inheritance pattern of their families was autosomal dominant. The clinical features of these cases are summarized in Table 2. There was a significant difference between the different probands. The youngest age at diagnosis was 2 years and 10 months, and the oldest age was 62 years (Supplementary Table 1). Two probands were on a diet to maintain good blood glucose control, and four probands were on insulin but with poor glycemic

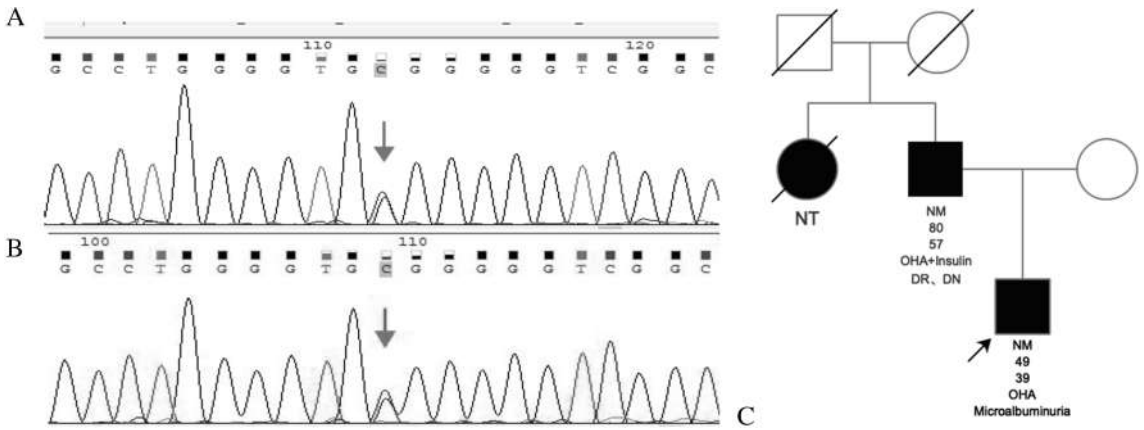

Fig. 1 DNA sequences of the *INS* mutation c.-332C > G found in the proband (**A**) and his father (**B**). **C** Indicates pedigrees of the proband. The solid symbol: diabetes status; empty symbol: normoglycemic subject. Arrow indicate the proband. The *INS* mutation c.-332C > G status is shown under each symbol: NM as heterozygote and NT as not tested. The text below indicates the following: present age, age of onset, treatment, diabetic complications. OHA: oral hypoglycemic agents. DR: diabetic retinopathy. DN: diabetic nephropathy

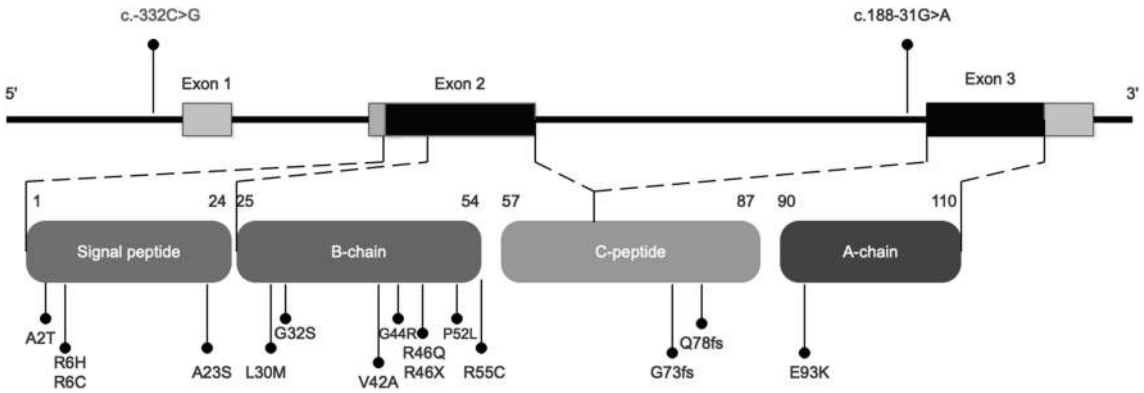

Fig. 2 A schematic of INS gene structure and the position of the pathogenic mutations of INS-MODY reported in previous studies and this study. Marked in red: the mutation of the proband found in this study (c.-332C>G)

Table 2 Clinical features of probands with INS pathogenic mutations of INS-MODY reported in previous study

Proband	Mutation	Age of diagnosis, years	BMI, kg/m^2	Treatment
1	A2T	31	/	OHA
2		47(IGT)	24.54	/
3		62	24.21	OHA
4		54	28.94	No
5		34	23	OHA
6		57	23.1	OHA
7	L30M	17	25.9	OHA + insulin
8	R55C	9	20.2	Insulin
9		10	20.8	Insulin
10		7	/	Insulin
11	R6H	20	24.9	OHA
12	R6C	15	24.1	OHA + insulin
13	V42A	6	15.2	Insulin
14	R46Q	20	23.9	No
15	E93K	11	/	Insulin
16	Q78fs	14	27.4	Insulin
17	G73fs	15	23.3	OHA + insulin
18	R46Q	13	21.4	Insulin
19	G32S	2 years 10 months	16	Insulin
20	A23S	6 years 8 months	16.4	Insulin
21	R46X	17	24.8	OHA
22		8	20.9	Insulin
23	G44R	17(IFG)	21.3	/
24		29	22.3	Insulin (pregnancy)
25	P52L	40	22.7	OHA
26	c.188-31G > A	3	24	Insulin

IGT, impaired glucose tolerance; *OHA*, oral hypoglycemic agents; *IFG*, impaired fasting glucose

control [HbA1c > 9% (74.86 mmol/mol)]. Complications of INS-MODY have been reported in only five families; diabetic retinopathy, neuropathy, and microalbuminuria were reported in a proband with a 30-year history of diabetes, while metabolic cataract, nephropathy, and neuropathy were reported in another patient with a frameshift mutation.

Discussion

This is the first report of a patient with early-onset diabetes due to a heterozygous mutation (c.-332C > G) in the promoter region of the *INS* gene. The compound heterozygous mutations c.-332C > G and c.-331C > G were first discovered in a pedigree of neonatal diabetes [8].

The most dominant *INS* mutations are located in the proinsulin domain and disrupt the oxidative folding of the protein, leading to the misfolding of proinsulin in the ER. Misfolded proinsulin accumulates in the ER, disrupts ER protein homeostasis, induces ER chronic stress, and leads to beta cell apoptosis [1, 10]. In addition, misfolded proinsulin can interact abnormally with co-expressed wild type proinsulin through the proinsulin dimerization interface, impairing the folding and ER export of wild type proinsulin, which reduces insulin production and leads to insulin-deficient diabetes [11, 12].

Mutations resulting from the deletion of *INS*, inactivation of promoters, and deletion of translation initiation can disrupt transcription and translation of the *INS* gene. Although both *INS* alleles have been shown to cause neonatal diabetes [8], these recessive *INS* mutations have also been reported to be associated with early-onset diabetes [13]. The c.-332C > G mutation is located between E1 and A1, and this sequence is conserved in a subpopulation of mammalian species. Multiple base mutations adjacent to this mutation impair *INS* promoter activity [14]. A previous study reported that the parents of a proband with neonatal diabetes carried the c.-332C > G mutation but did not have diabetes. However, it was not stated in the article whether the parents had performed glucose tolerance tests, and the reported proband had neonatal diabetes, it is possible that his parents were younger and thus not old enough to obtain abnormal glucose tolerance readings. In addition, previous studies have indicated that the mutations c.-331 (C > G, C > A) and c.-332C > G can decrease *INS* transcriptional activity by up to 90%. The proband in the current study carrying this heterozygous mutation (c.-332C > G) also had early-onset

diabetes. Together, these data suggest that the compound heterozygous mutation (c.-332C > G and c.-331C > G) can induce neonatal diabetes, while the c.-332C > G heterozygous mutation on its own can be a pathogenic mutation triggering early-onset diabetes in adults. Recessive *INS* mutations that cause neonatal diabetes might also function as dominant *INS* mutations that cause MODY phenotypes.

Previous reported cases have revealed that the clinical features of patients with *INS* mutations exhibit large inter- and intrafamilial variability, ranging from mild adult-onset hyperglycemia to an onset age of under 3 years old requiring insulin treatment. Even in patients with the same mutations or in the same pedigree, the onset age of diabetes ranged from childhood to middle age, and even some individuals without diabetes at the time of the study (Supplementary Table 1), suggesting that other genetic and environmental factors, including the degree of misfolded proinsulin accumulation and individual differences, may contribute to differences in the clinical features of INS-MODY. The proband of this study was found to have hyperglycemia at the age of 39 years and was overweight, exhibiting microalbuminuria, occasional hyperinsulinemia, hypertriglyceridemia, and low HDL-c levels, suggesting that diabetes in this pedigree results from the *INS* gene mutation against a background of insulin resistance. In addition, the patient's father exhibited later onset of diabetes, suggesting that environmental factors, such as age and lifestyle, might have contributed to the development of diabetes in these cases. Because the clinical phenotype of this heterozygous mutation in the promoter region is very similar to that of type 2 diabetes, it is difficult to diagnose such patients if genetic testing is not performed. Thus, screening a larger pool of patients with early-onset diabetes may reveal factors that could serve as indicators whether genetic testing should be performed.

In summary, this study highlights the utility of heterozygous mutations in the promoter region of the *INS* gene, and suggests that heterozygous mutations affecting the transcriptional activity of *INS* may increase the risk of early-onset diabetes in adults, indicating that genetic testing is needed in such patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01205-4.

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Data availability All datasets are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval The study protocol was approved by the Ethics Committee of Peking University People's Hospital (China). Written informed consent was obtained from the proband and his father.

Competing interests The authors declare no competing interests.

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LETTER TO THE EDITOR

Inclusion of diabetes plate method in Indian diabetes management guidelines—a public health imperative

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Sir,

Diabetes is a significant public health concern in India, with an estimated 77 million adults living with the disease in 2019 [1]. Poorly managed diabetes can lead to a range of health complications, including heart disease, stroke, kidney disease, and nerve damage. Additionally, the economic burden of diabetes is considerable, with the projected cost of diabetes care in India estimated to increase from $31 billion in 2015 to $101 billion by 2030 [2]. One of the effective ways to manage diabetes is by adopting a healthy diet, highlighting the need for individuals with diabetes to be mindful of their dietary habits to maintain their blood glucose levels within a normal range.

The diabetic plate method is a simple, evidence-based dietary strategy that can meet the specific needs of individuals with diabetes. This method aids in regulating blood sugar levels and promoting healthy eating habits in people with diabetes. The diabetes plate method has gained widespread adoption and recommendation by healthcare professionals and organizations, including the American Diabetes Association and the International Diabetes Federation [3].

The diabetes plate method involves dividing a standard 9-in. plate into three sections: [1] non-starchy vegetables: half of the plate is filled with non-starchy vegetables such as leafy greens, tomatoes, cucumbers, broccoli, and bell peppers [2]. Whole grains: one-quarter of the plate is filled with whole grains such as brown rice, millet, or chapatis made with whole wheat flour [3]. Lean protein: one-quarter of the plate is filled with lean protein sources such as dals (lentils), chana (chickpeas), rajma (kidney beans), paneer, chicken, or fish. Additionally, a serving of dairy product (dahi) is recommended.[3]

The diabetes plate method provides several advantages. Firstly, it offers a simple and easy-to-understand approach to meal planning that can be adapted to local and traditional foods in India, making it more accessible to people with limited access to nutrition education or resources. Secondly, it provides a visual guide to portion sizes, which can help people with diabetes to manage their blood sugar levels and prevent overeating. Thirdly, the diabetes plate method encourages a balanced intake of macronutrients—protein, carbohydrates, and fats—which can help people with diabetes to maintain a healthy weight, prevent insulin resistance, and manage their blood sugar levels. Finally, the diabetes plate method can be adapted to individual preferences and cultural norms, making it more feasible and sustainable for long-term use.

The inclusion of the diabetes plate method in the dietary guidelines for diabetes management in India can have several public health benefits, including standardized dietary recommendations across the country and consistent, evidence-based dietary advice from healthcare professionals to diabetic patients.

In conclusion, the diabetes plate method is a feasible, effective, and necessary dietary strategy for managing diabetes in India. Its potential impact on public health makes it crucial for policymakers and healthcare professionals to consider its inclusion in dietary management guidelines for diabetes in India. Given the high prevalence of diabetes in India, the adoption of this approach can potentially improve the health outcomes of millions of people with diabetes in the country.

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Author contribution

	Contributor 1	Contributor 2
Concepts	√	√
Design	√	√
Definition of intellectual content	√	√
Literature search	√	√
Data acquisition	√	√
Data analysis	√	√
Statistical analysis	√	√
Manuscript preparation	√	√
Manuscript editing	√	√
Manuscript review	√	√
Guarantor	√	√

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CASE REPORT

Acarbose associated pneumatosis cystoides intestinalis: A case report

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Abstract

Background Pneumatosis cystoides intestinalis is a rare intestinal disease characterized by single or multiple air cysts in the submucosa or subserosa of the intestinal wall. It is often accompanied by other gastrointestinal diseases. The clinical manifestations were nonspecific, and the diagnosis mainly depended on colonoscopy, imaging, and other auxiliary examinations.
Case presentation The reason for pneumatosis cystoides intestinalis's occurrence remains unclear. Enteral nutrition deficiency, especially the use of glucosidase inhibitors, may be involved in the pathogenesis of pneumatosis cystoides intestinalis in this patient. Here, we report a rare case of pneumatosis cystoides intestinalis in an elderly man who had been taking acarbose for diabetes for a long time. Colonoscopy of the patient was normal before taking acarbose.
Conclusion In patients with diabetes who are treated with glucosidase inhibitors for gastrointestinal symptoms, the possibility of pneumatosis cystoides intestinalis should be considered, and a thorough gastrointestinal examination should be performed in such patients.

Keywords Pneumatosis cystoides intestinalis · Acarbose · α-glucosidase inhibitor · Constipation · Intestinal obstruction

Introduction

Pneumatosis cystoides intestinalis (PCI) is a rare gastrointestinal disease. It is characterized by the presence of multiple inflated gas-bearing cysts in the submucosa and/or subserosa of the intestinal wall, which may be surrounded by inflammation and fibrosis. PCI is more common in the colon, followed by the small intestine. Colonic lesions are more common in the descending colon and sigmoid colon. The overall incidence of PCI was 0.03%, which was divided into primary or idiopathic (15%) and secondary (85%) [1]. PCI can occur in any age group, and the cause of the disease is unclear. Due to the low incidence and the neglect of clinicians, patients with this disease are often not treated in a timely and effective manner. Here, we report a rare case of PCI due to long-term acarbose administration.

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Case report

A 73-year-old male patient was admitted to the gastroenterology department of our hospital due to intermittent abdominal distension for 2 years and weight loss for half a year. He had a history of diabetes mellitus for 10 years, constipation for 10 years, dry and hard stools every 4 to 5 days, and colonoscopy 2 years ago showed no abnormalities. Two years ago, he was treated with metformin 0.5 g and acarbose 50 mg po tid, and his blood glucose was well controlled. His vital signs were stable when he was admitted, and liver and kidney function, blood routine, coagulation, and blood gas analysis showed no obvious abnormalities. Abdominal CT performed after admission revealed dilatation of the colon, with multiple saccular lesions on the wall beaded (Fig. 1a). Standing abdominal radiographs suggest intestinal obstruction. A colonoscopy revealed multiple grapestring air cysts in the colonic submucosa (Fig. 1b). Other examinations showed no obvious abnormalities. PCI was considered to be related to acarbose. Then, acarbose was stopped, insulin subcutaneous injection was given to lower blood glucose, and probiotics and lactulose were added to regulate intestinal flora and purgative therapy. The patient was discharged after improvement. Four months after discharge, blood glucose

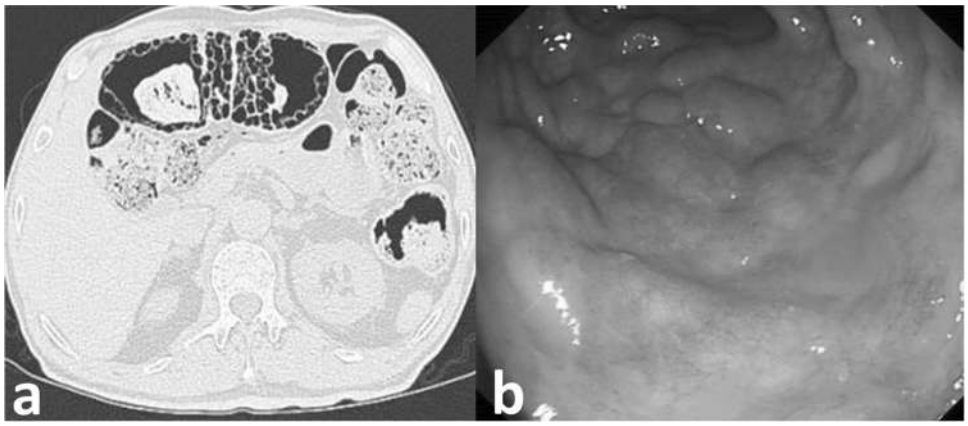

Fig. 1 CT scan indicated dilatation of the colon, with multiple saccular lesions on the wall beaded (**a**). Colonoscopy revealed multiple grapestring air cysts in the colonic submucosa (**b**)

was well controlled, defecation was unblocked, abdominal distension was relieved, and weight gained 10 kg.

Discussion

PCI is a rare gastrointestinal disease, and its etiology and pathogenesis are still unclear. The etiology of PCI can be divided into gastrointestinal disease, pulmonary disease, autoimmune system disease, infection, iatrogenic factor, drug-induced, and organ transplantation related [2]. But any one theory could not explain all the pathological processes of PCI, so it is considered that the pathogenesis of PCI is not caused by a single mechanism, but by the interaction of multiple mechanisms.

The clinical manifestations of PCI patients are mostly abdominal pain, abdominal distension, diarrhea, nausea, vomiting, hematochesis, and constipation, and the clinical manifestations are not specific [1], so the necessary auxiliary examinations are indispensable. CT is considered to be the most useful method for diagnosing PCI. It is more sensitive than X-ray in distinguishing intraluminal gas or submucosal fat. The main clinical manifestations were intestinal wall thickening, serosa or submucosa multiple cystic, beaded low density shadow, if the balloon rupture, pneumoperitoneum formation, visible intraperitoneal free gas shadow, but no signs of peritoneal irritation [3]. Submucosal PCI under colonoscopy showed multiple clusters of bumps of different sizes in the intestinal lumen, with translucent surface, congestion, edema, soft texture, and fluctuation feeling when the biopsy forceps was touched [4].

In this case, the patient with PCI was diagnosed by CT and colonoscopy because of constipation and intestinal obstruction. The colonoscopy before taking acarbose did not suggest abnormalities, but PCI was found by colonoscopy after taking the drug, drug-induced PCI needs to be highly considered. Acarbose, a α-glucosidase inhibitor (α-GI), is an oral hypoglycemic agent. The possible mechanisms by which acarbose causes PCI are as follows: α-glucosidase in the intestine is inhibited, which degrades disaccharides to monosaccharides, slows down the digestion and absorption of carbohydrates, and reduces postprandial hyperglycemia. The presence of undigested carbohydrates in the small and large intestine of patients taking α-GI promotes overgrowth of gut bacteria that ferment carbohydrates and produce nitrogen, hydrogen, oxygen, carbon dioxide, nitrous oxide, *n*-butane, isobutane, propane, and methane [1], leading to side effects such as bloating and flatulence. It is thought that excessive production of this gas increases intestinal pressure, allowing the gas to pass through the intestinal wall, leading to PCI [5]. In terms of treatment, some of the literatures have reported that the discontinuation of α-glucosidase inhibitors is the key to successful treatment [6–8]. Patients with obvious symptoms should be treated conservatively, including oxygen therapy, intestinal rest, and antibiotic therapy. When intestinal ischemia, intestinal obstruction, intestinal bleeding, or peritonitis is clinically suspected, emergency laparotomy should be performed in combination with laboratory data [9]. In recent years, some clinicians have tried to exhaust the cyst wall with biopsy clamp or injection needle, argon gas, or laser therapy under endoscope, but its safety and long-term efficacy need to be further studied.

Conclusion

PCI is rare, and its clinical manifestations are lack of specificity. Its diagnosis mainly depends on imaging examination and endoscopy, and it is easy to be misdiagnosed as multiple polyposis, intestinal tumors, and gastrointestinal perforation. Clinicians should deepen the understanding of this disease, so as to diagnose and treat patients more reasonably and avoid unnecessary surgical operations. In diabetic patients with autonomic neuropathy and bowel motility problems, colonoscopy should be considered before administering a glucosidase inhibitor to avoid secondary PCI.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Written informed consent was obtained from the patient.

Consent for publication Written informed consent was obtained from the patient.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

The effects of whey protein on anthropometric parameters, resting energy expenditure, oxidative stress, and appetite in overweight/obese women with type 2 diabetes mellitus: A randomized placebo controlled clinical trial

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Abstract

Background Insufficient data are available on the effects of long-term whey protein (WP) consumption on patients with type 2 diabetes mellitus (T2DM). So, in this study, we aimed to examine the effects of WP combined with individualized diet on anthropometric parameters, resting energy expenditure (REE), oxidative stress markers, and appetite among overweight/obese women with T2DM.

Methods In this 3-month double-blind placebo-controlled randomized clinical trial, 48 women with T2DM were randomly allocated into either WP group (one bread fortified by 20 g whey protein concentrates (WPC)) or placebo group (one unfortified bread) along with their individualized diets. At both pre- and post-intervention phases, physical activity, anthropometric parameters, REE, appetite, and serum levels of total antioxidant capacity (TAC) and malondialdehyde (MDA) as well as diet were assessed.

Results Thirty-five patients completed the trial. At the endpoint, there were no significant between-group differences for anthropometric parameters ($p > 0.05$), except for waist circumference (WC), which was lower in the WP group after adjusting for the confounders ($p = 0.040$). Serum level of MDA was significantly decreased in the WP group ($p = 0.022$). There were no significant within- or between-group changes for serum levels of TAC, REE, and appetite sensations ($p > 0.05$), except for the "hunger", which was lower in the WP group after adjusting for the confounders ($p = 0.045$).

Conclusion Regarding significant reduction in WC, serum levels of MDA, and feeling of hunger, consumption of the WPC fortified bread could be beneficial in women with T2DM.

Registration Number: IRCT20110123005670N26; Registration date: 2019/01/07.

Keywords Whey proteins · Diabetes mellitus · Oxidative stress · Anthropometry

Introduction

Diabetes mellitus (DM) is a group of diseases characterized by chronic high blood glucose levels [1]. The prevalence of DM around the world is about 425 million adults, which is predicted to reach about 629 million adults in 2045 [2]. Because of the chronic complications of DM (microvascular and macrovascular complications) and high medical costs for the treatment of these complications, controlling hyperglycemia and other disturbed metabolic parameters is vital for patients with DM [3-5].

Various evidence showed the relationship between obesity with insulin resistance and type 2 diabetes mellitus (T2DM) [6-8]. Similarly, adipocyte dysfunction in obesity results in elevated oxidative stress [9], with similar elevations found in T2DM [10, 11]. Thus, an increase in resting energy expenditure (REE) or the reduction in body weight or its related anthropometric parameters like waist circumference (WC), could be beneficial for the management of T2DM and its complications [12]. In this regard, control of appetite is one of the proposed possible strategies for obesity treatment and weight loss.

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Since the complications resulted from poor control of T2DM and the side effects of some pharmacologic agents for diabetes care are possible, so feasible lifestyle strategies are needed to improve outcomes in T2DM. In some recently performed trials, natural products made from micronutrients, plants, functional foods, and diverse supplements have been assessed for their potential therapeutic effects in the management of diabetes mellitus and its related complications [13-21].

Insulinotropic as well as glucose-lowering effects are attributed to some dietary factors like whey protein (WP) [22-24]. Of note, the components of cow milk's protein are WP (20%) and casein (80%). The components of WP are as follows: β-lactoglobulin, α-lactalbumin, glycomacropeptide (GMP), lactoferrin, immunoglobulins, bovine serum albumin, and lactoperoxidase [25]. Whey, as a by-product of the cheese-making process, is known as a functional food [26]. Different forms of WP include whey protein isolates (WPIs) and whey protein concentrates (WPCs) with more than 90% and 35–85% protein contents, respectively. In addition, whey protein hydrolysate (WPH) consists of proteins that are hydrolyzed by proteolytic enzymes [25, 27].

Some previous studies reported conflicting results regarding the effects of WP supplementation on body weight, oxidative stress, and appetite. In some studies, WP led to a reduction in body weight, WC, and body mass index (BMI) [12, 28]; however, the weight loss effect of WP has not been shown in some other studies [29]. The reduction of the REE has been shown in some studies [30], while some other studies reported an increased post-exercise REE after the consumption of WPI [31]. A meta-analysis of randomized controlled trials (RCTs) conducted on the WP effects on long and short-term appetite reported a significant reduction and no change in long and short-term appetite, respectively [32]. Recently, an 8-week trial showed appetite reduction after consumption of biscuits fortified with WPI [12]. Moreover, previous studies reported a decrease in some oxidation markers as well as no change in some other oxidative stress and antioxidant markers [33, 34].

Given the insufficient number of long-term clinical trials performed on the effects of WP on oxidative stress and metabolic parameters in patients with T2DM, we aimed to conduct this 3-month RCT for examining the long-term effects of the bread fortified with 20 g of WPC on anthropometric parameters, REE, oxidative stress markers [total antioxidant capacity (TAC) and malondialdehyde (MDA)], and appetite among overweight/obese women with T2DM. Considering the probable desire of patients or healthy subjects for the consumption of natural compounds as a part of their diet instead of powdered or capsulated supplements, due to the fact that bread is the staple food of people in Iran, and for the increment of our intervention's applicability, in this study, we administered WP as whole wheat bread fortified with WPC and designed dietary plans based on the specific characteristics of each participant.

Materials and methods

Study design and subjects

This double-blinded, placebo-controlled RCT was conducted between June 2019 and March 2020. The intervention was implemented for three months (90 days). In the current research, we recruited the patients from healthcare centers and polyclinics of Tabriz University of Medical Sciences and also from some other outpatient offices in Tabriz, Iran. At the beginning of the trial, 48 women with T2DM aged between 25 and 55 years old with a body mass index (BMI) of 25–40 kg/m^2, were enrolled in this study. The subjects with inflammatory, immunologic, pulmonary, neoplastic, and malabsorption diseases such as ulcerative colitis or Crohn's disease; uncontrolled thyroid, kidney, or liver disorders; as well as the patients who were taking non-steroidal anti-inflammatory drugs, glucocorticosteroid or hormonal drugs; and insulin recipients were not included in the study. Any change in type or dose of the administered drugs and change in diet or physical activity during the intervention period, smoking, pregnancy, breastfeeding, menopause, and allergy or intolerance to milk components were the other exclusion criteria of our study. Thereafter, the patients' basic characteristics including demographic information and disease history were recorded. Next, written informed consent was obtained from all the patients. The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethics code; IR.TBZMED.REC.1397.687) approved this study. This research was conducted according to the Declaration of Helsinki. The present trial was registered in the Iranian Registry of Clinical Trials (http://www.irct.ir, Registration Number: IRCT20110123005670N26).

Sample size

We used the mean (standard deviation [SD]) of fasting blood sugar (FBS) from a previous clinical trial [35], in order to determine sample size, based on a confidence level of 95% and power of 80% in two-sided tests. We calculated the sample size as 18 cases per group (the WP and placebo groups) utilizing the Pockock formula and considering a probably about 30% dropout rate, the sample size was increased to 24 cases in each of the groups.

Randomization and intervention

The included participants were randomly allocated by a research assistant (the first author) in a 1:1 ratio to either the WP group or the placebo group. We generated the sequence of the randomization by the Random Allocation Software, assuming a randomized block procedure of size 2 [age

(≤ 40 years vs. > 40 years) and BMI (≤ 32 kg/m^2 vs. > 32 kg/m^2)]. The participants, statistician, and investigators, other than the first author, were blinded to the intervention's allocation. Considering a desirable formulation for the dough of whole wheat flour, we used white flour (82% extraction rate) and whole wheat flour (96% extraction rate) in a ratio of 20:80, respectively. For achieving a desirable formulation for whole wheat flatbread, which was fortified with WPC for this research and based on the previous RCTs on WP supplementation [36-38], we used 20 g WP (WPC 80 instant; Sachsenmilch Leppersdorf GmbH, 01454 Leppersdorf, Germany) for the fortification of each bread.

One WPC fortified whole wheat flatbread (about 160 g) was daily provided to the patients in the WP group, while one whole wheat flat bread, which was not fortified with WPC (about 125 g), was provided for those in the placebo group, for a period of three-consecutive-month. WPC was the only difference between the ingredients of bread provided to the two groups. Accordingly, in each WP fortified bread, we replaced 20 g of flour with 20 g WPC. The macronutrient contents of both kinds of bread are shown in Table 1. Notably, the difference in weight of fortified and unfortified bread was due to WPC and higher amounts of water, which were used for the preparation of the fortified bread. A reference bakery (Athar Nan, Tabriz, Iran) prepared all these types of bread, and one of the investigators (the first author) supervised all steps of the preparation and baking processes. We provided placebo and WPC fortified bread to both groups every two weeks.

Considering the recommended dietary guidelines [1, 39] and the individual characteristics of each patient, low-calorie diets were designed by an experienced dietician for all the participants. As well, we recommended walking for at least 30 min a day to all patients. The patients were monitored every two weeks.

Anthropometric and REE measurements

One trained nutritionist performed the anthropometric and REE measurements once at baseline and once after three months. All the measurements were conducted in a fasting state. The weight and height of the participants were measured with a calibrated digital scale (Seca, Hamburg, Germany) and stadiometer to the nearest 0.1 kg and 0.1 cm, respectively. Thereafter, for calculating BMI, weight (kg) was divided by height squared (m^2). The smallest horizontal girth between the costal and iliac crests was measured as WC by a non-stretchable measuring tape to the nearest 0.1 cm. In addition, the widest circumference over the great trochanters was measured as hip circumference (HC). Next, the waist/hip ratio (WHR) was calculated. Although REE of some patients was measured via indirect calorimetry by Fitmate Pro (Rome, Italy), because of the indirect calorimetry device failure during the intervention period, we imputed the missing data using the fully conditional specification method. About 17% and 54% of the patients had missing values for indirect calorimetry measures both at baseline and after the intervention period, respectively. REE measurements were performed via indirect calorimetry in the morning before the patient was engaged in any moderate or vigorous physical activity and about 12 h after the ingestion of any food and drink. A rest period of about 5 to 10 min was considered before the measurements. Notably, the duration of REE test was 6 min.

Assessment of dietary intake, appetite, and physical activity

In order to estimate dietary intake, we used 24-h recalls at the pre- and post-intervention phases. The Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods was then used for analyzing the collected data on dietary intake. At the pre- and post-intervention phases, the patients completed a visual analog scale (VAS), which was 100 mm in length with some words used for expressing the most positive and the most negative rating at each end, for measuring appetite sensations (hunger, satiety, fullness, and desire to consume something sweet, fatty or salty) [40]. Afterward, for assessing the physical activity level of participants, a validated international physical activity questionnaire-short form (IPAQ-SF) [41] was used. Metabolic Equivalent Task minutes per week (MET-minutes/week) scores were also calculated in terms of the guidelines of data processing and analysis of the IPAQ [42]. According to these guidelines, those subjects achieving a minimum total physical activity of at least 600 MET-minutes/ week were considered to have a "moderate" physical activity level. The criterion for being classified as "high" physical activity level was achieving a minimum total physical activity of at least 3000 MET-minutes/ week. Those patients who did not

Table 1 Composition of WPC fortified and unfortified bread

Sample of bread	Energy (kcal/100 g)	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Fiber (g/100 g)
WPC fortified bread	223.7	37.72	14.02	1.86	5.37
Unfortified bread	251.91	50.14	8.9	1.75	6.14

WPC, Whey protein concentrate

meet the two above-mentioned criteria were considered to have a "low" physical activity level.

Laboratory assays

After 12-h overnight fasting, we collected 8 mL of blood samples from each patient in each gel separator tubes. For separating serum, the blood samples collected in gel separator tubes were centrifuged. Afterward, the serum levels of TAC and MDA were measured by appropriate kits ("Naxifer ^{TM}, Novin Navand Salamat Pishtaz Co, Urmia, Iran" and "Nalondi ^{TM}, Novin Navand Salamat Pishtaz Co, Urmia, Iran", respectively).

Statistical analyses

IBM SPSS Statistics software (IBM SPSS Statistics, Armonk, USA, version 26) was used for all statistical analyses. To perform analyses, a per-protocol (PP) approach was employed. Moreover, Kolmogorov-Smirnov test was run for assessing the normality of the data distribution. To assess between-group differences at baseline, independent samples t-test was used. Notably, a paired samples t-test was applied for assessing within-group changes. For the assessment of between-group differences of categorical variables, Fisher's exact test was run, and for comparing the two groups at the end of the study, an analysis of covariance (ANCOVA) test was used. In this study, we adjusted all the analyses for baseline values and confounding factors (i.e., age, diabetes duration, administered drugs, BMI, physical activity, and intake of energy, and macronutrients). p values < 0.05 were considered statistically significant.

Results

General characteristics of trial and dropouts

Of 48 patients enrolled in the study, 35 patients (17 in the placebo and 18 in the WP groups) completed the trial to the end. The flowchart of the study is shown in Fig. 1.

Demographic characteristics

The baseline characteristics of the study participants are shown in Table 2. At baseline, there were no significant differences between the two groups in terms of weight, height, BMI, age, marital status, physical activity level, and educational level. The mean age of the participants in the WP and placebo groups was 44.00 and 46.94 years old, respectively.

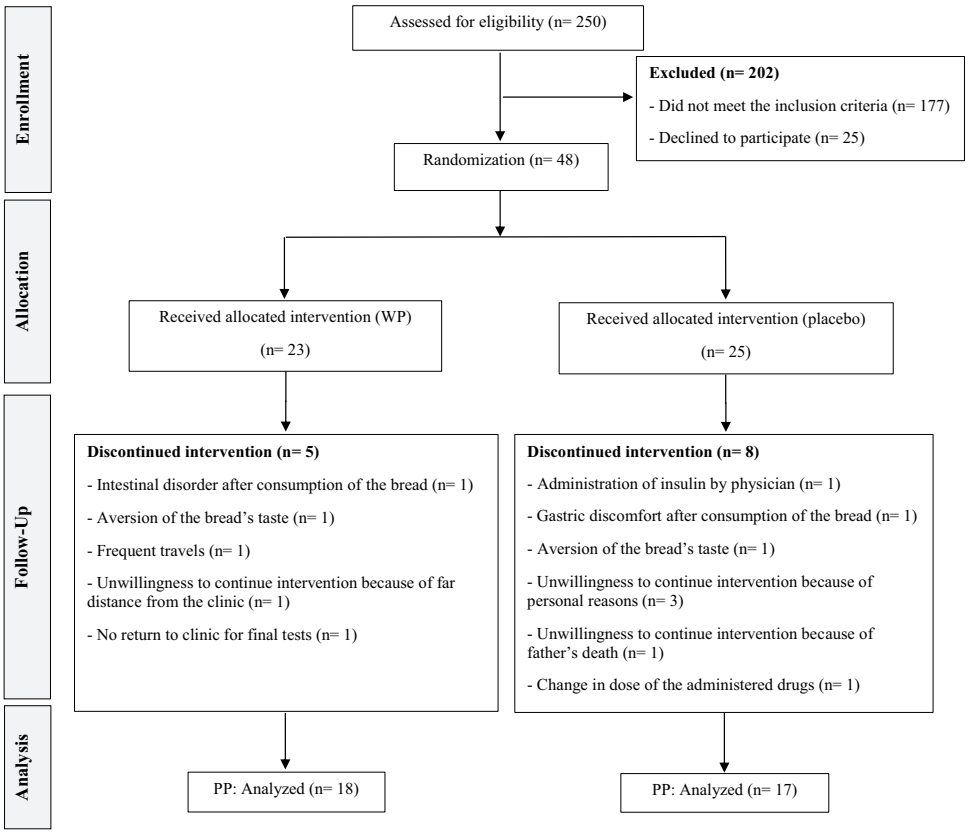

Fig. 1 Study flow diagram. WP: Whey protein; PP: Per protocol

Table 2 Baseline characteristics of the study participants

	WP ($n=18$)	Placebo ($n=17$)	p
Age (years)	44.00 (6.29)	46.94 (5.17)	0.142[a]
Weight (kg)	81.88 (12.84)	80.01 (16.05)	0.706[a]
Height (cm)	158.61 (7.70)	158.41 (6.44)	0.935[a]
BMI (kg/m^2)	32.54 (4.26)	31.66 (5.04)	0.579[a]
Marital status			
Single	1 (5.55)	1 (5.88)	1.000[b]
Married	16 (88.88)	15 (88.23)	
Divorced or widow	1 (5.55)	1 (5.88)	
Education			
Illiterate	1 (5.55)	0 (0.0)	0.562[b]
Diploma and lower	14 (77.77)	12 (70.58)	
Bachelors and higher	3 (16.66)	5 (29.41)	
Physical activity level			
Low	7 (38.88)	6 (35.29)	1.000[b]
Moderate	7 (38.88)	7 (41.17)	
High	3 (16.66)	4 (23.52)	
Drugs for glycemic control Metformin 23 (65.71) Diabezid 16 (45.71) Others (e.g., Zipmet, etc.,) 15 (42.85)	18 (100)	17 (100)	
Drugs for dyslipidemia Atorvastatin 16 (45.71) Others (fenofibrate, gemfibrozil) 3 (8.57)	9 (50)	10 (58.82)	
Drugs for hypertension Losartan 9 (25.71) Others (Metoral, Amlodipine, etc.,) 8 (22.85)	7 (38.88)	7 (41.17)	

WP, Whey protein; *BMI*, Body mass index

Age, weight, height, and BMI are presented as Mean (SD); other variables are presented as number (%)

[a] Independent samples t-test

[b] Fisher's exact test

Dietary intake and physical activity

As shown in Table 3, consumption of energy, protein, carbohydrates, and fat percent (percent of energy) have significantly decreased in the placebo group ($p<0.05$). A significantly lower protein intake was also observed in the placebo group after adjusting the confounders compared with the WP group. There were significant increase and decrease in protein percent (percent of energy) and carbohydrate intake in the WP group ($p<0.05$), respectively. Within- or between-groups' changes in physical activity (METs) of the patients were not significant during the study (Table 3).

Anthropometric indices, REE, and oxidative stress markers

There was no significant difference between the two groups in terms of anthropometric indices, REE, and oxidative stress markers at baseline (Table 4). As shown in Table 4, a significantly lower WC was observed in the WP group after adjusting the confounders compared to the placebo group ($p=0.040$). The serum level of MDA has significantly decreased in the WP group ($p=0.022$) (Fig. 2). There were no significant within- or between-group changes in terms of weight, BMI, HC, WHR, REE, and TAC serum levels of the patients throughout the study (Table 4).

Appetite sensations

As presented in Table 5, there were no significant within- or between-group changes in terms of the desire to eat, the feeling of fullness, the desire to eat sweet foods, the desire to eat salty foods, and the desire to eat fatty foods in the patients throughout the study. Regarding the feeling of hunger, within-group changes were significantly more in the placebo group compared to the WP group.

Table 3 Dietary intake and physical activity of the study participants throughout the study

Variable	Period	WP ($n=18$)	Placebo ($n=17$)	MD (95% CI), p
Energy (Kcal)	Baseline	1673.14 (679.45)	1808.47 (559.88)	-19.50 (-551.63, 512.62), 0.941[b]
	End	1469.12 (669.76)	1349.57 (463.66)	151.27 (-203.84, 506.39), 0.392[c], 0.164[d]
	MD (95% CI), P[a]	-204.02 (-587.31, 179.26), 0.276	- 458.89 (-700.83, -216.95), 0.001	
Protein (g)	Baseline	60.73 (27.40)	68.47 (18.04)	7.74 (-8.31, 23.80), 0.334[b]
	End	62.14 (30.96)	51.46 (19.83)	13.13 (- 4.67, 30.95), 0.143[c], 0.015[d]
	MD (95% CI), P[a]	1.41 (-15.91, 18.73), 0.865	-17.01 (-28.88, -5.13), 0.008	
Protein (Percent of energy)	Baseline	13.55 (3.43)	15.23 (2.92)	1.67 (- 0.52, 3.88), 0.130[b]
	End	16.33 (2.78)	15.00 (2.91)	1.39 (- 0.66, 3.46), 0.178[c], 0.222[d]
	MD (95% CI), P[a]	2.77 (0.74, 4.81), 0.010	- 0.23 (-2.44, 1.97), 0.824	
Carbohydrates (g)	Baseline	303.52 (142.66)	318.45 (101.84)	14.93 (-70.77, 100.63), 0.725[b]
	End	234.94 (97.94)	221.46 (74.54)	18.52 (-35.15, 72.20), 0.487[c], 0.584[d]
	MD (95% CI), P[a]	- 68.58 (-134.44, -2.72), 0.042	- 96.99 (-141.51, -52.47), < 0.001	
Carbohydrates (Percent of energy)	Baseline	65.94 (9.57)	69.11 (7.49)	3.17 (-2.76, 9.11), 0.285[b]
	End	62.61 (8.00)	64.64 (6.66)	-1.59 (- 6.78, 3.58), 0.535[c], 0.743[d]
	MD (95% CI), P[a]	-3.33 (-8.27, 1.61), 0.173	- 4.47 (-10.07, 1.12), 0.110	
Fat (g)	Baseline	37.94 (22.58, 58.87)	29.06 (17.49, 40.54)	- 0.08 (- 0.28, 0.10), 0.368[b]
	End	31.98 (18.61, 68.19)	26.78 (18.72, 45.79)	- 0.005 (- 0.17, 0.16), 0.950[c], 0.376[d]
	MD, P[a]	-5.96, 0.439	-2.28, 0.945	
Fat (Percent of energy)	Baseline	20.50 (9.06)	15.64 (6.14)	- 4.85 (-10.21, 0.50), 0.74[b]
	End	21.05 (8.03)	20.35 (5.40)	-1.08 (-5.68, 3.50), 0.633[c], 0.417[d]
	MD (95% CI), P[a]	0.55 (-3.67, 4.78), 0.785	4.70 (0.94, 8.46), 0.017	
Fiber (g)	Baseline	14.56 (10.01, 22.10)	15.43 (10.94, 28.74)	0.05 (- 0.12, 0.23), 0.539[b]
	End	18.62 (10.36, 22.08)	13.58 (7.83, 24.39)	0.009 (- 0.17, 0.19), 0.925[c], 0.935[d]
	MD, P[a]	4.06, 0.837	-1.85, 0.355	
PA (METs)	Baseline	685.50 (267.50, 2571.00)	840.00 (259.00, 2939.75)	0.12 (- 0.61, 0.86), 0.732[b]
	End	1071.00 (329.00, 2338.87)	1077.00 (675.00, 1968.00)	- 0.06 (- 0.38, 0.26), 0.709[c], 0.397[d]
	MD, P[a]	385.5, 0.374	237, 0.336	

WP, Whey protein; *PA*, Physical activity; *METs*, Metabolic equivalent tasks (MET-minutes/ week.)

Mean (SD) and Mean difference (95% CI) are presented for normally distributed data; Median (25^{th} and 75^{th} percentiles) and median differences are presented for data not normally distributed (fat, fiber, and PA). Not normally distributed data are analyzed after log transformation

[a] Paired samples t-test

[b] Independent samples t-test

[c] ANCOVA test, adjusted for baseline values (Model 1)

[d] ANCOVA test, adjusted for baseline values, age, diabetes duration, drugs, changes in BMI, intake of energy and macronutrients, and physical activity (Model 2)

Table 4 Anthropometric indices and resting energy expenditure of the study participants throughout the study

Variable	Period	WP ($n=18$)	Placebo ($n=17$)	MD (95% CI), p
Weight (kg)	Baseline	81.88 (12.84)	80.01 (16.05)	-1.86 (-11.83, 8.10), 0.706[b]
	End	81.28 (12.87)	79.95 (16.23)	- 0.52 (-2.17, 1,13), 0.526[c], 0.759[d]
	MD (95% CI), P[a]	- 0.59 (-1.85, 0.67), 0.335	- 0.06 (-1.16, 1.03), 0.902	
BMI (kg/m^2)	Baseline	32.54 (4.26)	31.66 (5.04)	- 0.88 (- 4.08, 2,32), 0.579[b]
	End	32.36 (4.56)	31.66 (5.02)	- 0.18 (- 0.81, 0.43), 0.543[c], 0.750[d]
	MD (95% CI), P[a]	- 0.17 (- 0.65, 0.29), 0.436	0.00 (- 0.41, 0.41), 1.00	
WC (cm)	Baseline	104.56 (12.15)	100.80 (11.16)	-3.76 (-12.35, 4.82), 0.378[b]
	End	103.87 (12.18)	102.00 (12.68)	-2.00 (- 4.29, 0.28), 0.084[c], **0.040*[d]**
	MD (95% CI), P[a]	- 0.68 (-2.50, 1.13), 0.434	1.20 (- 0.22, 2.62), 0.092	
HC (cm)	Baseline	115.00 (9.23)	112.60 (10.77)	-2.40 (- 9.75, 4.95), 0.510[b]
	End	114.06 (9.23)	112.13 (11.40)	- 0.46 (-2.47, 1.53), 0.637[c], 0.763[d]
	MD (95% CI), P[a]	- 0.93 (-2.07, 0.20), 0.101	- 0.46 (-2.19, 1.25), 0.571	
WHR	Baseline	0.90 (0.06)	0.89 (0.04)	- 0.01 (- 0.05, 0.02), 0.520[b]
	End	0.90 (0.05)	0.90 (0.06)	- 0.01 (- 0.03, 0.01), 0.252[c], 0.059[d]
	MD (95% CI), P[a]	0.001 (- 0.01, 0.01), 0.866	0.01 (- 0.002, 0.03), 0.079	
REE (Kcal/day)	Baseline	1410.45 (368.80)	1436.67 (352.18)	26.22 (-222.05, 274.51), 0.831[b]
	End	1402.06 (172.28)	1505.68 (241.94)	- 98.58 (-236.61, 39.44), 0.155[c], 0.163[d]
	MD (95% CI), P[a]	-8.38 (-190.30, 173.54), 0.924	69.00 (-104.09, 242.10), 0.411	

WP, Whey protein; *MD*, Mean difference; *BMI*, Body mass index; *WC*, Waist circumference; *HC*, Hip circumference; *WHR*, Waist to hip ratio; *REE*, Resting energy expenditure

Mean (SD) and Mean difference (95% CI) are presented for normally distributed data. * $p < 0.05$ was considered statistically significant.

[a] Paired samples t-test. [b] Independent samples t-test. [c] ANCOVA test, adjusted for baseline values (Model 1). [d] ANCOVA test, adjusted for baseline values, age, diabetes duration, drugs, changes in BMI, intake of energy and macronutrients, and physical activity (Model 2)

Discussion

In the present trial, we studied the long-term effects of WP by the administration of whole wheat bread fortified with 20 g WPC, and by considering that bread is a staple food of people in Iran.

The findings of this study showed that daily intake of the WPC fortified bread by women with T2DM for three months had beneficial effects on oxidative stress and anthropometric indices by decreasing MDA and WC, respectively. Moreover, it decreased hunger sensation changes. However, the other anthropometric and appetite parameters, as well as TAC and REE did not change significantly.

Regarding body weight, our result is inconsistent with the result of the Jakubowicz et al.'s study [35]. Correspondingly, they examined the effects of three different types of breakfasts as whey breakfast diet (WBdiet) containing 42 g protein of which 28 g were whey at breakfast; protein breakfast diet (PBdiet) containing 42 g protein from various protein sources (eggs, tuna, and soy); and carbohydrate breakfast diet (CBdiet) containing 17 g soy protein at breakfast, in adults with T2DM for 12 weeks. As a result, they found that the greatest reduction in body weight was achieved in WBdiet compared to the PBdiet and CBdiet. The differences between the findings of our study and those of the Jakubowicz et al.'s study might be due to the higher dose of WP in WBdiet in the Jakubowicz et al.'s study. Regarding WC, our findings are in agreement with the results of some studies [12, 33]. However, concerning weight and BMI, our results are inconsistent with the findings of those studies. Flaim et al. administered 20 g WPI before lunch and 20 g WPI before dinner, and Hassanzadeh-Rostami et al. assessed the effects of biscuits fortified with WPI and bran in overweight or obese adults for 8 weeks. As a result, both studies showed a significant change in weight, BMI, and WC. It seems that higher doses and different forms of WP as well as different supplementation modalities in those studies are the possible reasons for these inconsistencies.

The current study showed that the consumption of fortified bread with WPC led to no significant within- or between-groups changes for REE. Our result was in line with the finding of Fuglsang-Nielsen et al.'s study [43], but it was

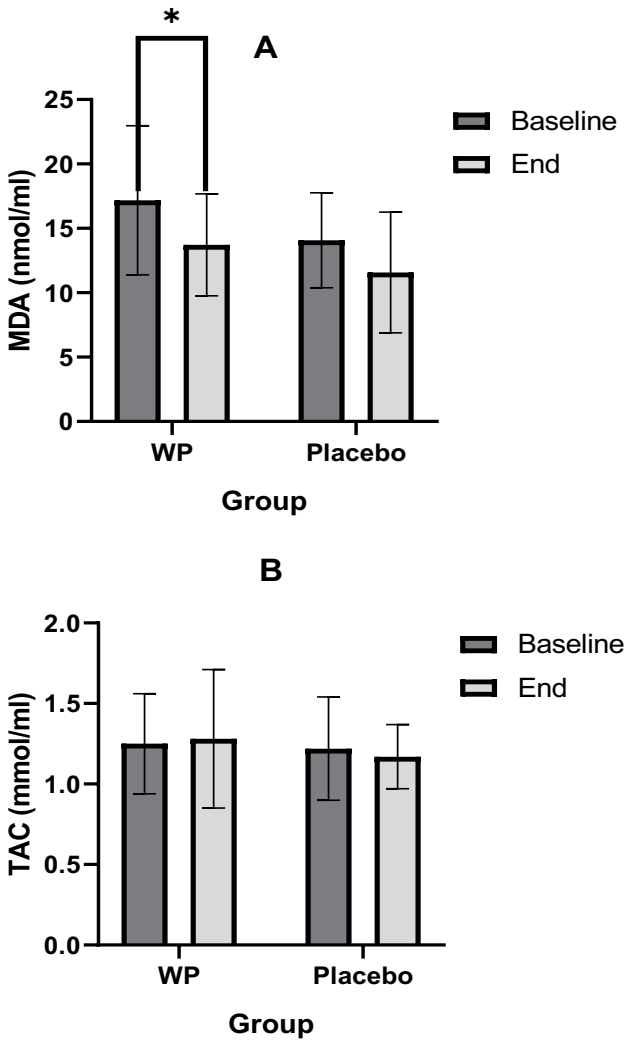

Fig. 2 A, Serum MDA levels at baseline and end of the intervention in women with T2DM; the serum levels of MDA significantly decreased in the WP group ($p=0.022$), the within-group change of MDA was close to significance in the placebo group ($p=0.058$). B, Serum TAC levels at baseline and end of the intervention in women with T2DM; there were no significant within-group changes in both WP and placebo groups ($p>0.05$). The values are mean ± SD. The mean values were significantly different (Paired samples *t*-test): *$p<0.05$. WP: Whey Protein; MDA: Malondialdehyde; TAC: Total antioxidant capacity; T2DM: Type 2 diabetes mellitus

inconsistent with those of some other studies [31, 44-46]. The difference between our results and findings of the Madzima et al.'s study seems to be due to the characteristics of the included participants; they included physically active college-aged men and declared that the increment of REE was related to healthy, physically active individuals [45]. The differences in dose and form of the administered WP, administration time and modality, as well as the procedure and equipment for REE measurement are the other probable reasons for the disagreement between our result and the findings of those studies.

The present study showed a significant decrease in MDA in the WP group but no significant effect was found on TAC levels. It has been shown that cysteine-rich WP could increase glutathione (GSH) [47-50], which favors the vitality and functionality of immune system cells and also plays a role in the detoxification of hydrogen peroxide by glutathione peroxidases (GSH-Px) [51]. Regarding TAC, our results were in agreement with the results of the Flaim et al.'s study. They reported that supplementation with 40 g/day WPI for 12 weeks did not affect the total antioxidant status of patients affected by T2DM or IFG [33]. As well, our results were in line with those of the Sadeghpour et al.'s study conducted on patients with acute ischemic stroke [52], except for within-group changes that were found in the WP group in our study. Accordingly, this difference may be related to less duration of the intervention, the different clinical statuses of the patients, and the administration of lipoic acid along with WP in the Sadeghpour et al.'s study. In a recently performed 3-month intervention study, Derosa et al. reported a significant improvement in oxidative stress markers (glutathione peroxidase and superoxide dismutase) of individuals with T2DM after WPI supplementation [34]. Besides the difference in the assessed markers, higher doses and different forms of WP in the Derosa et al.'s study may be the other possible reasons for the discrepancy between our results and their findings. El-Desouky et al. showed that WP treatment for 14 consecutive days, 20 h after γ-irradiation, led to a significant increase in TAC; however, it had no significant effect on MDA in γ-irradiation exposed rats [53]. Of note, our results were in contrast with their findings and this disparity may probably be related to the different design of the El-Desouky et al.'s study.

The results of our study showed no significant within- or between-group changes for parameters of appetite sensations except for "feeling of hunger", which was significantly lower in the WP group at both the beginning and the end of the intervention. Some acute studies showed that WP increases satiety and reduces food intake at the next meal [54, 55]. It seems that this effect of WP can be mediated by the stimulation of some gut hormones such as cholecystokinin (CCK), Peptide YY (PYY), and Glucagon-like peptide 1 (GLP-1) as well as by suppression of ghrelin [56-58]. Our findings were in line with those of Madzima et al. and Fuglsang-Nielsen et al.'s studies [43, 45]. However, Madzima et al. assessed the short-term appetite, while in our study, the long-term appetite was assessed. A meta-analysis of randomized controlled trials conducted on the effect of WP supplementation on long and short-term appetite showed a significant reduction in long-term appetite in combined appetite score, but no significant reduction was observed in short-term appetite [32]. It seems that the differences between our results and findings of the Mollahosseini et al.'s study might be due to higher doses of WP used in long-term studies that were included

Table 5 Appetite sensations of the study participants throughout the study

Variable	Period	WP ($n=18$)	Placebo ($n=17$)	MD (95% CI), p
Hunger	Baseline	2.00 (0.00, 4.25)	4.00 (3.00, 5.00)	0.24 (0.02, 0.47), 0.031[b]
	End	2.5 (0.00, 5.25)	5.00 (4.50, 6.00)	- 0.22 (- 0.45, 0.003), 0.053[c], 0.045[d]
	MD, P[a]	0.5, 0.399	1.00, 0.435	
Fullness	Baseline	6.52 (2.76)	6.46 (2.79)	- 0.20 (-2.17, 1.77), 0.838[b]
	End	7.17 (3.12)	5.40 (2.50)	1.48 (- 0.64, 3.61), 0.165[c], 0.221[d]
	MD (95% CI), P[a]	0.64 (-1.12, 2.41), 0.450	-1.06 (-3.39, 1.25), 0.342	
Desire to eat	Baseline	4.00 (2.00, 5.50)	5.00 (3.00, 6.00)	0.13 (- 0.03, 0.31), 0.117[b]
	End	5.00 (0.00, 6.25)	5.00 (3.00, 6.00)	- 0.09 (- 0.34, 0.15), 0.426[c], 401[d]
	MD, P[a]	1.00, 0.944	0.00, 0.444	
Desire to eat sweet foods	Baseline	5.50 (2.75, 8.00)	5.00 (3.00, 7.50)	- 0.06 (- 0.23, 0.10), 0.441[b]
	End	5.00 (2.75, 9.25)	5.00 (4.00, 6.00)	- 0.08 (- 0.25, 0.09), 0.346[c], 0.230[d]
	MD, P[a]	- 0.50, 0.262	0.00, 0.671	
Desire to eat salty foods	Baseline	4.00 (1.00, 5.00)	4.00 (1.00, 6.00)	- 0.01 (- 0.22, 0.18), 0.850[b]
	End	3.00 (2.00, 6.50)	3.00 (1.50, 5.00)	0.08 (- 0.07, 0.23), 0.301[c], 0.291[d]
	MD, P[a]	-1.00, 0.428	-1.00, 0.653	
Desire to eat fatty foods	Baseline	1.50 (0.00, 4.00)	2.00 (0.50, 5.00)	0.11 (- 0.10, 0.34), 0.296[b]
	End	1.50 (0.75, 4.00)	2.00 (0.00, 4.00)	0.07 (- 0.10, 0.24), 0.409[c], 0.237[d]
	MD, P[a]	0.00, 0.416	0.00, 0.363	

WP: Whey protein

Mean (SD) and Mean difference (95% CI) are presented for normally distributed data (Fullness); Median (25^{th} and 75^{th} percentiles) and median differences are presented for data not normally distributed. Not normally distributed data are analyzed after log transformation

[a] Paired samples t-test

[b] Independent samples t-test

[c] ANCOVA test, adjusted for baseline values (Model 1)

[d] ANCOVA test, adjusted for baseline values, age, diabetes duration, drugs, changes in BMI, intake of energy and macronutrients, and physical activity (Model 2)

in their meta-analysis as well as different methods proposed for appetite assessment. Our findings were inconsistent with those of two other studies [12, 46]. Kjølbæk et al.'s study employed a different method for appetite assessment [46]; they assessed the feeling of satiety using VAS score by passing 3 h from WP consumption. In addition, since they only explained the results of VAS score for satiety in the thirty-second week, it seems that they only compared the scores of both supplement and control groups in that week. The discrepancy between our results and the results of Hassanzadeh-Rostami et al.'s study may be related to the differences in methods and questionnaires used for appetite assessment. They used a 100 mm VAS including 4 questions and calculated a composite appetite score [12].

Strengths and limitations of the study

To the best of our knowledge, this study appears to be the first long-term RCT that used whole wheat bread fortified with WPC for examining the long-term effects of the WP on anthropometric parameters, REE, oxidative stress markers (TAC and MDA), and appetite among women with T2DM. The provision of dietary plans based on the individual characteristics of each patient can be considered the main strength of this study. Another strength of the present study was the every-two-week visits of the participants, which led to better monitoring of the patients and the increased motivation of participants. As well, there were some limitations in our trial, including subjective assessment of dietary intakes, because the accuracy of the obtained results via this method is dubious. In addition, it seems that the limitation on the consumption of other kinds of bread during the intervention period could lead to a reduction in patients' adherence after a while. Failure of the indirect calorimetry device also was another limitation of our study.

Conclusion

Based on our findings, in overweight/obese women with T2DM, daily consumption of whole wheat bread fortified with 20 g WPC for three months had significant beneficial effects on the feeling of hunger, oxidative stress, and anthropometric parameters by decreasing the MDA and WC, respectively. There were no side effects/ intolerance to WPC in the patients of present study, except two cases which, had gastrointestinal

complications and could not continue the trial. Subjective assessment of dietary intakes, because of the less accuracy; the limitation on the consumption of other kinds of bread during the intervention period which, may lead to a reduction in patients' adherence after a while; and failure of the indirect calorimetry device were the limitations of our study. Performing further studies by including a control group receiving no interventions, except individualized low-calorie diets, is recommended to more obviously clarify the possible beneficial effects of the intake of WPC-fortified bread on anthropometric, oxidative stress, and metabolic parameters in individuals with T2DM.

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Author contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by Maryam Nouri. Data analysis was performed by Maryam Nouri and Mohammad Asghari Jafarabadi. The first draft of the manuscript was written by Maryam Nouri and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability All data of present study are with the corresponding author.

Declarations

Ethics approval The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethics code; IR.TBZMED.REC.1397.687) approved this study. This research was conducted according to the Declaration of Helsinki. The present trial was registered in the Iranian Registry of Clinical Trials (http://www.irct.ir, Registration Number: IRCT20110123005670N26).

Research involving human participants All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (date of approval: 11.19.2019) (ethics code; IR.TBZMED.REC.1397.687)) and with the Helsinki Declaration of 1964 and later versions.

Consent to participate Informed consent or substitute for it was obtained from all patients for being included in the study.

Conflict of interest Authors declare no conflict of interest.

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ORIGINAL ARTICLE

Lack of *Faecalibacterium prausnitzii* and *Bifidobacterium* is associated with a higher risk of metabolic associated fatty liver disease in young-onset type 2 diabetes

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Abstract

Background The incidence of comorbidity between type 2 diabetes mellitus (T2DM) and metabolic-associated fatty liver disease (MAFLD) is high, and patients tend to be younger. When people develop metabolic diseases such as T2DM and MAFLD, the original homeostasis of the gut microbiota in the body is disrupted, and gut flora drift occurs. This study investigated the relationship between the number of gut flora and MAFLD in young-onset T2DM.

Methods This retrospective study analyzed 44 adolescent T2DM patients who were divided into a non-MAFLD group and a MAFLD group. Anthropometric measurements, clinical and biochemical markers, inflammatory markers, thyroid function assessments, and stool specimens were collected. Real-time PCR was performed to quantify several important gut flora constituents at the genus level. Student's t-test and the chi-square test were applied for group comparisons, and binary regression models were used to explore the relationship between gut flora and MAFLD in young-onset T2DM.

Results Among the 44 subjects, 26 (59.1%) were diagnosed with MAFLD, and 18 (40.9%) were not. Compared with the non-MAFLD group, body mass index (BMI), abdominal circumference, and levels of blood uric acid and thyroid stimulating hormone (TSH) in the MAFLD group were significantly increased, and age level and high-density lipoprotein cholesterol (HDL-C) were significantly decreased ($p < 0.05$). Compared with the non-MAFLD group, the abundance of *Faecalibacterium prausnitzii* and *Bifidobacterium* in the MAFLD group was significantly reduced, and the abundance of *Enterococcus* and *Lactobacillus* was significantly increased ($p < 0.05$). In the multivariate regression analysis, *Faecalibacterium prausnitzii* and *Bifidobacterium* were independent protective factors for MAFLD in young-onset T2DM, after excluding confounding factors.

Conclusion In young-onset T2DM, there was a difference in gut flora between patients with MAFLD and those without MAFLD. *Faecalibacterium prausnitzii* and *Bifidobacterium* were independent protective factors for MAFLD in young-onset T2DM.

Keywords Young-onset T2DM · Metabolic associated fatty liver disease · Gut flora · *Faecalibacterium prausnitzii* · *Bifidobacterium*

Introduction

Type 2 diabetes mellitus (T2DM) has emerged as one of the most severe health concerns worldwide and is traditionally considered to be a chronic disease of older individuals. However, due to changing lifestyles, including a sedentary lifestyle, sleep structure alterations, physical inactivity, and overeating, the largest increase in T2DM in this century has occurred among adolescents and adults under the age of 40. [1] We classified patients as having young-onset diabetes if they were diagnosed before the age of 40 years and as having late-onset diabetes if they were diagnosed at 40 years or older [2]. Metabolic-associated fatty liver disease (MAFLD) is a chronic liver disease that involves genetic susceptibility and metabolic and environmental factors, and its incidence rate has been increasing annually, affecting approximately 25% of the global population. [3] The diagnostic criteria for MAFLD are based on evidence of hepatic steatosis in addition to one of the following three criteria, namely, overweight/obesity, presence of type 2 diabetes mellitus, or evidence of metabolic

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dysregulation [4]. Metabolic disorders such as impaired glycemic control, insulin resistance (IR), and visceral obesity are considered to be the major risk factors for MAFLD, as well as T2DM. Therefore, the prevalence of MAFLD is high in T2DM patients. According to a meta-analysis that included 80 studies from 20 different countries, 47.3–63.7% of patients with T2DM are perceived to have MAFLD. [5] Huang et al. revealed that patients with MAFLD diagnosed by diabetes alone are more susceptible to hepatic fibrosis, which may eventually lead to cirrhosis and cardiovascular disease. [6]

The gut flora are considered the second genome in the human body. It has been established that crosstalk occurs between gut microbiota and human health, as the gut flora have the ability to aid in absorption and metabolism, fight against harmful bacteria, improve immunity, and fight tumors. [7] There is growing evidence that the gut microbiota can act as an endocrine organ involved in the dynamic regulation of the body's energy homeostasis and immune response. [8] When genetics, environmental factors, diet, and behavioral patterns change, the previously stable gut flora in the body drift. Recent years have shown that patients with T2DM and NAFLD exhibit drifting intestinal flora. A study examined the gut flora of people with normal glucose tolerance (NGT), prediabetes (pre-DM), and newly diagnosed T2DM. The results indicated that the abundance of butyrate-producing bacteria was higher in the NGT group than in the pre-DM group and that the abundance of the genus *Bacteroides* in the T2DM group was only half that of the NGT and pre-DM groups. [9] Loomba et al. found that among 86 patients with biopsy-proven MAFLD, those with advanced liver fibrosis had a higher abundance of the phylum Proteobacteria, those with stage 0–2 liver fibrosis had a higher abundance of the phylum Firmicutes, and 37 (of 40) predictors of advanced liver fibrosis were associated with gut flora [10]. Impaired intestinal barrier function and increased mucosal permeability in T2DM patients lead to increased absorption of lipopolysaccharide, which is the initiator of the inflammatory cascade and leads to chronic inflammation and insulin resistance by activating specific Toll-like receptor signaling pathways on the surface of hepatocytes, thereby accelerating the formation of MAFLD [11]. However, there is a lack of animal experiments and clinical studies on the changes in gut flora with the coexistence of MAFLD and T2DM, and we found many young-onset T2DM patients with MAFLD in clinical practice. The characteristics of gut flora drift in this group of patients are still unknown.

In this retrospective study, we collected clinical data from adolescent T2DM patients and compared the differences in several major gut flora (e.g., *Faecalibacterium prausnitzii*, *Escherichia coli*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Bacteroides*, and the phylum Firmicutes) between MAFLD patients and non-MAFLD patients. This study investigated the relationship between the number of gut flora and MAFLD in young-onset T2DM.

Materials and methods

Study participants

A total of 44 patients with T2DM who visited our hospital and received liver ultrasound from October 2020 to July 2021 were recruited for the study. The recruitment criteria were as follows: 1) adolescents and adults less than 40 years old, 2) sex not limited, 3) complete clinical data, and 4) informed consent. The exclusion criteria were as follows: 1) pregnant or lactating women; 2) acute complications such as diabetic ketoacidosis and hyperosmolar coma; 3) patients with acute and chronic infections; 4) patients with acute cardiac, hepatic, gastrointestinal, renal and cerebrovascular lesions; 5) patients with serious trauma or surgery within 6 months; 6) patients with malignant tumors and clinical connective tissue diseases; or 7) other serious endocrine metabolic diseases (such as hyperthyroidism, hypothyroidism, Cushing's syndrome). This retrospective study was approved by the Ethics Committee of XXX Hospital (No. 2020018), and the participants provided written informed consent for personal information collection. Finally, the participants were divided into a non-MAFLD group ($n=18$) and a MAFLD group ($n=26$) according to MAFLD diagnostic criteria. [4]

Real-time qPCR and microbial quantification

Early morning fecal samples were collected from all patients, and the fresh fecal samples (10 ± 5 g) were snap frozen in a -80 °C refrigerator within 2 h. Total microbial DNA was extracted from all stool specimens using a QIAamp DNA Stool Mini Kit (QIAGEN, Frankfurt, Germany). Quantitative real-time PCRs (qPCRs) were performed with FTC-3000TM real-time quantitative thermal cycler (Funglyn, Shanghai, China). All qPCRs were run with 3 replicates per DNA. Standard curves were set up by serially diluting the pMD18-T vector plasmid with the appropriate insert from 10^7 to 10^2 target gene copies μl^{-1} for every primer set. The standard curve was obtained using linear regression of threshold cycle numbers (ct) versus log copy numbers of targets. Real-time qPCRs were performed in 25 µl reaction mixtures that were composed of 12.5 µl SRBR Premix Ex Taq (Takara, Osaka, Japan), 1 µl of each forward and reverse primer, 5 µl of template DNA, and sterilized deionized water. The primers are described in Table 1. Melting curve analyses were performed from 60 to 96 °C with increments of 0.1 °C per cycle. The amounts of gut flora were log transformed for analysis.

Measurements

Baseline characteristics such as age, sex duration of diabetes, history of smoking, history of drinking, and family history of diabetes were collected by reviewing medical records.

Table 1 16S rRNA gene group-specific primer for quantitative real-time PCR

Target	Forward primers (5′–3′)	Reverse primers (5′–3′)
Total bacteria (16S V4V5)	GTGCCAGCMGCCGCGGTAA	CCGTCAATTCMTTTGAGTTT
Enterococcus	ACTCGTTGTACTTCCCATTGT	CCTTATTGTTAGTTGCCATCATT
Escherichia coli	GTTAATACCTTTGCTCATTGA	ACCAGGGTATCTAATCCTGTT
Firmicutes	TGAAACTYAAAGGAATTGACG	ACCATGCACCACCTGTC
Bacteroides fragilis	ATAGCCTTTCGAAAGRAAGAT	CCAGTATCAACTGCAATTTTA
Faecalibacterium prausnitzii	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA
Lactobacillus	GGAAACAGRTGCTAATACCG	CACCGCTACACATGGAG
Bifidobacterium	CTCCTGGAAACGGGTGG	GGTGTTCTTCCCGATATCTACA

Anthropometric measurements (height, weight, abdominal circumference) were collected according to a standard protocol. Measurements of routine blood tests, fasting plasma glucose (FPG), 2-h postprandial blood glucose (2hPG), glycosylated hemoglobin (HbA1c), triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum uric acid, serum creatinine, thyroid stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) were performed using standard laboratory methods.

Statistical analysis

IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Variables were examined for normality (normal plots), and nonnormally distributed variables were log transformed. The independent-samples t-test (for normally distributed variables) or the Mann−Whitney test (if nonparametric tests were required) was used to evaluate the significant differences in continuous variables, while the chi-squared test was used to evaluate the significant differences in categorical variables. Correlations of gut flora with BMI, abdominal circumference, FBG, and other variables were examined using Pearson correlation coefficients. Binary logistic regression analyses were used to evaluate the association between the amounts of gut flora and risk of MAFLD in young-onset T2DM after correction for potential confounders. A p value < 0.05 was considered statistically significant.

Results

Participant characteristics

A total of 44 participants were divided into a non-MAFLD group ($n = 18$) and a MAFLD group ($n = 26$). There was no statistically significant difference between the two groups in sex, DM course, history of smoking or alcohol consumption, or family history of diabetes ($p > 0.05$). The MAFLD group was younger than the non-MAFLD group ($p < 0.05$).

The MAFLD group had a larger BMI (28.97 ± 5.12 kg/m^2 versus 23.75 ± 3.95 kg/m^2, $p < 0.05$) and abdominal circumference (98.79 ± 13.24 cm versus 86.33 ± 9.00 cm, $p < 0.05$) than the non-MAFLD group. Regarding biochemical features, HDL-C in the MAFLD group was lower than that in the non-MAFLD group (0.97 ± 0.21 mmol/l versus 1.14 ± 0.32 mmol/l, $p < 0.05$), while the blood uric acid level was higher than that in the non-MAFLD group (460.66 ± 190.90 μmol/l versus 367.07 ± 91.89 μmol/l, $p < 0.05$). Regarding thyroid function, TSH was higher in the MAFLD group than in the non-MAFLD group (1.90 ± 0.88 mIU/l versus 1.47 ± 0.37 mIU/l, $p < 0.05$). No significant difference was observed in FPG or 2hPG between the two groups, although the values were relatively abundant in the MAFLD group compared to the non-MAFLD group ($p = 0.123$ and $p = 0.418$, respectively). HbA1c, other lipid profiles (TC, TG, and LDL-C values), and inflammation indicators (NLR, PLR) did not differ between the two groups. The participant characteristics are reported in Table 2.

Quantitative PCR analysis of gut flora

The MAFLD group had lower amounts of *Faecalibacterium prausnitzii* (8.76 ± 0.69 versus 9.45 ± 0.74, $p < 0.05$) and *Bifidobacterium* (6.56 ± 0.78 versus 7.35 ± 1.13, $p < 0.05$) and larger amounts of *Enterococcus* (7.27 ± 1.01 versus 6.66 ± 0.81, $p < 0.05$) and *Lactobacillus* (7.11 ± 0.70 versus 6.51 ± 1.03, $p < 0.05$). We did not observe any significant differences in the amounts of other gut flora (*Bacteroides fragilis*, *Escherichia coli*, Firmicutes) between the MAFLD groups and the non-MAFLD group (Table 3).

Correlation between gut flora and clinical data

Pearson correlation analysis was performed between the significantly different gut flora and related clinical indicators between the two groups. We reported a negative correlation between abdominal circumference, blood uric acid level, NLR, and the abundance of *Faecalibacterium prausnitzii.*

Table 2 Characteristics of young-onset T2DM

Characteristics	Non-MAFLD group ($n=18$)	MAFLD group ($n=26$)	*p value*
Sex, males, *n* (%)	12 (66.7%)	20 (76.9%)	$z=0.564$ $p=0.453$
Age (years)	35.4 ± 7.3	29.2 ± 7.3	$z=2.807$ $p=0.008$
Newly diagnosed diabetes, *n* (%)	3 (16.7%)	8 (30.8%)	$\chi^2=1.128$ $p=0.288$
Alcohol drinkers, *n* (%)	4 (22.2%)	2 (7.7%)	$\chi^2=1.907$ $p=0.167$
HBsAg positive, *n* (%)	1 (5.6%)	2 (7.7%)	$\chi^2=0.076$ $p=0.782$
Family history of diabetes, *n* (%)	13 (72.2%)	16 (61.5%)	$\chi^2=0.504$ $p=0.462$
BMI (kg/m^2)	23.75 ± 3.95	28.97 ± 5.12	$z=-3.631$ $p=0.001$
Abdominal circumference (cm)	86.33 ± 9.00	98.79 ± 13.24	$z=-3.469$ $p=0.001$
FPG (mmol/l)	12.04 ± 6.42	14.68 ± 4.70	$z=-1.573$ $p=0.123$
2hPG (mmol/l)	15.64 ± 6.55	17.04 ± 6.24	$z=-0.711$ $p=0.481$
HbA1c (%)	11.46 ± 3.29	11.54 ± 1.86	$z=-0.104$ $p=0.925$
TG (mmol/l)	3.40 ± 2.64	4.76 ± 4.17	$z=-1.222$ $p=0.229$
TC (mmol/l)	5.63 ± 1.87	5.81 ± 1.71	$z=-0.377$ $p=0.738$
HDL-C (mmol/l)	1.14 ± 0.32	0.97 ± 0.21	$z=2.229$ $p=0.049$
LDL-C (mmol/l)	3.57 ± 1.31	3.48 ± 0.97	$z=0.262$ $p=0.795$
SUA (umol/l)	367.07 ± 91.89	460.66 ± 190.90	$z=-1.926$ $p=0.037$
NLR	2.03 ± 0.77	2.05 ± 0.93	$z=-0.086$ $p=0.749$
PLR	98.91 ± 32.64	93.73 ± 29.94	$z=0.516$ $p=0.606$
TSH (mIU/l)	1.47 ± 0.37	1.90 ± 0.88	$z=-1.874$ $p=0.040$
fT3 (pmol/l)	3.94 ± 0.89	4.25 ± 1.01	$z=-1.017$ $p=0.315$
fT4 (pmol/l)	13.90 ± 1.75	14.24 ± 1.84	$z=-0.576$ $p=0.568$

2hPG, 2-h postprandial plasma glucose; *BMI*, body mass index; *FPG*, fasting plasma glucose; *fT3*, free triiodothyronine; *fT4*, free thyroxine; *HbA1c*, glycosylated hemoglobin; *HBsAg*, hepatitis B surface antigen; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *NLR*, neutrophil-to-lymphocyte ratio; *PLR*, platelet-lymphocyte ratio; *SUA*, serum uric acid; *TG*, triglyceride; *TSH*, thyroid stimulating hormone

We also found that the amount of *Enterococcus* was positively correlated with abdominal circumference; the amount of *Lactobacillus* was positively correlated with abdominal circumference, HbA1c, and LDL cholesterol; and the amounts of *Bifidobacterium* were negatively correlated with abdominal circumference and NLR (Table 4).

Association between the amounts of gut flora and risk of MAFLD in young-onset T2DM

Multivariate binary linear regression analysis was performed to assess the influence of gut flora on the risk of MAFLD (Table 5). The model was adjusted for age, sex, whether

Table 3 Comparison of the amounts of gut flora between the non-MAFLD and MAFLD group

Gut flora	log copies/gram stool		*p value*
	Non-MAFLD group	MAFLD group	
Total bacteria (16S V4V5)	11.16 ± 0.34	10.97 ± 0.69	$z = 0.978$ $p = 0.293$
Enterococcus	6.68 ± 0.85	7.27 ± 1.01	$z = -2.007$ $p = 0.045$
Escherichia coli	8.11 ± 0.88	7.85 ± 1.25	$z = 0.755$ $p = 0.455$
Firmicutes	10.23 ± 0.32	10.04 ± 0.55	$z = 1.363$ $p = 0.180$
Bacteroides fragilis	10.26 ± 0.47	9.87 ± 0.91	$z = 1.662$ $p = 0.104$
Faecalibacterium prausnitzii	9.45 ± 0.74	8.76 ± 0.69	$z = 3.145$ $p = 0.003$
Lactobacillus	6.51 ± 1.03	7.11 ± 0.70	$z = -2.267$ $p = 0.043$
Bifidobacterium	7.35 ± 1.13	6.51 ± 0.75	$z = 2.960$ $p = 0.010$

Table 4 Correlation of gut flora with metabolic indicators in young-onset T2DM

Variables	*Enterococcus*		*F. prausnitzii*		*Lactobacillus*		*Bifidobacterium*	
	r	*p value*	*R*	*p value*	*r*	*p value*	*r*	*p value*
BMI (kg/m2)	0.269	0.078	−0.189	0.219	0.237	0.122	−0.307*	0.042
AC (cm)	0.317*	0.036	−0.301*	0.047	0.309*	0.042	−0.384*	0.010
FPG (mmol/l)	−0.068	0.663	−0.188	0.221	0.021	0.894	−0.184	0.232
2hPG (mmol/l)	−0.193	0.209	−0.250	0.102	0.053	0.732	−0.045	0.770
HbA1c (%)	−0.170	0.270	0.218	0.156	0.374*	0.012	0.141	0.361
HDL-C (mmol/l)	−0.090	0.560	0.108	0.485	−0.155	0.315	0.249	0.102
LDL-C (mmol/l)	−0.180	0.243	0.062	0.689	0.371*	0.013	0.243	0.113
SUA (umol/l)	0.038	0.806	−0.432**	0.003	0.228	0.136	−0.213	0.166
NLR	−0.175	0.286	−0.407*	0.01	−0.045	0.785	−0.365*	0.023
PLR	0.005	0.977	−0.118	0.476	0.001	0.997	0.038	0.819
TSH (mIU/l)	0.165	0.285	0.044	0.778	0.240	0.117	−0.109	0.482
fT3 (pmol/l)	0.079	0.613	0.034	0.827	−0.240	0.121	0.074	0.637
fT4 (pmol/l)	0.130	0.444	−0.045	0.793	−0.149	0.378	−0.290	0.081

r = Pearson's correlation coefficient; * $p < 0.05$; **, $p < 0.001$

diabetes was newly diagnosed, and BMI. The results suggested that after adjusting for confounders, *Faecalibacterium prausnitzii* (OR = 0.197, 95% CI = 0.047–0.820) and *Bifidobacterium* (OR = 0.340, 95% CI = 0.121–0.959) remained as independent protective factors for MAFLD in adolescent T2DM. In other words, as the amounts of *Faecalibacterium prausnitzii* and *Bifidobacterium* increased, the probability of MAFLD decreased in young-onset T2DM.

Table 5 Association between the amounts of gut flora and risk of MAFLD in young-onset T2DM

	Multivariate OR (95% CI), *p* value	
	Unadjusted model	Adjusted model
Enterococcus	2.071 (0.961–4.467), 0.063	-
F. prausnitzii	0.191 (0.056–0.650), 0.008	0.197 (0.047–0.820), 0.028
Lactobacillus	2.332 (1.055–5.156), 0.036	2.255 (0.895–5.683), 0.085
Bifidobacterium	0.358 (0.162–0.794), 0.011	0.340 (0.121–0.959), 0.041

Adjusted model: adjusted for age, sex, whether newly diagnosed diabetes and BMI

Discussion

The main risk of T2DM is the damage to target organs caused by its chronic hyperglycemic state, and MAFLD is the main form of liver damage in T2DM. [12] For the past

few years, it has been shown that gut flora can participate in the development of T2DM, MAFLD, and other metabolic diseases by regulating host energy metabolism and improving the inflammatory response. [13–15] In this study, we compared the clinical characteristics and differences in gut flora between MAFLD and non-MAFLD patients in young-onset T2DM and explored the relationship between the amounts of gut flora and MAFLD in young-onset T2DM. We found that *F. prausnitzii* and *Bifidobacterium* were protective factors for MAFLD in young-onset T2DM, independent of age, sex, DM course, and other confounders.

F. prausnitzii is considered one of the most important members of *Firmicutes* and is one of the most abundant flora in the intestines of healthy individuals, and its absence inevitably induces gut microbiota disturbance. [16] Recent studies have shown that *F. prausnitzii* is strongly associated with intestinal inflammatory diseases such as ulcerative colitis and Crohn's disease; a LACK of *F. prausnitzii* is associated with the diseases and that its presence improves health benefits. [17, 18] *F. prausnitzii* induces the secretion of IL-10, an anti-inflammatory cytokine, in peripheral blood mononuclear cells (PBMCs). Munukka et al. reported that *F. prausnitzii*–treated mice had lower liver fat content, aspartate aminotransferase, and alanine aminotransferase and increased fatty acid oxidation and adiponectin signaling in the liver compared to high-fat control mice. [19] Its exact mechanism is related to an increase in adiponectin expression in visceral adipose tissue, an increase in insulin sensitivity, and decrease in inflammation in subcutaneous and visceral adipose tissue. Our study found that the amounts of *F. prausnitzii* were lower in the MAFLD population and were negatively correlated with SUA and NLR. SUA causes oxidative stress in mitochondria, activates the NLRP3 inflammatory complex, and exacerbates the hepatic inflammatory response and insulin resistance (IR), while NLR is considered a biomarker that reflects the inflammatory state of the body, and when NLR is increased, MAFLD patients are more likely to further develop hepatitis and liver fibrosis. [20] Martin et al. isolated strains of *F. prausnitzii* from the feces of healthy volunteers. [21] By studying their products, antibiotic resistance, immunomodulatory functions, and metabolic characteristics, the researchers found that *F. prausnitzii* has good anti-inflammatory effects and is a good candidate for the next generation of probiotics.

Bifidobacterium is extensively used as a probiotic, and 72 subspecies have been identified and sequenced that play an essential role in maintaining intestinal function and human homeostasis. [22] A metagenomics analysis of the duodenal microbiota of obese patients with T2DM revealed that the abundance of *Bifidobacterium* was significantly lower in obese individuals and obese patients with T2DM than in non-obese individuals. [23] Using a multiomics approach, Turroni et al. analyzed the interactions between four strains of human intestinal commensals (*Bifidobacterium bifidum PRL2010*, *Bifidobacterium adolescentis 22L*, *Bifidobacterium breve 12L*, and *Bifidobacterium longum* subsp. *infantis ATCC15697*) in the intestine of mice. [24] The results revealed that *Bifidobacterium* cooperated and established a symbiotic relationship with each other in a competitive environment through glycan harvesting, glycan breakdown, and crossfeeding behavior. The composition of the rat cecal microflora significantly changed with the introduction of *Bifidobacterium*, and the abundance of sugar-degrading flora such as *Lactobacillus* increased. Therefore, the intestinal degradation of plant carbohydrates and host polysaccharides was enhanced. Animal research demonstrated that probiotics from the combination of *Lactobacillus* and *Bifidobacterium* delayed the progression of high-fat-diet-induced NAFLD in rats, with significant reductions in body weight, serum free fatty acid, TG, ALT, IL-1β, and IL-18 levels; significant reductions in Gpr109a expression in liver and adipose tissue; and significant increases in butyric acid levels. [25] The aforementioned study suggested that *Bifidobacterium* was negatively associated with obesity, low-grade inflammation, insulin resistance, and glucose metabolism disorders. Our study also found that the amounts of *Bifidobacterium* were lower in MAFLD patients and were negatively correlated with BMI, abdominal circumference, and NLR, but not significantly correlated with glucose metabolism indicators (FBG, 2hPG, HAb1c).

Although they were not an independent risk factor for MAFLD in young-onset T2DM, we observed that the amounts of *Enterococcus* and *Lactobacillus* were higher in patients with MAFLD. *Enterococcus* are considered the second most common cause of nosocomial infections (following *Escherichia coli*). Based on mouse models and human data, Llorente et al. found that proton pump inhibitors can lead to liver inflammation and hepatocyte death by increasing the amounts of *Enterococcus* in the intestine and promoting their translocation. [26] Interestingly, as a probiotic, several studies have observed a positive correlation between *Lactobacillus* and obesity. [27–29] It has also been found that *Lactobacillus rhamnosus GG*, the most common probiotic, prevents the development of fatty liver by competing with the host intestine for fatty acids and inhibiting fatty acid metabolism and absorption in the intestine to inhibit liver fat accumulation. [30] Since dysbiosis of *Lactobacillus* is common in patients with T2DM and MAFLD, the mechanisms by which it acts on these two metabolic diseases need to be further explored.

In addition, exploring the clinical characteristics of patients with combined MAFLD in T2DM, we found that even among adolescents and adults less than 40 years old, patients with MAFLD were younger (35.4 ± 7.3 years versus 29.2 ± 7.3 years, $p = 0.008$), which may be related to the multiple burdens of study, career, and family in younger T2DM patients and more unhealthy living, eating, and sleeping

habits, thus increasing the metabolic disorder risk. We also found that adolescent T2DM combined with MAFLD was characterized by high BMI, high abdominal circumference, low HDL-C, and high SUA ($p < 0.05$). It is worth mentioning that TSH levels were higher in the MAFLD group. A cross-sectional study showed that in a T2DM population with HbA1c ≥ 7%, elevated levels of TSH were associated not only with the prevalence of MAFLD but also with the degree of MAFLD steatosis. [31] Existing research has shown the following mechanism: TSH acts on hormone-sensitive triglyceride lipase (HSL) and adipose triglyceride lipase (ATGL) to promote adipocyte differentiation and thus inhibit adipose tissue breakdown. In addition, TSH increases triglyceride synthesis by regulating the expression activity of sterol regulatory element-binding protein-1c (SREBP-1c) in hepatocytes. [32]

This study entailed some limitations. First, the sample size was small, and the results were limited by geographic constraints. Second, a detailed medication history was not taken, and the effect of glucose-lowering drugs on gut flora could not be excluded. Third, due to the retrospective nature of this study, a causative relationship between gut flora and MAFLD in young-onset T2DM could not be established. Therefore, in further research, we should design multicenter prospective studies based on large sample sizes and collect medication histories that may affect the gut flora.

Conclusion

In conclusion, our study revealed that the gut flora of MAFLD patients and non-MAFLD patients in young-onset T2DM were different. The amounts of *F. prausnitzii* and *Bifidobacterium* were lower and the amounts of *Enterococcus* and *Lactobacillus* were higher in the MAFLD group. *F. prausnitzii* and *Bifidobacterium* were protective factors for MAFLD in young-onset T2DM, independent of age, sex, DM course, and other confounders. These results suggest that we might prevent or treat MAFLD in young onset with T2DM by regulating the corresponding gut flora.

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

Declarations

Conflict of interest This retrospective study was approved by the Ethics Committee of XXX Hospital (No. 2020018), and the participants provided written informed consent for personal information collection..

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ORIGINAL ARTICLE

Correlating the role of *KCNJ11* polymorphism (rs5219) and T2DM: A case control study

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Abstract

Background Diabetes is one of the four major types of non-communicable disease which has reached the epidemic proportions leading to major public health problems and concern. Several studies have shown the impact of genetic variations on diabetes pathogenesis. *KCNJ11* gene has been associated with T2DM. Any variation in this gene disrupts the insulin release from β cells ultimately causing diabetes.

Aim The present research aims to resolve whether genetic variants of KCNJ11 have association with susceptibility to T2DM in the North Indian population.

Method PCR-RFLP technique was used to genotype 200 subjects for rs5219 genetic variant of *KCNJ11* gene. Student's *t* test and chi square test (χ^2) were used to evaluate continuous and categorical variables. Association of *KCNJ11*genotypes with T2DM was done by odds ratio (OR) and confidence interval (CI). All statistical analyses were performed using IBM SPSS-21 software.

Results Environmental factors such as smoking and lack of exercise increase the risk for T2DM (OR>1). The genotype frequency distribution for *KCNJ11* rs5219 SNP was in Hardy-Weinberg equilibrium (HWE) for both control (*p*-value-0.96) and T2DM case group (*p*-value-0.685). rs5219 was associated with T2DM in dominant genetic model (*p*-value- <0.001; OR-3.781) and recessive genetic model (*p*-value- <0.001; OR- 3.740). It was found that T allele was a risk allele that increases susceptibility for T2DM.

Conclusion This study elucidated that rs5219 genetic variant of *KCNJ11* may increase the susceptibility for T2DM and TT genotype might be involved in predisposing individuals for development of disease.

Keywords Type 2 diabetes mellitus · *KCNJ11* · Single nucleotide polymorphism · Genetic model

Introduction

Diabetes mellitus, a lifelong chronic metabolic disease is characterized by hyperglycemia. It results from either defects in insulin secretion, its action or both [1]. Globally 463 million people were diagnosed with this disease in 2019. The number is predicted to rise up to 745 million in 2045 (Diabetes Atlas). In 2021, around 77 million people are affected by this disease in India which is predicted to rise up to 134 million people by 2045 [2].

Glucose-induced insulin secretion from β cells of pancreas is regulated by K_{ATP} channel. *KCNJ11* gene localized on chromosome 11 encodes for four pores forming inward rectifier subunit (Kir6.2) of K_{ATP} channel. During fed state, elevated level of blood glucose increases the ATP/ADP ratio, causing K_{ATP} channel to close. The closure causes the depolarization of membrane of pancreatic β cells and voltage-gated calcium channel to open. This leads to an increase in calcium concentration inside the cells which in turn triggers the release of insulin [3]. Mutation in the gene (*KCNJ11*) either impairs the ATP binding site of Kir6.2 or stabilizes the open state of channel. This decreases the sensitivity of channel for its inhibition by ATP [4].

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Several association studies have reported various SNPs viz: rs5219, rs1800467, rs5215, rs241282930, rs2285676 of *KCNJ11* to be associated with T2DM [5–12], but rs5219 has received the most attention for its influence on glycemia [13]. In rs5219 SNP replacement of A to C alters the amino acid lysine to glutamine. This change reduces the ATP sensitivity of the channel and thus has its effect on secretion of insulin [14]. Though several studies have reported the association of rs5219 with disease [15, 16], the contradictory results in some population prevent to confirm it [17–19]. This implies that role of rs5219 in predisposing individual to T2DM is still divisive and warrants further investigation. Therefore the present study was undertaken to examine association of genetic variants of rs5219 with type 2 diabetes mellitus in North Indian population and to compare the levels of biochemical risk factor for susceptibility to diabetes with different genotypes.

Materials and methods

Subject selection

A total of 200 subjects: 100 control (healthy) and 100 T2DM patients (who were on follow-up treatment) were enrolled for the present study. The subjects geographically belonged and lived in the northern part of India, particularly the state of Haryana. The T2DM case subjects considered for the study were according to the diagnostic criteria laid down by the Indian Council of Medical Research (ICMR). Controls were recruited from outpatient department (OPD) of hospitals who were on regular body check-ups. Subjects diagnosed with T1DM and other severe diseases of kidney, liver, and coronary artery disease were excluded from the study. Pregnant and lactating mothers were also excluded.

Demographic characteristics

Demographic characteristics such as age, gender, alcohol, and smoking consumption habits were analyzed using a questionnaire. Information regarding weight and height of individuals were collected during sample collection. Body mass index (BMI) was appraised by dividing the weight/height (kg/m^2). Venous blood samples were drawn early in the morning from subjects after 10–12 h of overnight fasting in both EDTA and non-EDTA coated vials.

Genomic DNA extraction

Genomic DNA was extracted from Miller et al. [20], method with minor modifications. Extracted DNA was visualized in UV transilluminator using 1% agarose gel. Purity of extracted DNA samples was ascertained using A260/A280 ratio. The samples having the ratio in the range of 1.6 to 2.0 were considered for PCR-based analysis. The isolated DNA was stored at −20°C.

Polymorphism chain reaction-restriction fragment length polymorphism (PCR-RFLP) of SNP rs5219

Amplification of desired sequence of *KCNJ11* gene was carried out. A set of primers verified through virtual amplification by using in silico PCR online software was used. The sequence of primers used is shown in Table 1. The targeted amplicon was amplified with reaction mixture (25 µl) using forward and reverse primer (0.5 µl each), $MgCl_2$ (2.5 µl), dNTPs (0.5 µl), nuclease-free water (15.5 µl), Taq buffer (2.5 µl), Taq DNA polymerase (1 µl) and DNA (2 µl) as template. The PCR profile included denaturation initially for 5 min at 94°C, followed by 35 cycles of (a) 45 s at 94°C of denaturation, (b) 45 s at 63.2°C of annealing, (c) 45 s at 72°C of extension; (d) 5 min at 72°C of final extension. Amplified PCR products of 220 bp were resolved by electrophoresis (5V/60 min) in Tris Borate-EDTA (TBE) buffer containing 0.5 µg/ml of ethidium bromide using 1.5% agarose gel. Molecular size ladder of 100 bp (Genei) was used to ascertain the size of the bands. UV Transilluminator was then used for viewing and photograph the gel (Fig. 1).

The amplified amplicon of *KCNJ11* gene was digested in 10 µl aliquot with 2–5 units of Ban II restriction

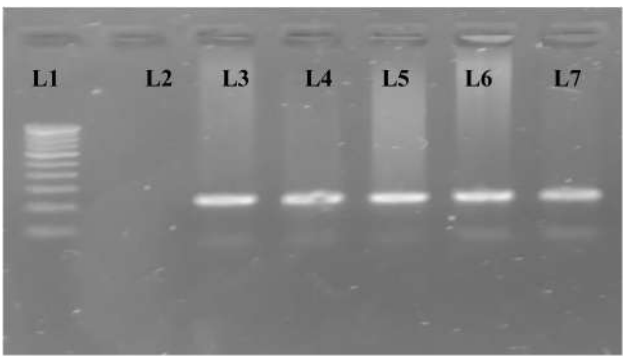

Fig. 1 PCR product of *KCNJ11* rs5219 SNP. Lane 1 shows 100 bp ladder, lane 2 shows blank, lanes 3, 4, 5 and 6 show 220 bp PCR product

Table 1 Primers, product size and restriction enzyme used

Gene	Variant	Primers	Product size	Restriction enzyme (RE)
KCNJ11	**rs5219**	**F-GAATACGTGCTGACACGCCT R-GCCAGCTGCACAGGAAGGACAT**	**220**	**Ban II**

endonuclease at 37°C for 2 h [3]. Restriction enzyme was verified using an online tool Restriction Mapper. The 10 µl reaction mixture consisted of: PCR product (8.5 µl), Ban II (0.5 µl), 10× buffer solution (1 µl). The digested fragments were resolved on 3% agarose gel stained with ethidium bromide and was observed on UV transilluminator. Wild type DNA shows two bands with 151, 69 bp, whereas mutated DNA shows three bands of 28, 41 and 151 bp, heterozygous mutated show four bands of 28, 41, 69 and 151 bp (Fig. 2).

Statistical analysis

The sample size of the present study was calculated by using online software OSSE (online sample size estimator). Sample size at 90% power came out to be 95 for control and T2DM case group respectively.The Hardy-Weinberg Equilibrium (HWE) ($p > 0.05$) for genotype and allele distribution was ascertained by employing $\chi 2$ goodness-of-fit test. Student's *t*-test was used to compare demographic and clinical characteristics of T2DM case and control groups. Chi-square ($\chi 2$) test was applied for comparison of allelic frequencies and genotype distribution. Association of *KCNJ11*genotypes with T2DM was done by odds ratio (OR) and confidence interval (CI) calculation. Data analyzed were defined statistically significant at *p*-value <0.05. All statistical analyses were carried out using IBM SPSS Statistics 21software.

Results

Analysis of demographic characteristics

Analysis of demographic characteristics revealed significant difference among two groups when their smoking status was considered. Smokers are highly susceptible to T2DM (OR-2.95; 95% CI-1.586-5.508, *p*- <0.001). The risk by demographic characteristics have been highlighted in Table 2. Vegetarian diet prevents the risk for diabetes with non-vegetarians being highly susceptible towards T2DM.

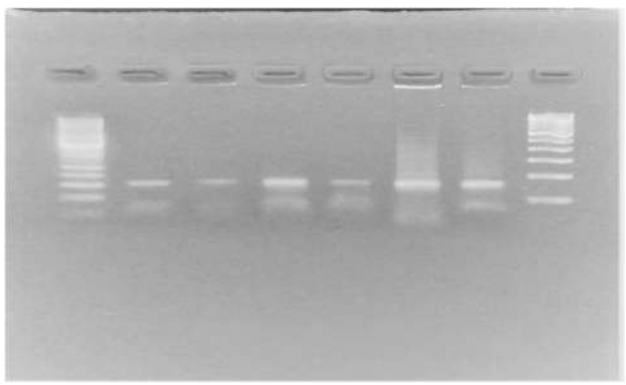

Fig. 2 Restriction digestion of *KCNJ11* rs5219 SNP products. Lane 1 shows 50 bp ladder, lane 8 shows 100 bp ladder, lanes 2, 3, 4, 5 and 7 show 151 and 69 bp band, lane 6 shows 151 and 41 bp band (28 bp band not visible)

Analysis of genotype

The genotype distribution of rs5219 for both control and case were in agreement with Hardy-Weinberg equilibrium (HWE) as calculated by χ^2 goodness of fit test (Table 3).

The genotype distribution frequency of CC, CT, and TT of rs5219 SNP of *KCNJ11* in T2DM group 19%, 54%, 27%, and for the T2DM cases 47%, 44%, and 9% respectively differs substantially with *p* value-0.000 (Table 4). It was observed that CC genotype was significantly higher in the control group whereas TT genotype was considerably higher in case group. Allelic frequency of T was found to be higher in T2DM cases (OR-2.613; 95% CI- 1.736–3.932; *p*- <0.001). Significant association was observed between SNP rs5219 and T2DM in recessive model: OR (3.740); 95% CI (1.656–8.447), *p*- value (< 0.001), and dominant model: OR (3.781); 95% CI (2.003–7.137), *p*- value (<0.000) (Table 4).

Effects of genotypes on clinical characteristics

Comparative analysis of rs5219 SNP genotypes with clinical parameters in cases and control was performed by using the dominant (CT+TT vs.CC) (Table 5) and recessive (TT vs. CC+CT) (Table 6) genetic model. In the recessive genetic model (TT vs. CC+CT), statistical significant

Table 2 Demographic characteristics

Demographic data	OR (95% CI)	*p* value
Gender (F/M)	0.961 (.551–1.675)	0.443
Smoking (Y/N)	2.95 (1.586–5.508)	<0.001*
Alcohol (Y/N)	1.084 (0.622–1.888)	0.198
Exercise (Y/N)	0.295 (0.160–0.544)	<0.001*
Diet (NonVeg/Veg)	2.364 (1.272–4.392)	0.003*

**p* value <0.05 considered as statistically significant; OR, odds ratio; CI, confidence interval

Table 3 Hardy-Weinberg equilibrium test

Genotype	Control	*p*-value	Case	*p*-value
CC	47	0.960	19	0.685
CT	44		54	
TT	9		27	

Table 4 *KCNJ11* rs5219 SNP genotype and allelic distribution frequencies

SNP(rs5219)		Cases (%)	Control (%)	OR(95% CI)	*p*-value
Genotype	CC	19	47		<0.001*
	CT	54	44		
	TT	27	9		
Alleles	T	108 (54)	62 (31)	2.613 (1.736–3.932)	<0.001*
	C	92 (46)	138 (69)		
Recessive model	TT	27	9	3.740 (1.656–8.447)	<0.001*
	CC+CT	73	91		
Dominant model	CT+TT	81	53	3.781 (2.003–7.137)	<0.001*
	CC	19	47		

*p-value <0.05 considered as statistically significant; OR, odds ratio; CI, confidence interval

Table 5 Analysis *of KCNJ11* rs5219 genotype distribution with clinical parameters under dominant model

Parameters	Case			Control		
	CT+TT	CC	*p*-value	CT+TT	CC	*p*-value
BMI (kg/m^2)	27.44 ± 3.30	27.85 ± 3.46	0.313	24.04 ± 3.68	24.47 ± 2.95	0.242
DBP (mmHg)	82.54 ± 9.48	84.89 ± 10.22	0.170	81.00 ± 9.96	81.28 ± 9.17	0.443
SBP (mmHg)	150.17 ± 14.71	141.36 ± 22.08	0.018*	124.7 ± 11.44	122.34 ± 11.87	0.157
PSF (mg/dl)	187.98 ± 61.17	185.26 ± 44.85	0.427	86.18 ± 7.45	87.78 ± 8.5	0.160
HbA1c (%)	7.50 ± 1.76	7.60 ± 1.51	0.405	5.43 ± 0.511	5.51 ± 0.50	0.228
TC (mg/dl)	189.74 ± 47.54	188.67 ± 39.89.74	0.464	154.69 ± 28.70	146.00 ± 36.90	0.094
TG (mg/dl)	193.45 ± 44.28	192.711 ± 49.21	0.474	110.24 ± 21.18	110.59 ± 21.86	0.467
HDL-C (mg/dl)	47.11 ± 17.22	44.01 ± 10.75	0.227	46.88 ± 9.30	49.37 ± 10.44	0.105
LDL-C (mg/dl)	104.57 ± 41.69	110.17 ± 35.87	0.295	57.72 ± 18.48	59.64 ± 22.07	0.319
VLDL-C (mg/dl)	35.93 ± 20.15	37.94 ± 19.11	0.347	24.21 ± 9.97	22.04 ± 9.00	0.129

Data shown as mean ± standard deviation, *p-value <0.05 considered as statistically significant

Table 6 Analysis of *KCNJ11* rs5219 SNP genotypes with clinical parameters under recessive model

Parameters	Case			Control		
	CC+CT	TT	*p*-value	CC+CT	TT	*p*-value
BMI(kg/m2)	27.52 ± 3.27	27.52 ± 3.50	0.499	24.34 ± 3.42	23.01 ± 2.36	0.129
DBP(mmHg)	82.56 ± 9.47	84.14 ± 10.10	0.233	81.21 ± 9.76	80.33 ± 6.98	0.397
SBP(mmHg)	142.30 ±17.46	141.37 ± 11.87	0.399	123.32 ± 11.73	126.33 ± 10.943	0.231
PSF(mg/dl)	180.42 ± 53.78	206.51 ± 66.23	0.023*	87.16 ± 7.94	84.66 ± 8.63	0.187
HbA1c(%)	7.45 ± 1.76	7.68 ± 1.56	0.276	5.47 ± 0.51	5.47 ± 0.40	0.497
TC(mg/dl)	187.03 ± 45.03	214.50 ± 55.22	0.006*	150.47 ± 32.54	152.0 ± 38.75	0.447
TG(mg/dl)	193.86 ±47.73	200.52 ± 38.85	0.274	109.52 ± 21.46	119.33 ± 19.62	0.095
HDL-C(mg/dl)	46.48 ± 11.79	46.63 ± 24.77	0.484	47.75 ± 10.15	43.47 ± 5.77	0.108
LDL-C(mg/dl)	101.89 ± 39.05	102.75 ± 39.31	0.461	58.34 ± 20.866	61.52 ± 11.22	0.327
VLDL-C(mg/dl)	36.35 ± 17.83	36.22 ± 24.97	0.488	22.95 ± 9.29	25.64 ± 12.14	0.216

Data shown as mean ± standard deviation, *p-value <0.05 considered as statistically significant

association was observed with regards to PSF,TC and LDL in TT genotype in T2DM patients with p value 0.023,0.006 and 0.017 respectively (Table 6). The levels of $HbA1_C$ and TG were found to be higher in TT genotype than the CC+CT genotype in the T2DM group but the difference was not statistically different (Table 6). Regarding dominant genetic model except SBP no dissimilarity was observed in clinical parameters among CC and CT+TT genotypes in both control group and case group (p-value <0.05) (Table 5).

Discussion

T2DM is a polygenic disorder that involves contribution of several genetic and environmental factors for its development due to dysfunctional insulin secretion resulting in impaired glucose metabolism. The K_{ATP} channel is involved in glucose-stimulated insulin secretion from pancreatic beta cells. K_{ATP} channel subunit Kir6.2 protein is encoded by *KCNJ11* gene. The activity of K_{ATP} channel is inhibited by ATP and stimulated by MgATP or Mg ADP. Any genetic variation in the *KCNJ11* gene can either reduce ATP's ability to inhibit the K_{ATP} channel's activity or increase MgATP ability to simultaneously stimulate the channel's function [4].

KCNJ11 gene variants at various loci have been demonstrated to be associated with diabetes in number of previous studies [8–12, 21, 22]. The present study investigated and elucidated the association of *KCNJ11* gene variant at rs5219 with T2DM (p value-<0.001). The frequency of the minor allele T (54%) is significantly higher in T2DM subjects (OR-2.613, p value- <0.001) than control subjects in this investigation, indicating that people with this genotype or allele are more likely to develop T2DM. The results of present study corroborated with findings of previous study conducted on Syrian [23] , Chinese Han [24], Iranian [25] and Indian population [9, 17, 26]. A meta-analysis by Wang et al. [27] and Gong et al. [13] also showed rs5219 SNP as risk factor for type 2 diabetes. Due to extensive diversity in the genetic and environmental makeup of the worldwide population, studies carried on Euro-Brazilian [28], South Asian [17], Iranian population [29, 30] Czech [31] and Moroccan population [18] were not able to replicate the result of associating rs5219 genetic variant with T2DM. In these studies mutated allele did not confer risk of T2DM. The current study also revealed relationship of TT genotype with increased risk of T2DM under both dominant (OR-3.781, p value- <0.001) and recessive (OR-3.740, p value-<0.001) genetic model. Similar to our results, Rizvi et al. [32] and Makhzoom et al. [23] also confirmed the association of rs5219 SNP under dominant (p value-0.022) and recessive genetic model (p value-0.035) respectively.

For assessing the presentation and progression of T2DM genotype distributions were correlated with clinical parameters under both dominant (Table 5) and recessive genetic model (Table 6). SBP (Table 5), PSF and TC (Table 6) clinical parameters were observed to be significantly higher in risk genotype. The T2DM subjects with TT genotype exhibited significantly higher levels of PSF in the studied population. These results are consistent with the reports of Bankura et al. [8] who have cited the association of *KCNJ11* rs5219 genetic variant with reduced secretion of insulin. The risk genotype TT genotype is associated with significantly higher levels of cholesterol in the present study (Table 6) which may be due to low cholesterol absorption efficiency associated with insulin resistance [33].The current study also showed association of rs5219 variant with SBP (Table 5) which is in coherence with study of Koo et al. [5].

Apart from genetic factors, plethora of lifestyle factors also contributes towards development of T2DM. The present study concluded the association of diet with risk of T2DM, with non-vegetarian diet increasing the risk. The study corroborates with findings of Chiu et al. [34], who associated lifelong vegetarians with 35% reduced risk for developing T2DM. The present study also observes the association of smoking with T2DM. Maddatu et al. [35] also confirm that smoking increases the risk of development of T2DM. Another lifestyle factor, exercise is also associated with T2DM, conferring decreased risk of T2DM in the current case-control study.

In summary, genetic variation in SNP rs5219 of *KCNJ11* gene is linked with increased risk for predisposing individual to T2DM with the CC genotype being protective and TT genotype conferring the risk for T2DM. This study also observes that controlling the lifestyle/environmental factors can control the risk of developing diabetes. Considering the sample size of present study, further studies with large sample size are needed. The results may be important in the ongoing efforts to identify individuals at increased risk of developing type 2 diabetes mellitus.

Conclusion

The results of the present study indicate that individuals with TT genotype of *KCNJ11* SNP rs5219 are more susceptible for T2DM than individuals with CC genotype in Indian population. T allele was more common in T2DM patients, thus it may be a risk factor in predisposing individual for type 2 diabetes mellitus. However, further studies with large sample size are required to evaluate the association of rs5219 genetic variant of *KCNJ11* as predisposing risk factor for T2DM.

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Authors contribution Archna Bhargave — Conceptualization, Methodology, Data collection and analysis, Writing of original draft for publication

Imteyaz Ahmed — Methodology, Data analysis

Anita Yadav — Conceptualization

Ranjan Gupta — Conceptualization, Methodology, Resources, Data analysis and editing of original draft

All authors have read and approved the final draft.

Declarations

Ethics approval The study protocol was approved by the Institutional Human Ethical Committee Kurukshetra University, Kurukshetra Haryana.

Consent to participate Informed written consent was obtained from all the participating individuals.

Consent for publication The participants provided the written consent to publish their data.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Impaired glucolipid metabolism in gestational diabetes mellitus with T variation of TCF7L2 rs7903146: A case–control study

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Abstract

Background Transcription factor 7-like 2 (TCF7L2) rs7903146 polymorphism has been shown to display a significant association with gestational diabetes mellitus (GDM). But the effects of TCF7L2 rs7903146 on glucose and lipid metabolism are not clear.

Objective The purpose of this study was to assess the role of TCF7L2 rs7903146 genotypes on glycolipid metabolism in GDM.

Methods In total, 484 individuals (239 in GDM group and 245 in control group) were included in the final analysis from January 2015 to February 2022. Their baseline demographics, plasma lipid concentration in the first trimester and third trimester, blood glucose values of the OGTT during gestational 24–28 weeks, glycosylated hemoglobin, fasting plasma glucose and fasting insulin in third trimester, 1 min Apgar scores, 5 min Apgar scores, glucose values of cord blood, and umbilical artery pH were collected. TCF7L2 rs7903146 genotypes were analyzed by polymerase chain reaction-Sanger sequencing.

Results The frequencies of TCF7L2 rs7903146 genotype were found to have no significant differences between the two groups; however, the plasma lipid concentrations during the first trimester were higher in GDM group than control group. In GDM group, women carried the risk allele (T) in TCF7L2 rs7903146 displayed the significantly higher glucose values at 1-h during OGTT, and the higher TG and lower fasting insulin levels than those in non-carriers.

Conclusion Our results indicate that the risk allele (T) in TCF7L2 rs7903146 plays an important role in the abnormality of glucose and lipid metabolism in GDM women. For the risk allele(T) carriers of TCF7L2 rs7903146, low-fat and low-sugar diets, exercise interventions can be carried out at an early stage, and insulin therapy should be considered when their blood glucose were inadequately controlled.

Keywords Gestational diabetes mellitus · Polymorphism · TCF7L2 rs7903146 · Glucose metabolism · Lipid metabolism

Introduction

Gestational diabetes mellitus (GDM) is defined as abnormal glucose tolerance with onset or first recognition during pregnancy. GDM has been shown to be associated with adverse perinatal outcomes: for mothers who are diagnosed with GDM, there are increased risks of preeclampsia, cesarean and type 2 diabetes (T2D); newborns are at increased risks of respiratory distress syndrome, premature birth, macrosomia, shoulder dystocia, and even death. Globally, the incidence of GDM has gradually increased [1]. Therefore, early detection, diagnosis, and treatment are the keys to improve maternal and fetal adverse outcomes. It is well-known that the islet β-cell dysfunction and insulin resistance are the main pathologic mechanisms of GDM [2]. Islet β-cell dysfunction leads hyperglycemia, which in turn exacerbates islet β-cell dysfunction. On the other hand, hyperglycemia plays a key role in maternal dyslipidemia, while abnormal lipid metabolism can cause insulin resistance, leading to a vicious cycle [3, 4]. Therefore, glucose metabolism disorders, lipid metabolism disorders, and their interactions may be closely related to the incidence of GDM.

Genetic factors, especially gene polymorphisms related to islet β-cell function are risk factors for GDM [5, 6]. Many studies had reported that higher frequency of risk transcription factor 7-like 2 (TCF7L2) rs7903146 allele (T) was found in GDM women [6–9]. Lu J and colleagues found that women harboring TCF7L2 rs7903146 TT genotypes displayed significantly higher 1-h blood glucose values than CC genotypes during OGTT; these results suggested that TCF7L2 rs7903146 SNP had significant effects on glucose homeostasis

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[10]. Other studies showed that TT genotypes in TCF7L2 rs7903146 were associated with decreased insulin secretion [11, 12]. In islet β-cells, TCF7L2 gene encodes a transcription factor with a high mobility group (HMG) domain; activated by WNT signal, β-catenin in the cytoplasm is transferred into the nucleus, interacts with TCF7L2, and forms a transcriptional complex; then, the transcriptional complex initiates transcription of target genes after raising a range of activating factors [13, 14]. However, whether TCF7L2 rs7903146 risk allele regulates 1-h blood glucose levels in Chinese population has not been investigated in detail.

Recently, it has been reported that disorder of lipid metabolism was a risk factor for GDM [15]. In order to maintain the normal pregnancy needs, including providing fuels and nutrients to the fetus, the maternal blood lipid levels need to be elevated physiologically [16]. In fact, hypertriglyceridemia is a stimulating factor for insulin resistance, while insulin resistance is one of the underlying mechanisms of GDM [17]. Therefore, excessively elevated blood lipid levels during pregnancy can affect blood glucose metabolism. Recent study reported that embelin attenuated adipogenesis and lipogenesis through Wnt/β-catenin signaling pathway, involved increasing nuclear protein levels of β-catenin and TCF7L2 [18]. However, few reports have evaluated the effect of TCF7L2 rs7903146 polymorphism on lipid metabolism in GDM in Chinese population.

Therefore, the aim of the present study was to determine whether TCF7L2 gene polymorphism rs7903146 influences the glucose and lipid metabolism in Chinese GDM women.

Methodology

Study subjects

The genotype frequency of rs7903146 polymorphic form of TCF7L2 gene in 1000 genome data was used to calculate the required sample size [19]. The sample size was estimated using the GPower v3.1.9.2 for survival analysis, with a significance level of 0.05 (two-tailed) and a power of 0.9. The effect sizes were calculated with Cohen's d: an effect size of 0.2 corresponding to a small effect and an effect size of 0.8 corresponding to a large effect [20]. Combined with the actual situation, the minimum sample size of this study was 200 which calculated with an effect size of 0.325.

A total of 484 women in their 24–28 gestational weeks were enrolled between January 2015 and February 2022; GDM were diagnosed if one or more of the following criteria from the International Association of Diabetes and Pregnancy Study Groups guideline or Chinese Society of Gynecology and Obstetrics guideline: fasting plasma glucose (FPG) 5.1–6.9 mmol/L, 1-h OGTT plasma glucose ≥ 10.0 mmol/L, or 2-h OGTT plasma glucose 8.5–11.0 mmol/L [21, 22]. The GDM group was composed of patients who were diagnosed as GDM during the second trimester. The control group was constituted by euglycemic women. The exclusion criteria were pre-pregnancy diabetes, hyperlipidemia, other endocrine or metabolic diseases, severe liver or kidney diseases, and malignant tumors.

Baseline demographics and clinical characteristics were extracted, including maternal age, pre-pregnancy body mass index (BMI), gestational weeks at delivery, newborn birth weight, 1 min Apgar scores, 5 min Apgar scores, OGTT plasma glucose levels, lipid levels in the first trimester and third trimester, glycosylated hemoglobin (HbA1c), FPG, and fasting insulin before delivery.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University, China, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

DNA extraction

Peripheral blood samples from pregnant women and umbilical cord blood samples were collected. DNA extractions were performed using the Blood DNA Extraction Kit (Heasbio, Guangzhou, China). After the extraction, the DNA samples were stored at − 20 °C.

DNA amplification

The TCF7L2 rs7903146 polymorphism was detected by polymerase chain reaction (PCR) using GC-rich PCR Master Mix (Thermo Scientific, Shanghai, China). The following primers were designed using Primer 3 software: forward primers were (5′-GCCGTCAGATGGTAATGCAG-3′) and reverse primers were (5′-CCCAAGCTTCTCAGTCACAC-3′). The cycling program was as follows: initial denaturation at 95 ℃ for 5 min, 35 cycles including denaturation at 95℃ for 30 s, annealing at 58℃ for 30 s, elongation at 72℃ for 30 s, and a final elongation step at 72℃ for 10 min.

PCR products identifying by electrophoresis

Successful amplification was determined by 1.2% agarose gel electrophoresis at 100 V for 40 min. Single strip of each product was shown in UV light visualization.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 20) and R language (v3.4.1). The frequency

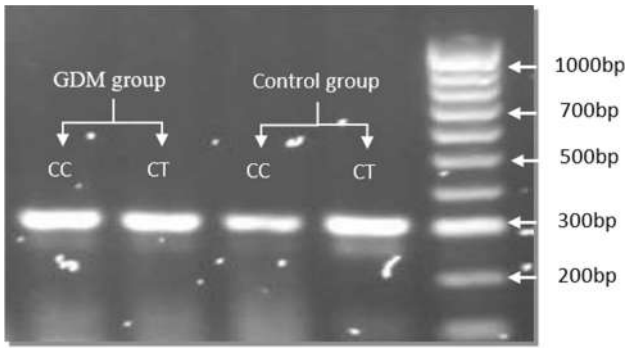

Fig. 1 Representative picture of agarose gel electrophoresis of PCR product of rs7903146

distributions were tested for Hardy–Weinberg equilibrium (HWE) by exact chi-square test. The baseline demographics and clinical characteristics were described as mean and standard deviation (SD). Independent sample *t*-test was applied in quantitative data between two groups. The qualitative data were analyzed with Pearson's chi-square test. Correction for multiple comparisons was performed using FDR-correction. A value of $p < 0.05$ (two-tailed) was considered statistically significant.

Results

Genotype distributions of TCF7L2 rs7903146

A single strip of each product was shown in UV light visualization (Fig. 1). After PCR amplification, products were sequenced by Sanger sequencing. Genotypes CC and CT were found in TCF7L2 rs7903146; no TT genotypes were found in this population (Fig. 2).

Baseline demographics, clinical characteristics, and genotype frequencies of the participants

The baseline demographics of participants are summarized in Table 1. There were no significant differences in maternal age, pre-pregnancy BMI, gestational weeks at delivery, and newborn birth weight between the two groups.

At the first trimester, plasma triglyceride (TG) and total cholesterol (TC) concentrations were significantly higher in GDM group than those in control group, while there were no significant differences in TG and TC values at the third trimester between the two groups. No significant differences were found in high-density lipoprotein (HDL), low-density lipoprotein (LDL), HbA1c, FPG (before delivery), fasting insulin, 1 min Apgar scores, 5 min Apgar scores, and umbilical artery pH between the two groups. Cord blood glucose levels were lower in GDM group than those in control group. No significant differences were found in the T allele frequencies between the two groups (Table 2).

Influence of TCF7L2 rs7903146 genotype on glycolipid metabolism of GDM women

The baseline characteristics of GDM patients according to TCF7L2 rs7903146 genotypes are shown in Table 3. In the first trimester, no significant differences were found in blood lipid levels between the carriers of risk allele (T) and non-carriers. In the second trimester, OGTT 1-h blood glucose levels were significantly higher in the carriers of risk allele (T). Furthermore, significantly elevated TG and decreased fasting insulin concentrations were detected in the carriers of risk allele (T) during the third trimester.

We also extracted the characteristics of control participants, and the data are shown in Table 4. There were no significant differences in OGTT 1-h blood glucose levels at the second trimester and TG and fasting insulin levels at

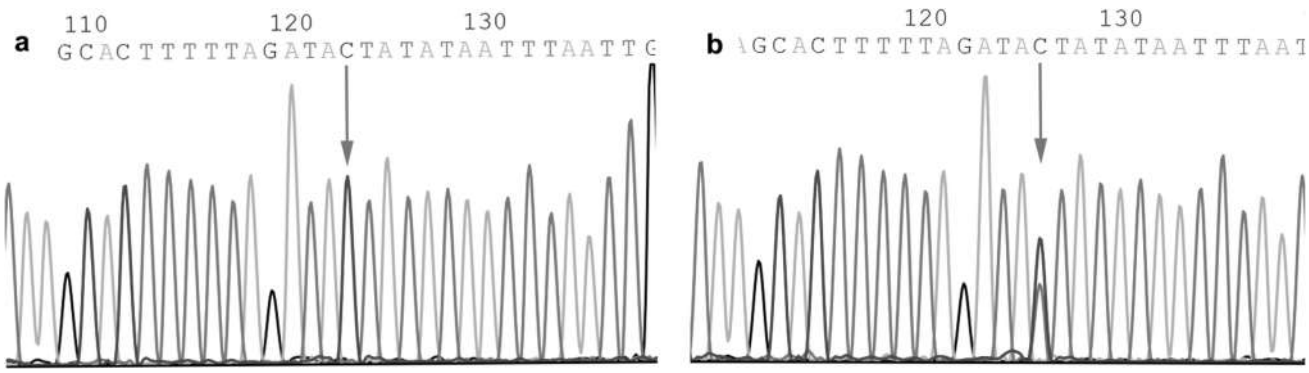

Fig. 2 Representative picture of DNA sequencing result of wild-type genotype CC (**a**) and heterozygous genotype CT (**b**)

Table 1 Baseline demographics of the participants

	GDM group	Control group	*p* value
Cases	239	245	-
Age (years)	31.50 ± 4.28	31.31 ± 4.22	0.620
BMI(kg/m^2)	21.60 ± 2.63	21.17 ± 2.43	0.062
Gestational weeks at delivery	38.98 ± 1.21	38.84 ± 2.09	0.366
Newborn birth weight (kg)	3.11 ± 0.41	3.10 ± 0.40	0.650

the third trimester between the carriers of risk allele (T) and non-carriers in control group.

Discussion

In present study, no TT genotypes were found in the all selected participants and no significant difference was found in genotype distribution between GDM group and control group (Table 2). There may be three possible reasons. Firstly, the frequencies of the risk allele (T) of TCF7L2 rs7903146 vary in different countries, races or regions. According to 1000 genome data, the minor allele frequency (MAF) of TCF7L2 rs7903146 was 2% in East Asian, 32% in European, and 30% in South Asian populations [19]. Some studies have shown that CC/CT/TT genotype frequency of TCF7L2 rs7903146 was related to GDM, while others found that CC/CT genotype of TCF7L2 rs7903146 was susceptibility to GDM [23–26]. Secondly, it may be attributed that 90% of the participants in our study were Cantonese, and the MAF of TCF7L2 rs7903146 was 2.93%(Table 2), which was close to the MAF in Southern Han Chinese (2.86%) [19]. Thirdly, it might be that our sample size was relatively small. Therefore, a larger sample size study was further needed.

In our study, we found that GDM group showed a significantly higher TG and TC levels in the first trimester than control group, which indicated that hyperlipidemia might induce

Table 2 Clinical characteristics and genotype frequencies

		GDM group	Control group	*p* value
Cases		239	245	-
First trimester	TC (mmol/L)	5.31 ± 1.20	5.09 ± 1.01	0.033
	TG (mmol/L)	1.74 ± 0.79	1.55 ± 0.74	0.005
	HDL (mmol/L)	1.87 ± 0.81	1.89 ± 0.32	0.663
	LDL (mmol/L)	2.86 ± 0.96	2.79 ± 0.86	0.415
Second trimester	OGTT-0H (mmol/L)	4.54 ± 0.58	4.26 ± 0.37	< 0.001
	OGTT-1H/(mmol/L)	9.82 ± 1.40	7.44 ± 1.31	< 0.001
	OGTT-2H/(mmol/L)	8.85 ± 1.31	6.48 ± 1.07	< 0.001
Third trimester	TC (mmol/L)	6.01 ± 1.21	5.88 ± 1.30	0.243
	TG (mmol/L)	2.09 ± 0.90	2.09 ± 0.87	0.998
	HDL (mmol/L)	1.83 ± 0.43	1.85 ± 0.36	0.576
	LDL (mmol/L)	3.24 ± 1.10	3.34 ± 1.04	0.304
	HbA1c (%)	5.15 ± 0.48	5.12 ± 0.34	0.514
	FBG (mmol/L)	4.97 ± 0.87	4.76 ± 0.81	0.192
	Fasting insulin (mU/L)	9.62 ± 5.49	9.69 ± 5.70	0.894
Delivery	1 min Apgar score	9.96 ± 0.19	9.96 ± 0.30	0.827
	5 min Apgar score	9.98 ± 0.14	9.99 ± 0.19	0.574
	Glucose level in cord blood	5.31 ± 1.83	5.90 ± 1.38	< 0.001
	Umbilical artery pH	7.25 ± 0.07	7.24 ± 0.08	0.112
Allele(*n*/%)	C	464 (97.07%)	477 (97.35%)	0.847
	T	14 (2.93%)	13 (2.65%)	
Genotype (*n*/%)	CT	14 (5.86%)	13 (5.31%)	0.845
	CC	225 (94.14%)	232 (94.69%)	
Hardy–Weinberg equilibrium		0.218	0.182	-

All continuous variables were represented as mean and standard deviation. Independent sample *t*-test was performed to compare the quantitative data between GDM and Control subjects. Qualitative data were analyzed with Pearson's chi-square test. $p < 0.05$ was considered significant

Allele frequencies fit the Hardy–Weinberg genetic equilibrium

TG triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1c* glycated hemoglobin, *FBG* fasting blood glucose

Table 3 Characteristics of GDM group

		CT group	CC group	*p* value[#]
Cases		14	225	
Age (years)		33.14 ± 4.13	31.40 ± 4.28	0.390
BMI (kg/m^2)		20.69 ± 2.53	21.66 ± 2.63	0.390
Gestational weeks at delivery		38.62 ± 1.87	39.01 ± 1.15	0.390
Newborn birth weight (kg)		3.04 ± 0.56	3.12 ± 0.40	0.642
First trimester	TC (mmol/L)	5.32 ± 1.67	5.31 ± 1.17	0.963
	TG (mmol/L)	1.78 ± 0.86	1.74 ± 0.78	0.933
	HDL (mmol/L)	1.64 ± 0.59	1.88 ± 0.83	0.400
	LDL (mmol/L)	2.92 ± 1.43	2.85 ± 0.93	0.933
Second trimester	OGTT-0H (mmol/L)	4.78 ± 0.80	4.53 ± 0.57	0.390
	OGTT-1H (mmol/L)	11.99 ± 1.47	9.68 ± 1.28	0.011
	OGTT-2H (mmol/L)	9.04 ± 1.31	8.84 ± 1.31	0.697
Third trimester	TC (mmol/L)	6.05 ± 1.08	6.01 ± 1.22	0.949
	TG (mmol/L)	3.90 ± 0.92	1.98 ± 0.77	0.011
	HDL (mmol/L)	1.60 ± 0.49	1.84 ± 0.43	0.225
	LDL (mmol/L)	3.60 ± 1.04	3.21 ± 1.10	0.390
	HbA1c (%)	5.30 ± 0.23	5.14 ± 0.49	0.390
	FBG (mmol/L)	5.24 ± 0.93	4.85 ± 0.87	0.390
	Fasting insulin (mU/L)	6.45 ± 3.71	9.82 ± 5.53	0.183
Delivery	1 min Apgar score	9.93 ± 0.27	9.96 ± 0.19	0.642
	5 min Apgar score	9.93 ± 0.27	9.98 ± 0.13	0.390
	Glucose level in cord blood	4.60 ± 1.49	5.35 ± 1.85	0.390
	Umbilical artery pH	7.23 ± 0.11	7.25 ± 0.07	0.390

TG triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1c* glycated hemoglobin, *FBG* fasting blood glucose.

[#]Data were corrected for FDR by Benjamini–Hochberg program.

GDM by injuring the endothelium [27–29]. But no differences about TG and TC levels were found in the third trimester. And with regard to neonatal outcomes, such as 1 min Apgar scores, 5 min Apgar scores, or umbilical artery pH value, there was no significant difference between GDM group and control group, except that the mean glucose concentrations in cord blood were significantly lower in GDM group (Table 2), and neither group experienced serious hypoglycemia. The possible reason may be related to the well-controlled blood glucose levels of GDM patients in the present study is that, the GDM patients received standard treatment for lifestyle adjustment and their blood glucose were well controlled during pregnancy [30].

In this study, women carrying risk allele (T) showed significantly higher 1-h blood glucose levels in OGTT than those carrying the non-risk genotypes in GDM group (Table 3), while no difference was found in control group (Table 4). This finding was consistent with the Potasso's findings which showed that TCF7L2 rs7903146 T carriers presented significantly higher OGTT 1-h glucose levels and were more likely to require insulin therapy [31]. Compared with the 2-h blood glucose concentration, 1-h blood glucose had a stronger correlation with β-cell dysfunction [32]. The underlying mechanism included early insulin response disorder and insulin resistance [33]. Shah et al. found that a genetic T variant harbored in TCF7L2 rs7903146 impaired glucose tolerance through effects on glucagon as well as insulin secretion [34]. Therefore, it is hypothesized that, for GDM patients which already have existing insulin resistance, carrying risk allele (T) can further affect insulin production, which in turn induces early insulin response disorders and leads to glucose increase at 1 h. In the control group, despite the presence of T risk genes affecting glucose homeostasis, there was no OGTT 1-h glucose increase due to the absence of insulin resistance. So the T variation in TCF7L2 rs7903146 may be very important on regulating 1-h blood glucose levels which is the key indicators in Chinese population, because 1-h blood glucose level on OGTT was a strong predictor of future risk for T2D [35]. But how the risk allele (T) in TCF7L2 rs7903146 regulates islet function on 1-h blood glucose levels needs to be further studied.

Notably, neither in GDM group nor control group, there were no significant differences of TG and TC levels in the first trimester between the risk allele (T) carriers and the non-carriers. But in GDM group, there were significantly higher TG levels in the risk allele (T) carriers than the non-carriers in the third trimester, while in the control group, there were no significant differences. As we all know, lifestyle adjustment is the first line of treatment for women in GDM management [36]. However, the risk allele

Table 4 Characteristics of control group

		CT group	CC group	*p* value[#]
Cases		13	232	
Age (years)		32.31 ± 4.97	31.25 ± 4.18	0.980
BMI (kg/m^2)		20.00 ± 2.47	21.24 ± 2.42	0.980
Gestation week (weeks)		38.86 ± 1.74	38.84 ± 2.12	0.982
Newborn birth weight (kg)		3.02 ± 0.54	3.10 ± 0.40	0.980
First trimester	TC (mmol/L)	5.19 ± 0.60	5.09 ± 1.03	0.980
	TG (mmol/L)	1.75 ± 0.71	1.54 ± 0.74	0.980
	HDL (mmol/L)	1.87 ± 0.19	1.89 ± 0.32	0.980
	LDL (mmol/L)	2.95 ± 0.90	2.78 ± 0.86	0.980
Second trimester	OGTT-0H (mmol/L)	4.21 ± 0.37	4.26 ± 0.37	0.980
	OGTT-1H (mmol/L)	7.91 ± 1.12	7.41 ± 1.31	0.980
	OGTT-2H (mmol/L)	6.79 ± 0.91	6.46 ± 1.07	0.980
Third trimester	TC (mmol/L)	5.96 ± 0.75	5.87 ± 1.33	0.980
	TG (mmol/L)	2.27 ± 0.67	2.08 ± 0.88	0.980
	HDL (mmol/L)	1.74 ± 0.32	1.86 ± 0.36	0.980
	LDL (mmol/L)	3.46 ± 0.91	3.33 ± 1.05	0.980
	HbA1c (%)	5.13 ± 0.33	5.13 ± 0.34	0.982
	FBG (mmol/L)	4.81 ± 0.43	4.77 ± 0.82	0.980
	Fasting insulin (mU/L)	9.89 ± 5.72	9.68 ± 5.71	0.982
Delivery	1 min Apgar score	10.00 ± 0.00	9.97 ± 0.307	0.980
	5 min Apgar score	10.00 ± 0.00	9.99 ± 0.20	0.980
	Glucose level in cord blood	5.36 ± 1.27	5.93 ± 1.38	0.980
	Umbilical artery pH	7.22 ± 0.10	7.24 ± 0.08	0.980

TG triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1c* glycated hemoglobin, *FBG* fasting blood glucose.

[#]Data were corrected for FDR by Benjamini–Hochberg program.

(T) of TCF7L2 rs7903146 could influence changes in BMI and total body fat during lifestyle intervention [37, 38], which might partially explain such differences in our study. Similarly, in GDM group, we also found that the risk allele (T) carriers had lower fasting insulin level in the third trimester than the non-carriers, and the FBG levels also appeared as an elevated trend, despite no statistical significance (Table 3). Therefore, these results suggested that appropriate new strategies including insulin therapy, were needed to be introduced for controlling blood glucose in GDM patients who carry risk allele (T) of TCF7L2 rs7903146, as early as possible [31].

Conclusion

In summary, our studies have shown that TCF7L2 rs7903146 polymorphism effected glucose and lipid metabolism in GDM women. Further investigations are needed to unravel the mechanism by which TCF7L2 rs7903146 affects glycolipid metabolism. For the risk allele (T) carriers of TCF7L2 rs7903146, low-fat and low-sugar diets, exercise interventions can be carried out at an early stage, including before pregnancy or early pregnancy, and insulin treatment should be used as soon as possible when the blood glucose cannot be controlled well.

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Author contribution Changping Fang is the guarantor of this work and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; Shuzhen Wu and Zijing Zhang Wu were involved in data collection and data management; Jun Zhang and Qi Tian conceived and designed the study; Lingling Wu was involved in conceptualization, methodology, supervision, writing, reviewing, and editing. All authors read and approved the final version of the manuscript for publication.

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Data availability All data and material for this article is available upon reasonable request.

Declarations

Ethics approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen.

Competing interests The authors declare no competing interests.

Consent to participate Informed consent had been obtained from all the participants prior to the inclusion into the study.

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ORIGINAL ARTICLE

The relationship between neuron-specific enolase, high sensitivity C reactive protein, and diabetic peripheral neuropathy in Chinese patients with type 2 diabetes: A prospective nested case–control analysis

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Abstract

Background Diabetic peripheral neuropathy (DPN) involves a very complex pathogenesis, and there is no neuro-specific marker for risk prediction. Neuron-specific enolase (NSE), a key enzyme in glycolysis, has a broad spectrum neurotrophic and neuroprotective effect on neurons, and also cause injury and inflammatory response of peripheral nerves. The relationship between neuron-specific enolase, highly sensitive c-reactive protein (hsCRP), and diabetic peripheral neuropathy remains unclear. We aimed to investigate whether elevated serum NSE and hsCRP levels increased the risk of DPN in patients with type 2 diabetes.

Materials and methods In this prospective nested case–control study, a total of 1072 eligible subjects with type 2 diabetes constituted the follow-up cohort. Demographic data and parameters including serum NSE and hsCRP were collected at baseline. Two neuropathy screening scales (MNSI and MDNS) were used to assess DPN during follow-up period. Nerve conduction studies were performed at the end of follow-up. Conditional logistic regression was used to inspect the risk factors of the incidence of DPN.

Results During an average follow-up period of 5.1 years, 176 subjects developed DPN. Serum NSE and hsCRP levels at baseline were significantly higher in DPN group than in matched non-DPN groups ($p < 0.001$). NSE was positively correlated with age and hsCRP ($p < 0.001$). The amplitude of sensory nerve action potential and compound muscle action potential of the lower extremity nerves were significantly decreased in the high tertile of NSE. After adjustment for matching and confusing factor, conditional logistic regression showed the risk of DPN in the high tertile of NSE level was still 3.176 times higher than that in the low tertile of NSE level ($p < 0.001$).

Conclusion Elevated serum NSE levels predicted the high incidence of DPN in Chinese patients with type 2 diabetes for an average of 5.1 years, which may be associated with increased neuroinflammatory response caused by high NSE levels, but further studies are needed.

Keywords Neuron-specific enolase · High sensitivity C-reactive protein · Type 2 diabetes · Diabetic peripheral neuropathy

Abbreviations

NSE	Neuron-specific enolase
hsCRP	High sensitivity C-reactive protein
T2DM	Type 2 diabetes mellitus
DPN	Diabetic peripheral neuropathy
BMI	Body mass index
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
TC	Total cholesterol
TG	Triacylglycerol
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
HbA1c	Glycosylated hemoglobin A1c
FPG	Fasting plasma glucose
eGFR	Estimated glomerular filtration rate
ACR	Urine albumin-creatinine ratio
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration

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MNSI	Michigan Neuropathy Screening Scale
MDNS	Michigan Diabetic Neuropathy Score Scale
ORs	Odds ratios
CI	Confidence interval

Introduction

The incidence of type 2 diabetes is increasing globally in recent decades. Diabetic peripheral neuropathy (DPN) is one of the main microvascular complications in patients with type 2 diabetes [1]. The prevalence and incidence of DPN in patients with type 2 diabetes varies widely according to the criteria and methods used to define neuropathy [2]. DPN is often ignored by patients due to its insidious onset, large heterogeneity of clinical features, and varying severity of symptoms. DPN is the leading cause of diabetic foot and even amputation, which seriously threatens the health and life safety of patients [3]. Therefore, early prediction and diagnosis of DPN is of great significance for improving the quality of life of patients and reducing the high disability and mortality of DPN.

Neuron-specific enolase (NSE) is a glycolytic enzyme, which mainly exists in neuronal cells, neuroendocrine cells, and related tumor cells [4]. It can promote the survival of neurons in the midbrain and spinal cord under hypoxia, and has a broad spectrum neurotrophic and neuroprotective effect on neurons in central nervous system [5]. Since the pathological characteristics of DPN are neurodegeneration, neuron, and nerve remyelination, the glucose metabolism process and the level of NSE in nerve tissue may have corresponding dynamic changes [6]. NSE may act as a broad-spectrum neurotrophic factor to participate in and guide axon extension and modification of damaged myelin sheaths [7]. In addition, NSE may also be involved in the activation of inflammatory cytokines, chemokines, and other inflammatory mediators, resulting in pathological damage of peripheral nerve tissue [8]. As a commonly used inflammatory marker in clinical practice, high sensitivity C reactive protein (hsCRP) can conveniently provide valuable information about the inflammatory state [9]. By monitoring the expression of NSE and hsCRP, it is possible to analyze the relationship between NSE and inflammatory response in peripheral nerve injury.

In a cross-sectional study, serum NSE levels were higher in patients with diabetes and diabetic neuropathy than in healthy individuals, regardless of blood glucose levels [10]. In another study, serum NSE mRNA expression levels in patients with diabetic neuropathy were significantly lower than those in DM patients and normal individuals [11]. These studies on the correlation between NSE and DPN are cross-sectional studies, with conflicting results and unclear causality.

The onset of DPN involves a variety of abnormal pathophysiological processes. In the pathogenesis of type 2 diabetes, it is not clear whether different expression levels of NES and hsCRP have a promoting effect on the incidence of DPN. The purpose of this study is to explore the potential value of serum NSE and hsCRP in predicting DPN in patients with type 2 diabetes.

Materials and methods

Study design and participant

This was a prospective nested case–control study in China. A total of 1868 individuals with type 2 diabetes were recruited in the diabetes treatment centers of three hospitals affiliated to the PLA Rocket Force Characteristic Medical Center from January 1, 2013 to December 1, 2015. Type 2 diabetes was diagnosed based on the 1999 diagnostic criteria for diabetes of the World Health Organization [12]. Individuals ($n=796$) presenting with special clinical diseases at baseline were excluded, including diagnosed diabetic neuropathy ($n=663$); peripheral neuropathy other than diabetic origin ($n=102$); psychiatric disorders ($n=11$); pregnant women ($n=9$); malignant tumor ($n=8$); and drugs and toxins related neuropathy ($n=3$). A total of 1072 eligible subjects with type 2 diabetes constituted the follow-up cohort. The participants' flow chart is shown in Fig. 1.

Data collection

Demographic and physical examination data at baseline were obtained from the subjects. The items included gender, age, height, weight, smoking history, duration of diabetes, blood pressure, and diagnosed diabetic retinopathy. Body mass index (BMI) was calculated as height divided by the square of weight (kg/m^2). The blood pressure, including systolic blood pressure (SBP) and diastolic blood pressure (DBP), was measured three times and the average value was taken. Diabetic retinopathy diagnosed at baseline was assessed by a specialist ophthalmologist using fundus microscopy based on the presence of hard or soft exudates, microaneurysms, bleeding, and new blood vessels in the retina [13].

The data from the laboratory measurement at baseline were collected and evaluated. Blood and urine samples in the morning were collected after the subjects fasted for at least 8 h. The blood samples were centrifuged at 3500 rpm at 4 °C for 10 min and stored at 80 °C until analysis. The fasting serum NSE concentration was determined by electrochemiluminescence immunoassay. Measuring interval was < 16.3 ng/mL (95%); 15.7–17.0 ng/mL (95% confidence interval). Plasma samples were measured in duplicates with intra-assay CV of 2.06% and inter-assay CV of 5.00% for

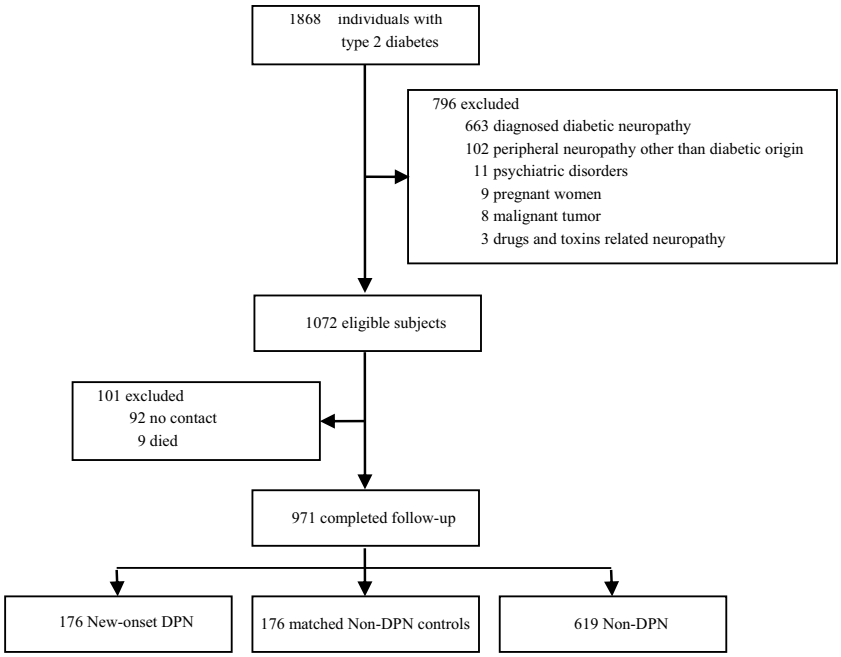

Fig. 1 The participants' flow chart

NSE. The glycated hemoglobin was determined by high-performance liquid chromatography, and the value is a percentage (HLC-723G7, Tosoh Corporation, Japan). Levels of total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (Scr), and fasting plasma glucose (FPG) were measured using an automatic biochemical analyzer (Hitachi 7600 chemical analyzer). C-peptide levels were measured using the chemiluminescence method with an ADVIA Centaur XP automatic analyzer (Siemens Healthcare Diagnostics). Urine albumin concentration was measured by immunoturbidimetric method, urine creatinine concentration was measured by alkaline picric acid, and urine albumin-creatinine ratio (ACR) was calculated by albumin (mg)/creatinine (g). HsCRP were analyzed by enhanced immunoturbidimetric method. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (https://www.niddk.nih.gov).

During follow-up period, patients were called to diabetes medical center of the hospital at a scheduled time each year for formal evaluation by trained medical personnel. DPN was regarded as the primary endpoint of the study and defined using the Michigan Neuropathy Screening Scale (MNSI) and the Michigan Diabetic Neuropathy Score Scale (MDNS). MNSI is recommended to assess the presence of peripheral neurological impairment in patients with diabetes in cohort trials [14]. The inspection content consists of sensory and motor dysfunction such as pain, numbness, and muscle weakness, as well as neurological abnormalities detected by ankle reflexes, a 128-Hz tuning fork vibration perception test, and a 10-g nylon rope tactile test [15]. The items in the study were carried out by trained doctors and nurses.

According to the tertiles of serum NSE levels, 20–25 patients were randomly selected from each group. After obtaining the consent of the patients, nerve conduction function was measured at the end of the follow-up. The electromyography was performed with the Danish Keypoint electromyography instrument by two professional doctors. In a quiet environment, the room temperature was 25–28 °C, and the surface temperature of the patient's limbs was maintained above 32 °C. The detected nerves included bilateral median nerve, ulnar nerve, common peroneal nerve, tibial nerve, superficial peroneal nerve, and sural nerve. Sensory nerve action potential (SNAP) amplitude, latency and sensory nerve conduction velocity (SCV), compound muscle action potential (CMAP) amplitude, latency and motor nerve conduction velocity (MCV) were measured separately.

Statistical analysis

The statistical analyses were performed using the SPSS Statistics software (SPSS Statistics version 25 for Windows; IBM, New York). Case and control patients were determined by 1:1 case–control matching. Matching criteria: same gender, age difference less than 2 years. Continuous variables with normal distribution were expressed as mean ± SD and analyzed using Student's *t*-test or ANOVA, respectively. Data for continuous but non-normally distributed variables were expressed as medians and interquartile ranges and analyzed using non-parametric tests. Categorical data

were expressed as percentages and analyzed using chi-square tests. Bivariate associations of NSE with clinical variables were analyzed using Spearman rank correlation analyses. Serum NSE concentration was categorized by tertiles, the low tertile as the reference category, trend test for ANOVA was used to observe the linear trend between groups. Only parameters significant in univariate analysis or known confounding factors to DPN at baseline were added sequentially into the multivariable models. Conditional logistic regression was used to calculate odds ratios (ORs) for incident of DPN and 95% confidence interval (CI) for each risk factor. All statistical tests were two-sided with a level of significance being < 0.05.

Results

After an average follow-up period of 5.1 years, among these 1072 subjects, 971 (90.578%) cases completed the study, 92 cases (8.582%) were lost to follow-up because of no contact or refusal, and 9 (0.840%) cases died. A total of 176 participants with type 2 diabetes developed incident of DPN. Characteristics at baseline of DPN patients and non-DPN patients completed follow-up are shown in Supplementary Table 1.

A total of 176 control patients matched to DPN cases were identified according to 1:1 gender and age matching. Patients with DPN had longer duration of type 2 diabetes; higher ACR,hsCRP and NSE [12.790 (11.043–15.030) vs 10.150 (8.875–11.905) ng/L, $t = -8.039$, $p < 0.001$]; and more diagnosed diabetic retinopathy at baseline than the matched non-DPN. There were no significant differences in smoking history, HbA1c, FPG, peptide, SBP, DBP, TC, TG, HDL-C, LDL-C, and eGFR between the two groups ($p > 0.05$). The baseline characteristics of the two groups were shown in Table 1.

The level of serum NSE at baseline was positively correlated with age and hsCRP ($p < 0.001$). There was no correlation between NSE and BMI, duration of type 2 diabetes, ACR, HbA1c, FPG, C-peptide, SBP, DBP, TC, TG, HDL-C, LDL-C, eGFR, etc. ($p > 0.05$) (Table 2).

Serum NSE at baseline was divided into three groups by tertiles level, respectively, as NSE < 10.175 g/L; 10.175 ≤ NSE ≤ 12.805 g/L; and NSE > 12.805 g/L. With the increase of NSE level, the number of DPN cases,

Table 1 Baseline demographics and clinical characteristics of patients with or without DPN ($N = 352$; 1:1 matching)

Variables	DPN ($n = 176$)	Non-DPN ($n = 176$)	$t/z/x^2$	p value
Males/females	90/86	90/86	–	–
Age (years)	59.959 ± 8.752	60.241 ± 8.692	− 0.304	0.762
BMI (kg/m^2)	24.978 ± 2.720	25.349 ± 2.491	− 1.336	0.182
Duration of T_2DM (years)	8.500 (6.825–11.000)	6.500 (4.000–7.800)	− 8.836	< 0.001*
Smoking history [n (%)]	17 (9.656)	26 (14.773)	2.146	0.143
Diabetic retinopathy [n (%)]	34 (19.318)	17 (9.656)	6.627	0.010*
DBP (mmHg)	77.430 ± 8.474	77.110 ± 7.343	0.211	0.712
SBP (mmHg)	124.730 ± 12.313	124.980 ± 10.338	0.370	0.833
TG (mmol/L)	1.932 ± 1.181	1.854 ± 0.872	0.699	0.485
TC (mmol/L)	4.448 ± 0.890	4.514 ± 0.760	0.751	0.453
LDL-C (mmol/L)	2.779 ± 0.712	2.853 ± 0.642	− 1.015	0.311
HDL-C (mmol/L)	1.390 ± 0.391	1.377 ± 0.369	0.317	0.751
HbA1c (%)	8.827 ± 1.580	8.648 ± 1.666	1.031	0.303
FPG (mmol/L)	9.172 ± 1.837	9.508 ± 1.995	− 1.643	0.101
C-peptide 0 min (U/L)	2.230 (1.690–2.965)	2.180 (1.813–2.665)	− 0.625	0.532
eGFR (ml/min/1.73 m^2)	91.000 (82.250–99.000)	91.000 (77.250–99.000)	− 1.109	0.267
ACR (mg/g)	2.220 (1.253–3.858)	1.680 (1.053–2.870)	− 2.215	0.027*
NSE (ng/mL)	12.790 (11.043–15.030)	10.150 (8.875–11.905)	− 8.039	< 0.001*
hsCRP (mg/L)	1.938 ± 0.915	1.537 ± 1.098	3.722	< 0.001*

BMI, body mass index; *T_2DM*, type 2 diabetes mellitus; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *TC*, total cholesterol; *TG*, triacylglycerol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *HbA1c*, glycosylated hemoglobin A1c; *FPG*, fasting plasma glucose; *eGFR*, estimated glomerular filtration rate; *ACR*, urine albumin-creatinine ratio; *NSE*, neuron-specific enolase; *hsCRP*, high sensitivity C reactive protein

Values are mean ± SD for normally distributed continuous variables and median (interquartile range 25–75%) for non-normal distribution data, analyzed using Student's t-test. Values are n (%) for categorical variables and assessed using Pearson's chi-square test

*Significant, $p < 0.05$

Table 2 The Spearman rank correlation of serum NSE with anthropometric and biochemical parameters

Clinical characteristic	Spearman rank correlation	*p* value
Age (years)	0.116	0.030*
Duration of T2DM (years)	0.088	0.098
BMI (kg/m2)	0.036	0.501
SBP (mmHg)	−0.041	0.443
DBP (mmHg)	−0.021	0.690
TG (mmol/L)	0.036	0.499
TC (mmol/L)	0.022	0.675
LDL-C (mmol/L)	0.003	0.959
HDL-C (mmol/L)	−0.091	0.087
HbA1c (%)	0.084	0.117
FPG (mmol/L)	0.034	0.528
C-peptide 0 min (U/L)	−0.036	0.495
eGFR (ml/min·1.73 m^2)	−0.057	0.284
ACR (mg/g)	0.035	0.514
hsCRP (mg/L)	0.264	< 0.001*

The correlation between variables was analyzed by Spearman rank correlation analysis

*Significant, $p < 0.05$

hsCRP, duration of type 2 diabetes, and age at baseline also increased; other parameters as HbA1c, FPG, C-peptide, TC, TG, LDL-C, HDL-C, eGFR, and ACR showed no significant differences (Table 3).

The nerve conduction studies data, including SNAP amplitude, latency and SCV, CMAP amplitude, latency and MCV in each groups, were showed in Table 4. With increasing levels of NSE, the CMAP amplitude of common peroneal nerve and tibial nerve decreased significantly, as well as the SNAP amplitude of superficial peroneal nerve and sural nerve ($p < 0.05$). SCV and MCV were slightly lower than normal, and there was no significant difference among the three groups. No obvious difference was found in the amplitude and latency of CMAP and SNAP in median and ulnar nerves of upper limbs.

In the conditional logistic regression, after adjusting for confounding factors at baseline such as age, gender, BMI, ACR, HbA1c, hsCRP, and diagnosed diabetic retinopathy, serum NSE at baseline ($\beta = 0.045$, SE = 0.013, $p = 0.001$) and duration of type 2 diabetes ($\beta = 0.077$, SE = 0.01, $p < 0.001$) remained significant predictors of incidence of DPN. By incorporating confounding factors into the model successively, the risk of DPN in the group with the high tertile of NSE level was still 3.176 times higher than that in the group with the low tertile of NSE level ($p < 0.001$) (Tables 5 and 6).

Discussion

This study was a prospective nested case–control study to investigate the potential value of serum NSE and hsCRP in predicting the risk of DPN in patients with type 2 diabetes.

Table 3 Mean value and *p* value of each index according to the tertiles level of baseline serum NSE

Baseline characteristics	First tertile	Second tertile	Third tertile	F/x^2	*p* for trend
Cases number (PDN/non-PDN)	24/92	65/53	87/31	65.526	< 0.001*
Age (years)	59.122	59.797	61.364	3.904	0.049*
Duration of T2DM (years)	7.092	7.408	8.078	5.812	0.016*
BMI (kg/m2)	25.154	24.829	25.507	1.096	0.296
Smoking history [*n* (%)]	20 (17.241%)	14 (11.864%)	9 (7.627%)	5.025	0.025*
Diabetic retinopathy [*n* (%)]	18 (15.517%)	8 (6.780%)	25 (21.186%)	1.549	0.213
SBP (mmHg)	125.500	123.540	125.530	0.001	0.976
DBP (mmHg)	77.720	76.830	77.260	0.195	0.659
TG (mmol/L)	1.838	1.839	2.000	1.439	0.231
TC (mmol/L)	4.508	4.433	4.502	0.003	0.957
LDL-C (mmol/L)	2.862	2.796	2.791	0.630	0.428
HDL-C (mmol/L)	1.379	1.444	1.328	1.092	0.297
HbA1c (%)	8.511	8.904	8.793	1.754	0.186
FPG (mmol/L)	9.275	9.347	9.398	0.237	0.627
C-peptide 0 min (U/L)	2.295	2.277	2.199	0.959	0.328
eGFR (ml/min·1.73 m^2)	90.480	89.360	88.260	1.394	0.239
hsCRP (mg/L)	1.554	1.620	2.034	13.268	< 0.001*
ACR (mg/g)	3.018	3.617	3.819	1.220	0.270

First tertile: NSE < 10.175 g/L; second tertile: 10.175 ≤ NSE ≤ 12.805 g/L; third tertile: NSE > 12.805 g/L

Values are mean for each item. Trend test for ANOVA was used to observe the linear trend

*Significant, $p < 0.05$

Table 4 Nerve conduction studies at the end of follow-up according to the tertiles level of baseline serum NSE

Nerve conduction parameters (mean)		First tertile	Second tertile	Third tertile	*F*	*p* for trend
n (PDN/non-PDN)		11/12	12/13	12/12		
CMAP amplitude (mV)	Median	4.622	4.560	4.533	2.009	0.161
	Ulnar	4.487	4.500	4.400	2.153	0.147
	Common peroneal	3.974	3.756	3.488	6.600	0.012*
	Tibial	3.965	3.788	3.471	6.885	0.011*
Latency (ms)	Median	3.509	3.436	3.392	2.724	0.103
	Ulnar	3.239	3.228	3.192	0.441	0.509
	Common peroneal	4.465	4.488	4.433	0.200	0.656
	Tibial	4.778	4.656	4.621	3.158	0.080
MCV (m/s)	Median	46.649	46.152	45.458	2.619	0.110
	Ulnar	45.917	45.660	44.904	2.271	0.136
	Common peroneal	34.852	34.636	35.429	0.655	0.421
	Tibial	33.496	33.296	33.179	0.179	0.674
SNAP amplitude (uV)	Median	15.348	15.100	14.729	1.479	0.228
	Ulnar	13.274	12.852	12.388	2.090	0.153
	Superficial peroneal	4.009	3.940	3.179	5.919	0.018*
	Sural	4.200	3.848	3.425	6.280	0.015*
Latency (ms)	Median	3.278	3.172	3.158	2.375	0.128
	Ulnar	3.026	2.924	2.871	2.759	0.101
	Superficial peroneal	3.922	3.896	3.679	3.229	0.077
	Sural	3.844	3.720	3.608	3.143	0.081
SCV (m/s)	Median	43.461	43.444	42.908	0.706	0.404
	Ulnar	43.017	42.628	42.313	0.815	0.370
	Superficial peroneal	39.683	39.336	39.246	0.380	0.540
	Sural	40.187	39.792	39.342	1.839	0.180

First tertile: NSE < 10.175 g/L; second tertile: 10.175 ≤ NSE ≤ 12.805 g/L; third tertile: NSE > 12.805 g/L

SNAP, sensory nerve action potential; *SCV*, sensory nerve conduction velocity; *CMAP*, compound muscle action potential; *MCV*, motor nerve conduction velocity

Values are mean for each item. Trend test for one-way ANOVA was used to observe the linear trend

*Significant, $p < 0.05$

During an average 5.1-year follow-up period, the risk of DPN in patients with type 2 diabetes increases with baseline serum NSE levels. After matching and adjusting the main risk factors at baseline, including age, gender, BMI, duration of type 2 diabetes, hsCRP, HbA1c, ACR, and diagnosed diabetic retinopathy, the risk of DPN in the high tertile of NSE level was still 3.176 times higher than that in the low tertile of NSE level ($p < 0.001$). As far as we know, this is the first report to clarify the potential link between NSE and the development of clinical DPN in patients with type 2 diabetes in a prospective nested case–control study.

Enolase is a key enzyme in the glycolysis process, catalyzing the dehydration of 2-phosphoglycerate to phosphoenolpyruvate. Among the isoenzymes of enolase, a soluble acid protease that belongs exclusively to neuroendocrine tissues and neurons is also called NSE. NSE may have diagnostic and predictive value in differential diagnosis, disease monitoring, curative effect evaluation and recurrence prediction of nervous system, endocrine system diseases and tumors, etc. [16, 17]. Until now, NSE-related research mainly focuses on the mechanism of central nervous system neurons and neuroendocrine tumors, and its role in the pathophysiology of DPN is still unclear. The expression level and type of enolase in peripheral glial cells (Schwann cells, etc.) may change under abnormal glucose metabolism and stress [18]. Typically, Schwann cells only expresses the non-neuronal form of enolase. Long-term abnormal glucose metabolism leads to Wallerian degeneration or demyelination of peripheral neuropathy. The abnormity in Schwann cells metabolic activity may lead to an increase in NSE synthesis to adapt to the increased energy requirements of cell metabolism. Compared to other types of isoenzymes, NSE is more stable and has neurotrophic and neuroprotective effects on damage to axons and Schwann cells. It is beneficial to promote functional recovery after nerve injury. NSE is involved in the activation of glycolysis pathway and the

Table 5 Conditional logistic regression analyzed the risk factors of DPN

Factors	β	SE	OR (95% CI)	p value
Age (years)	−0.006	0.009	0.994 (0.977–1.011)	0.492
Gender (male/female)	−0.052	0.155	0.949 (0.700–1.286)	0.736
BMI (kg/m^2)	−0.040	0.031	0.961 (0.904–1.021)	0.2000
Duration of T_2DM (years)	0.077	0.015	1.080 (1.049–1.112)	< 0.001*
Diabetic retinopathy (yes vs no)	0.145	0.205	1.156 (0.774–1.726)	0.480
HbA1c (%)	0.016	0.049	1.016 (0.924–1.117)	0.744
ACR (mg/g)	−0.003	0.013	0.997 (0.971–1.024)	0.837
hsCRP (mg/L)	0.069	0.076	1.072 (0.929–1.236)	0.342
NSE (ng/mL)	0.045	0.013	1.046 (1.019–1.074)	0.001*

Only significant factors in the univariate analysis or known confounding factors were successively added to the model

*Significant, $p < 0.05$

increase of metabolite expression level. At certain physiological levels, it can help to maintain and restore the function of Schwann cells in damaged nerve fibers and has positive significance for the repair of damaged nerves after peripheral nerve injury [19, 20]. In addition, NSE may also cause axon damage by mediating the activation of neurodegeneration pathways and promoting the activation of inflammatory cytokines, chemokines, and other inflammatory mediators [8]. Therefore, the dynamic observation of NSE expression in peripheral blood can be used to infer the injury and repair of peripheral nerves as well as the level of inflammatory response, which can provide clinical reference for the evaluation of metabolism and function of peripheral nerves.

The relationship between NSE and DPN have been investigated in some literatures. In a cross-sectional study conducted by Li et al. [10], it was found that serum NSE levels in diabetic patients were slightly higher than those in normal glycemic patients (9.1 [1.5] vs 8.7 [1.7], $p = 0.037$).

Diabetic patients with neuropathy had significantly higher serum NSE levels than diabetic patients without neuropathy (10.8 [2.8] vs 9.1 [1.5], $p \leq 0.001$). But in the study of Sandhu et al. [11], the NSE mRNA level was significantly higher in diabetic group (no neuropathy or retinopathy) ($n = 22$) than in healthy subjects ($n = 26$), and the NSE mRNA level was lower in diabetic neuropathy group ($n = 24$) than in diabetic control group (no neuropathy or retinopathy) ($n = 22$). The limitation of the projects was that they were all cross-sectional studies. Some studies included a small number of cases; differences between ethnic groups might affect the final conclusions. Moreover, the conclusions were not consistent in different study populations, which had no practical reference significance for elucidating the causal relationship between NSE and the incidence of DPN.

Our study showed that the baseline serum NSE level of patients in the DPN group was significantly higher than that of the matched non-DPN group [12.790 (11.043–15.030) vs 10.150 (8.875–11.905) ng/L, $t = -8.039$, $p < 0.001$], although NSE in both groups was within the normal range or slightly exceeded the normal value. Older ages at baseline were independently associated with NSE in this study. Studies have shown that the content of NSE in cerebrospinal fluid increases with age, on average by 1% per year [21]. The level of NSE in peripheral blood also increased with age, which may be similar to that of NSE in cerebrospinal fluid.

Table 6 Conditional logistic regression analyzed relationship between incident DPN and NSE

Model	OR (95% CI)			p value
	NSE < 10.175	10.175 ≤ NSE ≤ 12.805	12.805 < NSE	
Model 1	1	2.650 (1.659–4.233)	3.693 (2.343–5.821)	< 0.001*
Model 2	1	2.557 (1.600–4.086)	3.318 (2.095–5.257)	< 0.001*
Model 3	1	2.640 (1.646–4.237)	3.284 (2.070–5.210)	< 0.001*
Model 4	1	2.633 (1.641–4.224)	3.176 (1.995–5.055)	< 0.001*

Note: *OR*, odds ratio; *CI*, confidence interval

Model 1: adjusted for age, sex, and BMI

Model 2: based on model 1, duration of T_2DM was further corrected

Model 3: based on model 2, HbA1c, ACR, and diabetic retinopathy were further corrected

Model 4: hsCRP was further corrected on the basis of model 3

*Significant, $p < 0.05$

Secondly, we also found no correlation between NSE and HbA1c at baseline; the relationship between hyperglycemia and NSE was controversial. The literature reports that the level of NSE was independent of hyperglycemic metabolic status (fasting glucose, HbA1c, course of disease, and type of diabetes) and other potential confounding factors affecting NSE levels (such as age, sex, and renal status) [10, 11]. However, the mechanism by which blood glucose levels affect NSE remains unclear. High blood glucose levels may cause a manageable increase in the physiological level of NSE; this suggest that NSE may play a more important role in the pathogenesis of DPN. Thirdly, inflammation is one of the important mechanisms of neuropathy. Oxidative stress and inflammation of the nervous system can be induced in the context of persistent hyperglycemia and ischemia or hypoxia, increasing the risk of peripheral neuropathy [22]. At the same time, the expression level of enolase in nerve tissue is upregulated to increase the repair and survival of peripheral nerve tissue. We speculated that the expression level of NSE may fluctuate in different development stages of diabetes; NSE in peripheral nerve tissue may also be controlled, which is related to the functional state of peripheral nerve tissue. In this study, we selected hsCRP as a marker of inflammation. We found a significant correlation between the baseline NSE and hsCRP, which provides evidence for the involvement of NSE in inflammatory response, but the specific mechanism of action remains unclear. Interestingly, no independent association was found between hsCRP and the incidence of DPN. It is currently believed that NSE has a biological half-life of about 24 h in body fluids, and increased serum levels of NSE may trigger the activation of different cellular pathways leading to neuroinflammation. The pro-inflammatory effects of NSE may involve the activation of MMP-9 and NSE-mediated activation of PI3K and MAPK pathways, leading to the release of inflammatory cytokines and chemokines that contribute to the development of neuropathy [23, 24]. In order to clarify the mode and significance of NSE's involvement in neuropathic inflammatory mechanisms of DPN, it may be necessary to select more appropriate markers of inflammation.

The most significant finding in this study is that elevated serum NSE levels at baseline is a risk factor for DPN, independent of other known risk factors for DPN. Older ages and duration of type 2 diabetes are common risk factors for DPN, which have been confirmed in ours and other studies [25, 26]. HbA1c has a higher predictive value of DPN in patients with type 1 diabetes than in type 2 diabetes, active hypoglycemic therapy has been shown to be effective in delaying the onset of DPN in type 1 diabetes, but the effect on DPN in type 2 diabetes is not obvious, which is related to the complexity pathogenesis of DPN in type 2 diabetes. Modifiable cardiovascular risk factors are associated with the incidence of neuropathy, including elevated TG levels, BMI, and hypertension, etc. [27]. No statistical significance was found about these indexes in our study.

In nerve conduction studies, with the increase of NSE level, the CMAP amplitude of the motor nerve and the SNAP amplitude of the sensory nerve of the lower limbs were decreased significantly ($p < 0.05$). The peripheral neuropathy in these patients with type 2 diabetes may be dominated by axonal injury in the distal nerves of the lower limbs, mainly small nerve fibers, and the axonal injury in the high tertiles level of NSE is more significant than that in the low NSE tertiles. Due to the high content of NSE in the axons of peripheral nerves, long-term neuropathy leads to Wallerian degeneration, axonal destruction, and NSE leakage increased. SNAP and CMAP amplitude, which reflect the degree of axonal damage, can be significantly reduced [28, 29]. At the same time, the demyelination of nerve tissue was not obvious. Therefore, there was no significant change of latency, SCV, and MCV in sensory and motor nerve detection. For patients with type 2 diabetes, the low tertile expression level of NSE in nerve tissue has neurotrophic and protective effects on diabetic peripheral nerve tissue, and the high tertile expression level is related to abnormal metabolic status, inflammatory response, and the pathological damage of peripheral nerve tissue. Persistent metabolic abnormalities or irreversible damage of Schwann cells in peripheral nerves indicate the possibility of DPN in the future. The study shows that the risk of DPN in the group with the high tertile of NSE level was still 3.176 times higher than that in the group with the low tertile of NSE level ($p < 0.001$). This provides support for the possibility that NSE becomes a neuro-specific marker to predict DPN in type 2 diabetes.

There are some limitations in this study. Only Chinese patients with type 2 diabetes were included in this study, which may not be fully representative of the general population. Therefore, we should be cautious when extrapolating the conclusions. In addition, due to the limited sample size and the complexity pathogenesis of DPN, confounders and the influencing factors to NSE were not all included in the study. Third, the diagnosis of DPN is mainly based the neuroscale, and only some patients have nerve conduction studies; the relationship between NSE and the severity of DPN was also not discussed. Lastly, the dynamic changes of NSE during the disease were not fully considered; further adjustments should be made in future studies.

Conclusion

This prospective nested case–control study identified a potential association between NSE and the development of

clinical DPN in patients with type 2 diabetes. The results indicate that elevated serum NSE levels increased neuro-inflammatory responses and had a predictive value for the occurrence of DPN in patients with type 2 diabetes for an average of 5.1 years. NSE may become a neuro-specific marker for DPN and provide a useful reference for the early diagnosis and treatment of DPN.

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

Declarations

Ethics approval and consent to participate This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethics committee of this medical center (2012). Each participant signed the informed consent voluntarily.

Conflict of interest The authors declare no competing interests. Data availability statement.

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ORIGINAL ARTICLE

Early effect of exenatide treatment on atherogenicity in patients with type 2 diabetes mellitus

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Abstract

Objective Diabetes mellitus is a chronic metabolic disease often associated with hyperlipidemia. High low-density lipoprotein cholesterol (LDLc), high triglyceride, and low high-density lipoprotein cholesterol (HDLc) form the atherogenic lipoprotein profile. In this study, we examined how exenatide, a glucagon-like peptide 1 (GLP-1) analog, affects lipid profile and atherogenic indices in patients with diabetes.

Methods 100 patients diagnosed with type 2 diabetes mellitus (T2DM) participated in this retrospective study. Clinical and laboratory data of the patients were obtained before exenatide treatment and at the 12th week. From the lipid profile, Atherogenicity Plasma Index (AIP), Castelli Risk Index I (CRI-I), Castelli Risk Index II (CRI-II), Atherogenic Coefficient (AC), triglyceride (TG)/HDLc, TG- Glucose index (TyG) and TyG-Body Mass Index (BMI) data were calculated.

Results There was a significant improvement in body weight (BW), BMI, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), and conventional lipid profile after exenatide treatment. Statistically, significant decreases were observed in atherogenicity indices TyG index, TyG-BMI index, CRI-I, CRI-II, AIP, and AC indices ($p < 0.05$). This improvement in TG/HDLc, TyG index, CRI-I, CRI-II, AIP and AC indices was independent of HbA1c and BMI. Especially in patients with BMI ≥ 40 kg/m^2, TyG-BMI index (p:0.01), a statistically significant decrease was observed in TyG index, TyG-BMI index, CRI-I, and AIP values in patients with HbA1c $\geq 8\%$ (p:0.001, p:0.016, p:0.047, p:0.008).

Conclusion In addition to its commonly known effects such as lowering FPG levels and weight loss, exenatide has been observed to have a positive effect on traditional lipid profiles and atherogenicity-related indices. In addition to its antidiabetic effect, it should be considered in diabetic patients in treatment options for atherosclerotic cardiovascular prevention.

Keywords GLP-1 analogs · Exenatide · Type 2 diabetes mellitus · Atherogenicity

Introduction

It is estimated that 450 million individuals worldwide have type 2 diabetes mellitus (T2DM), and by the year 2040, that number will rise to 640 million [1]. The close relationship of T2DM with insulin resistance and obesity has brought a new perspective to treatment in recent years. The insufficient weight loss effect of oral antidiabetic drugs and insulin analogs, which have been in use for a long time, especially in diabetic obese patients, has led to the emergence of glucagon-like peptide-1 (GLP-1) agonist drugs. GLP-1 is a polypeptide hormone released from L cells in the intestinal wall. While GLP-1 inhibits glucagon release from the pancreas, it increases insulin release. As a result, blood sugar control is achieved. In addition to these effects, GLP-1 slows gastric emptying and reduces gastric acid secretion. These effects result in decreased appetite and weight loss [2]. GLP-1 receptor agonist drugs are grouped as short-acting (lixisenatide and exenatide) and long-acting (dulaglutide, liraglutide, albiglutide, and exenatide). In addition, a new glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist drug called tirzepatid has been used in recent years [3, 4]. Among these drugs, exenatide is available in a short-acting form for 2–4 hours (twice a day) and a long-acting form for up to 1 week (once a week) [5, 6].

According to the World Health Organization (WHO) report, overweight and obesity affect 60% of adults in

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European countries. According to this report, obesity has been increasing rapidly over the years in the countries in the region and obesity is not expected to regress in any country until 2025 [7]. Obesity poses a significant risk for the development of T2DM. As a result of BMI exceeding 35 kg/m^2, the risk of developing T2DM increases between 49 and 93 times. Every 1 kg of weight loss in obese individuals reduces the conversion of impaired glucose tolerance to T2DM by approximately 16% [8]. Concomitant hypertension and atherogenic hyperlipidemia in diabetic obese individuals constitute metabolic syndrome (MS). Atherosclerosis in patients with MS is the main cause of cardiovascular morbidity and mortality. Low-density lipoprotein cholesterol (LDLc) is considered the lipoprotein with the highest risk of atherosclerosis. In addition, LDLc is the cholesterol most closely associated with insulin resistance. In recent years, lowering LDLc has been seen as the most important measure for the prevention of atherosclerosis and cardiovascular mortality [9, 10]. While atherosclerosis is best detected by invasive imaging methods, studies on non-invasive atherogenic indices have attracted attention in recent years. Atherogenicity Plasma Index (AIP), Castelli Risk Index I (CRI-I), Castelli Risk Index II (CRI-II), Atherogenic Coefficient (AC), triglyceride (TG)/ high-density lipoprotein cholesterol (HDLc), TG-Glucose index (TyG) and TyG-Body Mass Index (BMI) are some of them [11–14]. In studies, positive effects of GLP-1 agonists on lipid profile and positive effects on atherogenic cardiovascular diseases were investigated with large patient populations through invasive studies [15, 16]. However, the number of studies on non-invasive atherogenic indices of GLP-1 analogs is limited. The early effects of exenatide on new atherogenic indices were investigated in this study.

Material and method

Patient selection

This study was conducted on 100 obese (BMI ≥ 35 kg/m^2) patients diagnosed with T2DM who applied to our hospital between January 2019 and June 2021. The flow chart for patient admission is shown in Fig. 1. Exenatide therapy began with 5 µg subcutaneous (sc) given twice daily, and

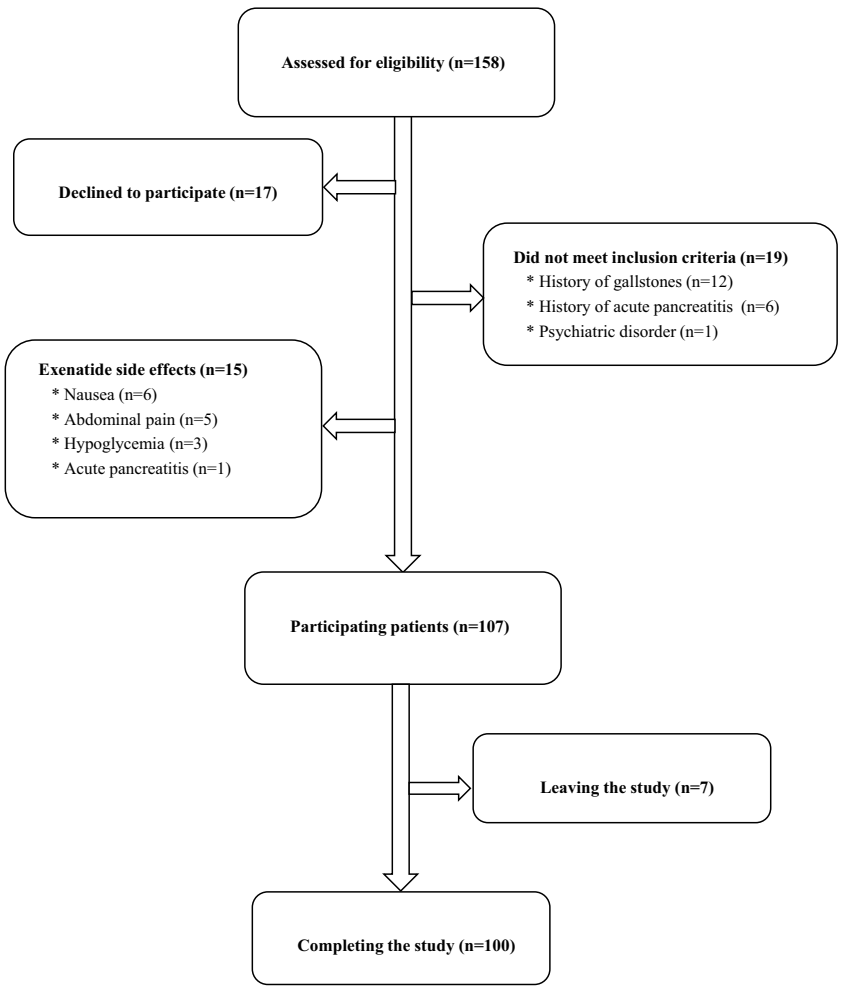

Fig. 1 Flow diagram

after 4 weeks, 10 µg sc twice daily. The examination of the patients was completed at the beginning and the end of 12 weeks.

Patient exclusion criteria

- Volunteers under the age of 18
- Patients with a previous medical history of gallstones
- Patients with a past medical history of acute pancreatitis
- Patients who develop side effects during the use of exenatide and cannot complete the 3-month treatment period (for reasons such as nausea, abdominal pain, and dizziness)
- Patients with psychiatric disorders
- Patients with history of or family history of medullary thyroid carcinoma, patients with multiple endocrine neoplasia syndrome type 2
- Patients with microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary artery disease, cerebrovascular accident, peripheral artery disease) complications
- Patients who declined to contribute for the research

Clinical and biochemical measurements

All patients' ages, genders, and drug regimens were documented at the start of the trial. For all patients, body weight (BW), height, BMI, and biochemical tests were recorded on the hospital database at baseline and 12 weeks. HbA1c test was performed by the HPLC method in the Trinity Biotech device. Biochemical parameters, including FPG, TC, HDL-c, LDL-c, TG, creatinine, AST, ALT and albumin, were measured using the Beckman Coulter AU5800 device. Non-high-density lipoprotein cholesterol (non-HDLc) was calculated with the formula total cholesterol (TC)–HDLc. TyG index was calculated with the formula Ln(fasting TG [mg/dl] x fasting plasma clucose (FPG) [mg/dl])/2. TyG-BMI index was calculated with the formula TyG x BMI. CRI-I was calculated with the formula TC/HDLc. CRI-II was calculated with the formula LDLc/HDLc. AIP was calculated with the formula Log10 (TG/HDLc). AC was calculated with the non-HDLc / HDLc formula.

Statistical analysis

SPSS version 26 application (IBM Corporation) was used to examine the information gathered for our study. The distribution of data was evaluated by Kolmogorov-Smirnov test. In the form of categorical data, numbers (n) and percentages (%) were used. Paired Samples t-test (normal) and Wilcoxon test (non-normal) were used in dependent groups. In the comparison of independent groups, the independent samples t-test was used for data with normal distribution and Mann Whitney U test was used for data not distributed normally. The threshold for statistical significance (P) was set at 0.05. We evaluated the levels of atherogenic indices affected by BMI and HbA1c with multivariate linear regression analysis.

Informed consent

Approval for our research was obtained from the Aksaray University Faculty of Medicine Clinical Research Ethics Committee with the number 2021/08–08. The Declaration of Helsinki of the World Medical Association guided the execution of this study. All participants received signed informed permission after being made aware of the research protocol.

Results

25 (or 25%) of the article's patients were men, and 75 (or 75%) were women. The average patient age was 52.2 year. After 12 weeks of exenatide treatment, major reductions were seen in the BW, BMI, FPG, HbA1c, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) parameters of the patients ($p < 0.001$, $p < 0.001$, p: 0.004, $p < 0.001$, $p < 0.001$, p:0.002). Albumin raised in a meaningful way (p:0.03). Statistically significant decreases were observed in TC, TG, LDLc, and non-HDLc data within the conventional lipid profile (p:0.003, $p < 0.001$, p:0.008, $p < 0.001$). No appreciable alterations were found in the HDL-c data (Table 1). 32 patients included in our study were using one antilipidemic drug and 68 patients were not using antilipidemic drugs. There was no antilipidemic drug change in any patient during the study period. In our study, no statistically significant difference was found in atherogenic index changes (Δ) between patient groups using antilipidemic drugs and those not using antilipidemic drugs ($p > 0.05$). As seen in Table 1 after three months of treatment, atherogenicity indices TG/HDLc ($p < 0.001$), TyG index ($p < 0.001$), TyG-BMI index ($p < 0.001$), CRI-I ($p < 0.001$), CRI- II (p:0.003), AIP ($p < 0.001$) and AC ($p < 0.001$) showed statistically significant decreases. In addition, this improvement in TG/HDLc, TyG index, CRI-I, CRI-II, AIP, and AC indices was found to be independent of HbA1c and BMI (Table 4).

The participants were separated into two categories based on their BMIs, which were < 40 kg/m^2 and ≥ 40 kg/m^2. Statistically significant reductions in BW ($p < 0.001$), BMI ($p < 0.001$), and HbA1c (BMI < 40 kg/m^2; $p < 0.001$, BMI ≥ 40 kg/m^2; p:0.001) parameters in both groups after 12 weeks of exenatide treatment detected. The decrease in FPG was not significant having BMI <40 kg/m^2 (p:0.125), but it was significant having BMI ≥ 40 kg/m^2 (p:0.02). A significant decrease in ALT (BMI < 40 kg/m^2; p:0.037, BMI ≥ 40 kg/m^2; p:0.003) and AST (BMI < 40 kg/m^2;

Table 1 Demographic, clinical data, and atherogenicity indexes

Parameters	Basal	3. month	Δ	*p*
Age (years)	52.2 ± 1			
Weight (kg)	111.1 ± 1.9	102.9 ± 1.9	-8.23 ± 6.37	<0.001*
BMI (kg/m^2)	42.7 ± 0.7	39.4 ± 0.7	-3.35 ± 3.09	<0.001*
FPG (mg/dl)	151.5 (69–393)	134.5 (74–394)	-11.5 (-226–242)	0.004*
HbA1c (%)	7.8 (5.5–13.9)	7 (5-13.4)	-0.6 (-6,6–6,2)	<0.001*
Creatinine (mg/dl)	0.6 ± 0.1	0.7 ± 0.2	0.02 ± 0.14	0.146
ALT (U/L)	21.5 (8–117)	19 (5–69)	-2 (-58–20)	<0.001*
AST (U/L)	20.5 (12–111)	19 (10–58)	-2 (-59–44)	0.002*
Albumin (g/dl)	4.1 ± 0.03	4.2 ± 0.02	0.06 ± 0.3	0.033*
Traditional lipid profile/parameters				
TC (mg/dl)	200.5 (112–367)	183 (86–402)	-4 (-107–94)	0.003*
TG (mg/dl)	172 (73–1295)	141 (52–1063)	-22.5 (-420–292)	<0.001*
LDLc (mg/dl)	121 ± 3.4	112 ± 3.9	-8.96 ± 33.19	0.008*
HDLc (mg/dl)	48.1 ± 1	48.8 ± 1	-0.7 ± 8	0.383
Non–HDLc (mg/dl)	151 (81–318)	134.5 (52–353)	-7.5 (-110–89)	<0.001*
Atherogenicity indexes				
TG/HDLc	3.51 (1.27–26.43)	2.82 (0.78–21.69)	-0.38 (-9.68–8.07)	<0.001*
TyG index	9.50 ± 0.67	9.20 ± 0.67	-0.31 ± 0.68	<0.001*
TyG-BMI index	406.90 ± 69.10	363.50 ± 69.90	-43.48 ± 39.79	<0.001*
CRI-I	4.33 ± 0.984	4.01 ± 0.99	-0.32 ± 0.78	<0.001*
CRI-II	2.51 (1.08–5.8)	2.24 (0.64–4.63)	-0.17 (-2.46–2.37)	0.003*
AIP	0.56 ± 0.253	0.47 ± 0.244	-0.09 ± 0.19	<0.001*
AC	3.33 ± 1.033	3 ± 0.99	-0.34 ± 0.79	<0.001*

* *p* value <0.05 was considered statistically significant

Δ: value change

BMI: Body Mass Index

FPG: Fasting Plasma Glucose

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

TC: Total cholesterol

TG: Triglyceride

LDLc: Low-density lipoprotein cholesterol

HDLc: High-density lipoprotein cholesterol

Non-HDLc: Non-high density lipoprotein cholesterol

TyG index: TG-Glucose index (TyG)

CRI-I: Castelli Risk Index I

CRI-II: Castelli Risk Index II

AIP: Atherogenicity Plasma Index

AC: Atherogenic Coefficient

p:0.022, BMI ≥ 40 kg/m^2; *p*:0.031) parameters was found after treatment. When the traditional lipid profile was examined, a notable decline was discovered in the TC (*p*:0.005), TG (*p*:0.018), LDLc (*p*:0.028), and Non-HDLc (*p*:0.003) parameters having BMI <40 kg/m^2. Significant reductions in TG ($p < 0.001$) and non-HDLc (*p*:0.02) were found with treatment having BMI ≥40 kg/m^2, but there was no discernible difference in any further metrics. Statistical analysis of TyG index (*p*:0.024), TyG-BMI index ($p < 0.001$), CRI-I (*p*:0.02), AIP (*p*:0.015) and AC (*p*:0.008) parameters having BMI <40 kg/m^2 significant decreases were observed, but no significant changes were found in TG/HDLc and CRI-II parameters. In the group with BMI ≥40 kg/m^2, TG/HDLc ($p < 0.001$), TyG index ($p < 0.001$), TyG-BMI index ($p < 0.001$), CRI-I (*p*:0.005), CRI-II (*p*:0.016), AIP ($p < 0.001$) and AC (*p*:0.01) parameters decreased statistically significantly. Exenatide reduced BW, BMI, and TyG-BMI index, and

these reductions were more pronounced in the group with BMI ≥40 kg/m^2 than in the group with BMI 40 < kg/m^2 (p:0.008, p:0.016, and p:0.01, respectively) (Table 2).

Groups with HbA1c <8% and ≥ 8% were created from all patients. There was a major reduction in BW ($p < 0.001$) and BMI ($p < 0.001$) data after treatment across both groups.

Table 2 Demographic data, clinical data, atherogenic indexes of BMI <40 kg/m^2 and BMI ≥40 kg/m^2 patients

	BMI <40 kg/m^2 (n:45)				BMI ≥40 kg/m^2 (n:55)				p^{Δ}
Parameters	Basal	3. Month	Δ	p	Basal	3. Month	Δ	p	
Age (years)	52.1 ± 10.6				52.3 ± 9.1				
Weight (kg)	98.7 ± 11.96	92.2 ± 12.56	-6.46 ± 3.57	<0.001*	121.3 ± 17.20	111.6 ± 18.72	-9.67 ± 7.7	<0.001*	0.008*
BMI (kg/m^2)	36.6 ± 1.62	34.1 ± 2.27	-2.59 ± 1.43	<0.001*	47.7 ± 6.15	43.7 ± 6.65	-3.97 ± 3.86	<0.001*	0.016*
FPG (mg/dl)	154 (75-393)	141 (74-394)	-12 (-226–242)	0.125	149 (69-359)	129 (78-362)	-7 (-204–158)	0.02*	0.69
HbA1c (%)	8.2 (5.6–13.6)	7.4 (5–11.6)	-0.5 (-6.6–1.4)	<0.001*	7.8 (5.5-13.9)	6.9 (5.2-13.4)	-7 (-5.7–6.2)	0.001*	0.57
Creatinine (mg/dl)	0.65 (0.43-1.38)	0.68 (0.4-1.47)	0.01 (-0.29–0.7)	0.232	0.66 (0.33–1.08)	0.62 (0.38–1.3)	0.01 (-0.44–0.3)	0.44	0.72
ALT (U/L)	21 (8-76)	19 (7-69)	-1 (-23–20)	0.037*	22 (9-117)	19 (5-59)	-2 (-58–18)	0.003*	0.7
AST (U/L)	20 (12–48)	21 (13–111)	-2 (-16–11)	0.022*	21 (13-111)	20 (10-58)	-2 (-59–44)	0.031*	0.88
Albumin (g/dl)	4.13 ± 0.36	4.26 ± 0.21	0.13 ± 0.28	0.005*	4.2 ± 0.26	4.21 ± 0.26	0.01 ± 1.3	0.735	0.06
Traditional lipid profile/parameters									
TC (mg/dl)	196 (128–280)	203 (112–367)	-9 (-103–66)	0.005*	203 (112-367)	188 (116-402)	-2 (-107–94)	0.152	0.23
TG (mg/dl)	183 (73-554)	147 (52-422)	-16 (-420–67)	0.018*	154 (80-1295)	133 (57-1063)	-27 (-363–292)	<0.001*	0.39
LDLc (mg/dl)	119.6 ± 31.74	109.3 ± 37.58	-10.24 ± 30.21	0.028*	122.2 ± 36.11	114.3 ± 39.64	-7.91 ± 35.68	0.106	0.73
HDLc (mg/dl)	47.2 ± 9.26	46.8 ± 8.47	-0.4 ± 7.1	0.707	48.8 ± 10.08	50.4 ± 10.19	1.6 ± 8.6	0.173	0.21
Non-HDLc (mg/dl)	148 (92–274)	132 (52–234)	-8 (-109–65)	0.003*	152 (81-318)	140 (64-353)	-7 (-110–89)	0.02*	0.52
Atherogenic indexes									
TG/HDLc	3.9 (1,5-20.6)	3.2 (0.7-12)	-0.09 (-9.68–3.28)	0.079	3.4 (1.2-26.4)	2.5 (1.2-21.6)	-0.66 (-7.93–8.07)	<0.001*	0.15
TyG index	9.53 (8.14–10.9)	9.4 (8.1–10.4)	-3 (-2.01–0.91)	0.024*	9.37 (8.03-11.49)	9.14 (8.03-11.81)	-0.27 (-2.79–1.19)	<0.001*	0.49
TyG-BMI index	350.7 ± 29.376	317.8 ± 32.659	-32.89 ± 26.67	<0.001*	453 ± 57.298	400.8 ± 70.389	-52.14 ± 46.41	<0.001*	0.01*
CRI-I	4.17 (2.66–7.48)	4 (2.12–6.39)	-1.7 (-2.89–1.35)	0.02*	4.12 (2.76-7.49)	3.94 (2.21-8.2)	-0.29 (-2.27–0.73)	0.005*	0.83
CRI-II	2.61 ± 0.88	2.39 ± 0.903	-0.22 ± 0.77	0.062	2.55 ± 0.735	2.29 ± 0.702	-0.26 ± 0.76	0.016*	0.82
AIP	0.58 ± 0.262	0.51 ± 0.245	-0.07 ± 0.19	0.015*	0.55 ± 0.248	0.44 ± 0.241	-0.11 ± 0.2	<0.001*	0.31
AC	3.17 (1.66–6.48)	3 (1.12–5.39)	-0.26 (-2.89–1.35)	0.01*	3.12 (1.44-6.49)	2.94 (1.21-7.2)	-0.29 (-2.27–1.61)	0.002*	0.99

* p value <0.05 was considered statistically significant

Δ: Value change

p^{Δ}: Statistical significance level of value changes

BMI: Body Mass Index

FPG: Fasting Plasma Glucose

ALT: alanine aminotransferase

AST: aspartate aminotransferase

TC: total cholesterol

TG: triglyceride

LDLc: low-density lipoprotein cholesterol

HDLc: high-density lipoprotein cholesterol

Non-HDLc: Non-high density lipoprotein cholesterol

TyG index: TG-Glucose index (TyG)

CRI-I: Castelli Risk Index I

CRI-II: Castelli Risk Index II

AIP: Atherogenicity Plasma Index

AC: Atherogenic Coefficient

In the group with HbA1c <8%, the level of ALT (p:0.039) and AST (p:0.039) data significantly dropped after treatment, nevertheless, there was no appreciable drop in the FPG and HbA1c values. In the group with HbA1c ≥8%, there was a significant decrease in FPG ($p < 0.001$), HbA1c ($p < 0.001$), ALT (p:0.002) and AST (p:0.021) data. There was a significant decrease in TG (p:0.039) and non-HDLc (p:0.015) in the group with HbA1c <8%, but there was no significant change in TC, HDLc, and LDLc. The decrease in TC (p:0.033), TG ($p < 0.001$), LDLc (p:0.037), and non-HDLc (p:0.005) was statistically significant in the group with HbA1c ≥8%, but there was no significant change in HDLc. When evaluated in terms of atherogenic indices, a significant increase was found in TyG-BMI index ($p < 0.001$), AIP (p:0.046), and AC (p:0.035) in the group with HbA1c <8%, nevertheless, there were no appreciable alterations in TG/HDLc, TyG index, CRI-I, or CRI-II. In the group with HbA1c ≥8%, all atherogenic indices (TG/HDLc $p < 0.001$, TyG index $p < 0.001$, TyG-BMI index $p < 0.001$, CRI-I $p < 0.001$, CRI-II p:0.008, AIP $p < 0.001$ and AC p: 0.001) significantly decreased. Exenatide substantially more effectively reduced FPG, HbA1c, TyG index, TyG-BMI index, CRI-I and AIP in the group with HbA1c ≥8% than in the group with HbA1c <8% ($p < 0.001$, $p < 0.001$, p:0.001, p:0.016, p: 0.047 and p:0.008) (Tables 3 and 4).

Discussion

Our study examined the 12-week short-term outcomes of exenatide in 100 overweight diabetic patients treated at our hospital. An important finding of our study is that treatment with exenatide leads to significant short-term improvements in glycemic parameters and lipid profiles of obese diabetic patients. The most remarkable point of the article is that the improvements in these parameters reduce the atherogenic burden calculated by atherogenic indices. Although studies have not clearly elucidated the mechanism, the lipid-lowering effect of exenatide is evident. Studies focus on the reduction of chylomicron synthesis and stimulation of lipoprotein lipase, which is the main cause of intravascular TG clearance. Some other studies focus on the increase of chylomicron metabolism due to the improvement of insulin sensitivity induced by weight reduction. The decrease in chylomicron synthesis in enterocytes with little or no GLP-1 receptor suggests that there may be other receptors that have not yet been identified. Studies show that the decrease in chylomicron synthesis occurs independently of delayed gastric emptying and increased insulin secretion. In various studies, disruption of lymphatic lipid flow from the thoracic duct or the stimulation of peroxisome proliferator-activated receptor alpha by GLP-1 receptor agonist is thought to decrease TG [15]. It is thought that GLP-1 agonists have some direct cardiovascular effects besides the anti-atherosclerotic effects provided by the improvement in the lipid profile. Some of the mechanisms that produce this effect are to decrease matrix metalloproteinase-2 (MMP-2) levels and inhibit vascular smooth muscle cells. However, studies show conflicting results for cardiovascular effects in the clinical experience of GLP-1 agonists in patients. Despite the contradictions, some of the accepted effects are a reduction in myocardial infarction, a reduction in all-cause cardiovascular mortality, and a reduced risk of stroke. In these investigations, GLP-1 agonists were not linked to a decline in heart failure (HF) [16].

In patients with MS, the atherogenic lipoprotein profile features high LDLc, hypertriglyceridemia, and low HDLc. This profile is closely associated with increased cardiovascular disease (CVD), increased morbidity, and greater mortality in MS patients. In individuals with CVD and T2DM, elevated LDLc seems like an important predictor of cardiovascular events [17]. In a study comparing liraglutide and placebo group, a decreasing trend was found in the TG level in the group using liraglutide, even if it didn't matter. Moreover, the LDLc level substantially increased in this research, although the HDLc level did not alter much [18]. In another study comparing semaglutide with the placebo group, significant decreases were found in TC, TG, VLDL, and HDLc levels in the group using semaglutide [19]. In a study comparing lixisenatide with glargine insulin, lixisenatide was superior for improvement in lipid profile [20]. Similarly, in our study, exenatide provided significant reductions in TC, LDLc, TG and non-HDLc in the early 12-week period in obese patients with T2DM. Exenatide was shown to have a similar effect on lipid profiles in groups with and without adequate blood sugar management. Likewise, the improvement in the lipid profile did not differ in the groups separated by BMI. Results of GLP-1's agonists on cardiovascular adverse events, both through improvement in lipid profile and improvement in direct vascular structure, have been investigated in studies. In the EXSCEL study, no superiority was found over placebo in cardiovascular mortality rates, myocardial infarction, stroke, and hospitalization rates for heart failure in patients using exenatide, but there was no significant difference in safety [21]. In the SUSTAIN-6 study, in the group receiving semaglutide, there was a decrease in non-fatal myocardial infarction and non-fatal stroke, but the risk of cardiovascular death was similar to the placebo group [22]. Liraglutide drastically reduced cardiovascular mortality and all-cause mortality in the LEADER trial participants as compared to the placebo group. In this study, the frequency of hospitalization for myocardial infarction, stroke, and heart failure was insignificantly lower in the liraglutide group compared to the placebo [23]. A study comparing albiglutide with the placebo group showed superiority over placebo for cardiovascular mortality, myocardial infarction,

Table 3 Demographic data, clinical data, atherogenic indexes of HbA1c <8% and HbA1c ≥8% patients

	HbA1c <8% (n:52)				HbA1c ≥8% (n:48)				p^{Δ}
Parameters	Basal	3. Month	Δ	*p*	Basal	3. Month	Δ	*p*	
Age (years)	51.1				53.5				
Weight (kg)	115.5 (80-155)	107.5 (75-149)	-7 (-31–3.8)	<0.001*	101.5 (79-166)	94.1 (74-165)	-7.8 (-43–-1)	<0.001*	0.87
BMI (kg/m^2)	44 ± 8.35	40.6 ±7.89	-3.4 ± 3.49	<0.001*	41.3 ± 5.51	38 ± 5.78	-3.29 ± 2.61	<0.001*	0.82
FPG (mg/dl)	126 (69-319)	122.5 (78-362)	-1.5 (-183–158)	0.831	196 (100-393)	152 (74-394)	-45 (-226–242)	<0.001*	<0.001*
HbA1c (%)	6.8 (5.5-7.9)	6.5 (5-13.4)	-0.1 (-2.2–6.2)	0.451	9.5 (8–13.9)	7.9 (5.3–13.1)	-1.45 (-6.6–1.1)	<0.001*	<0.001*
Creatinine (mg/dl)	0.67 (0.33-1.38)	0.67 (0.38-1.47)	-0.03 (-0.44–0.3)	0.242	0.63 (0.38–1.08)	0.61 (0.38–1.34)	0.01 (-0.29–0.7)	0.33	0.86
ALT (U/L)	22.5 (10-117)	19.5 (5-69)	-2 (-58–20)	0.039*	20.5 (8-76)	17 (7-55)	-1.5 (-36–17)	0.002*	0.43
AST (U/L)	21.5 (13-111)	19.5 (13-52)	-2 (-59–11)	0.039*	19 (12-48)	19 (10-58)	-2 (-17–44)	0.021*	0.66
Albumin (g/dl)	4.2 (3-4.8)	4.28 (3.5-4.9)	-0.1 (-0.89–0.82)	0.153	4.2 (3.3–4.9)	4.28 (3.6–4.7)	0.06 (-0.35–1.1)	0.04*	0.92
Traditional lipid profile/parameters									
TC (mg/dl)	198 (128–288)	186.5 (116–303)	-5 (-82–94)	0.04*	204,5 (112-367)	182,5 (86-402)	-3.5 (-107–54)	0,033*	0.82
TG (mg/dl)	153.5 (73–465)	144.5 (57–298)	-8.5 (-338–67)	0.07	185,5 (73-465)	136,5 (52-1063)	-30 (-420–292)	<0,001*	0.008
LDLc (mg/dl)	120,3 ± 28,77	113,8 ± 36,19	-6.5 ± 28.69	0,108	121,8 ± 39,31	110,2 ± 41,38	-11.63 ± 37.6	0,037*	0.44
HDLc (mg/dl)	50,1 ± 8,69	50,1 ± 9,83	0.04 ± 8.56	0,974	45,9 ± 10,36	47,3 ± 9,19	1.42 ± 7.33	0,187	0.39
Non-HDLc (mg/dl)	148.5 (85–274)	137 (74–222)	-9.5 (-99–89)	0.01*	159 (81-318)	133.5 (52-353)	-6.5 (-110–35)	0.005*	0.99
Atherogenic indexes									
TG/HDLc	3.18 (1.27-10.57)	2.75 (1.38-8.7)	-0.07 (-6.83–3.28)	0.135	3.98 (1.55-26.43)	2.86 (0.78-21.69)	-0.85 (-9.68–8.07)	<0.001*	0.006
TyG index	9.19 ± 0.467	9.09 ± 0.534	-0.1 ± 0.59	0.242	9.9 ± 0.663	9.35 ± 0.765	-0.55 ± 0.7	<0.001*	0.001*
TyG-BMI index	404.5 ± 75.911	370.1 ± 76.461	-34.37 ± 40.75	<0.001*	409.6 ± 61.782	356.3 ± 62.221	-53.35 ± 36.63	<0.001*	0.016*
CRI-I	4.11 ± 0.795	3.94 ± 0.887	-0.17 ± 0.69	0.081	4.56 ± 1.114	4.09 ± 1.095	-0.48 ± 0.84	<0.001*	0.047*
CRI-II	2.44 ± 0.623	2.32 ± 0.736	-0.12 ± 0.58	0.132	2.72 ± 0.941	2.36 ± 0.863	-0.37 ± 0.91	0.008*	0.12
AIP	0.49 ± 0.197	0.44 ± 0.189	-0.04 ± 0.16	0.046*	0.64 ± 0.283	0.5 ± 0.292	-0.15 ± 0.22	<0.001*	0.008*
AC	3.03 (1.44–6.09)	2.85 (1.12–5.39)	-0.17 (-2.12–1.61)	0.02*	3.43 (1.76-6.49)	3.03 (1.21-7.2)	-0.4 (-2.89–0.8)	0.001*	0.17

* *p* value <0.05 was considered statistically significant

Δ: Value change

p^{Δ}: Statistical significance level of value changes

BMI: Body Mass Index

FPG: Fasting Plasma Glucose

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

TC: Total cholesterol

TG: Triglyceride

LDLc: Low-density lipoprotein cholesterol

HDLc: High-density lipoprotein cholesterol

Non-HDLc: Non-high density lipoprotein cholesterol

TyG index: TG-Glucose index (TyG)

CRI-I: Castelli Risk Index I

CRI-II: Castelli Risk Index II

AIP: Atherogenicity Plasma Index

AC: Atherogenic Coefficient

and stroke. This study also emphasized that albiglutide is not inferior to placebo in terms of safety [24]. The common conclusion of these studies is that GLP-1 agonists do not differ from placebo in cardiovascular safety. On the other hand, positive results have been obtained in cardiovascular adverse events, but not all results support each other.

Table 4 The multivariate linear regression analysis of atherohenic indexes

		Adj. R^2	SC β	t	*p*	95% CI		VIF
						LB	UB	
Δ TG/HDLc	Δ BMI	0.058	-0.162	-0.806	0.422	-0.426	0.180	4.228
	Δ HbA1c		0.085	0,679	0.499	-0.198	0.404	1.645
Δ TyG index	Δ BMI	0.626	0.010	0.082	0.934	-0.053	0.057	4.228
	Δ HbA1c		0.034	0.432	0.666	-0.043	0.066	1.645
Δ TyG-BMI index	Δ BMI	0.813	0.660	15.058	<0.001	7.389	9.633	1.019
	Δ HbA1c		0.042	0.779	0.438	-1.336	3.060	1.546
Δ CRI-I	Δ BMI	0.029	-0.072	-0.722	0.472	-0.068	0.032	1.019
	Δ HbA1c		0.068	0.555	0.580	-0.070	0.125	1.546
Δ CRI-II	Δ BMI	0.004	-0.108	-1.065	0.290	-0.076	0.023	1.019
	Δ HbA1c		0.061	0.486	0.628	-0.073	0.121	1.546
Δ AIP	Δ BMI	0.136	-0.100	-1.058	0.293	-0.018	0.005	1.019
	Δ HbA1c		0.093	0.799	0.426	-0.014	0.032	1.546
Δ AC	Δ BMI	0.063	-0.125	-1.273	0.206	-0.082	0.018	1.019
	Δ HbA1c		0.017	0.137	0.891	-0.091	0.105	1.546

Adj. $R^{2:}$ Adjusted R Spuare
SC β: Standardized Coefficients Beta
95% CI: Confidence interval
LB: Lower bound
UB: Upper bound
VIF: Variance inflation factor
Δ: Value change
BMI: Body Mass Index
FPG: Fasting Plasma Glucose
TyG index: TG-Glucose index
CRI-I: Castelli Risk Index I
CRI-II: Castelli Risk Index II
AIP: Atherogenicity Plasma Index
AC: Atherogenic Coefficient

Cardiovascular mortality is mostly attributed to atherosclerotic heart disease. It is necessary to identify asymptomatic patients at risk for atherosclerosis before encountering the negative consequences of atherosclerotic disease. Some atherogenic indices have been put forward to reveal this risky patient group [13]. In a study conducted to investigate some of these indices, it was shown that the CRI-I, CRI-II, AIP, and AC indices, which are thought to predict atherogenicity among individuals with coronary artery disease that has been diagnosed by angiogram, were significantly higher than the healthy control group [25]. In a study with ApoE-deficient experimental mice, vascular endothelial aging and atherosclerotic plaque growth were detected in mice exposed to chronic stress. A significant reduction in plaque lipid accumulation and atherosclerotic lesion formation was found in chronically stressed mice administered a GLP-1 agonist, exenatide, compared to chronically stressed mice not administered exenatide [26]. In another study, a significantly higher reduction in carotid intima-media thickness (with Doppler ultrasonography) was noted in the patient population receiving the GLP-1 analog liraglutide treatment, independent of the improvement in BW, BMI, and lipid profile, compared to the control group. Thus, the regression of atherosclerosis was demonstrated with liraglutide treatment [27]. Significant regressions were observed in all of the TG/HDLc, TyG index, TyG-BMI index, CRI-I, CRI-II, AIP, and AC atherogenic indices including in our study after exenatide treatment. Morover, exenatide treatment revealed a significant difference in atherogenicity reduction in the BMI $40 \geq kg/m^2$ group compared to the BMI $40 < kg/m^2$ group. Similarly, atherogenic index change due to exenatide treatment was more successful in patients with HbA1c ≥8% than in patients with HbA1c <8%.

Limitations

The minimal number of volunteer patients necessitated the single-centered and retrospective nature of our investigation. This circumstance constrains the findings of our

investigation. Patients without microvascular and macrovascular complications were included in our study. Since it was a retrospective study, invasive atherogenic imaging was not performed because it was not indicated for our patients. The control group was not included in our study because our research plan was designed according to the changes in the pre-treatment and post-treatment times. In this study, we examined the short-term effects of exenatide on atherogenicity indices. Therefore, this study gives limited results about the atherogenic changes that develop in the chronic period. There is a need for randomized controlled studies that include prospective invasive and non-invasive atherogenic parameters and evaluated in terms of cardiac event outcomes in long-term follow-up.

Conclusions

In conclusion, the GLP-1 analog exenatide caused a significant reduction in atherogenicity indices defined in the literature. Exenatide showed these positive effects more prominently having HbA1c ≥8%. Given the elevated risk of cardiovascular problems in obese individuals with uncontrolled diabetes, it is advised to use GLP-1 analogs early on. Our findings should be substantiated by randomized controlled prospective trials in which imaging techniques and invasive procedures can verify exenatide's beneficial effects on atherogenicity.

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Declarations

Ethics clearance This study was approved by the Aksaray University Faculty of Medicine clinical research ethics committee (Date: 19.08.2021, Decision no: 2021/08-08). The Declaration of Helsinki (1964) of the World Medical Association guided the execution of this study.

Conflict of interest The authors have no conflict of interest to declare regarding the content of this article.

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CORRECTION

Correction to: Basic fibroblast growth factor alleviates metabolic abnormalities in the heart of streptozotocin-induced diabetic rats

Yinli Huang[1] · Wei Dong[1] · Minjie Lin[1] · Hongchang Gao[2] · Hong Zheng[1,2]

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VISION STATEMENT

To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

MISSION STATEMENT

1. Promotion of excellence in diabetes care to make India the Diabetes Care Capital
2. Empowerment of persons living with diabetes
3. Support for diabetes research
4. Dissemination of information and knowledge in diabetes care
5. Advocacy for the cause of diabetology

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Research Grants upto INR 200000 to support outstanding thesis/ research work by first year MD/DNB/ PHD students/Research fellows from India.

Eligibility Criteria
All Postgraduates in First year MD, DM /DNB from any of the institutions in the country are eligible to apply

How to apply?
Upload your Research proposals on the RSSDI Online Research Grant Platform.

Research proposal should have following proofs-

1. A supporting letter from your guide/ head of department stating that this is a bonafide project for your thesis and also mentioning the dates of you joining the program and expected date of graduation. The guide must also state that he/she will stand guarantee for the work done
2. A detailed budget
3. Thesis proposal approved by the department/appropriate institutional authority
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Selection Process
Proposals will be reviewed by the research committee of the RSSDI.

Disbursement of Grant
20% of the grant amount will be disbursed initially. 30% of payment after receiving your project status report and utilisation of sanctioned amount, 25% on further completion and pending 25% on final submission of your project. All reports must be uploaded on the RSSDI Online Research Grant Platform.

Responsibility:
All grant awardees are expected to present their work at RSSDI Annual Conference during research presentation's session. Failure to file progress reports annually and when requested by the RSSDI and failure to present progress at RSSDI Annual conference may result in the forfeiture of the grant.

All awardees are expected to follow the tenets of responsible and ethical conduct of research. Unethical or fraudulent use of RSSDI research funds will warrant adverse action from the society including forfeiture of grant, black listing in the society's databases and other legal recourses that are available to the society.

Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

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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.

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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.
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(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

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4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
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8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
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11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
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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
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