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EDITORIAL

Muscle health — the expanding horizon of diabetes care

S. V. Madhu[1]

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Sarcopenia is an age-related degenerative skeletal muscle disorder. It is characterized by a progressive and generalized decrease in muscle mass, and muscle strength and function. There are many bidirectional links between diabetes mellitus and sarcopenia, and the existence of one may increase the risk of developing other. Hence, muscle health in relation to diabetes mellitus has been the focus of recent attention of physicians as well as researchers.

Diabetes mellitus is associated with insulin resistance, inflammation, accumulation of advanced glycosylated end products, increased oxidative stress, and several micro- and macrovascular complications. All these factors can cause cellular dysfunction and death of myocytes leading to loss of skeletal muscle mass, strength, and function and development of sarcopenia [1]. Abnormalities in protein and lipid metabolism as well as vascular and mitochondrial dysfunction can also compromise muscle mass, strength, quality, and function [2].

Sarcopenia is widely prevalent globally and is of particular concern in south Asians and Indians who are an aging population already faced with the problem of low protein intakes. According to a recent meta-analysis, patients with type 2 diabetes mellitus have an increased risk of sarcopenia compared with euglycemic subjects. The prevalence of sarcopenia among Asians aged ≥60 years using the Asian Working Group for Sarcopenia criteria was 15.9% in diabetic and 10.8% in non-diabetic individuals. Type 2 diabetes mellitus patients were found to have lower muscle performance and strength but similar muscle mass compared with euglycemic subjects [3]. Another study showed high HbA1c levels, prediabetes, diabetes, and diabetes-associated complications were all associated with an increased risk of sarcopenia [4]. Similarly, the prevalence of sarcopenia in Japanese and Chinese adults (aged ≥65 and >60 years, respectively) with type 2 diabetes mellitus was reported to be 15% using the AWGS definition [5, 6]. The Korean sarcopenic obesity study reported 15.7% among diabetic individuals [7]. Similar data from south Asia are lacking. However, these studies suggest that strategies for the management of sarcopenia are required in Asian elderly patients, especially with diabetes.

In the current issue, Pineda et al. [8] from Venezuela report that about one-fourth of elderly diabetic patients, 65 years or older, had sarcopenia using the European Working Group on Sarcopenia in Older People (EWGSOP) criteria. More importantly, nearly 50% of the diabetic patients had decreased muscle strength and those with poor glycemic control were at a higher risk for sarcopenia. The rate of sarcopenia and sarcopenia components being reported in a Latin American study population is higher than earlier studies in literature even from Asia. However, this could have been influenced by the much longer duration of diabetes in these subjects that could have adversely affected muscle mass and function. Nevertheless, it reinforces the need to screen for sarcopenia in diabetic populations across the world and the need to develop strategies to preserve muscle function in long standing elderly patients with diabetes. With more and more reports of impairment of bone health in diabetic patients, the need to prevent falls and maintain good muscle function becomes all the more important.

Sarcopenia also contributes to development and progression of diabetes mellitus. The low muscle mass as well as local inflammation secondary to inter- and intramuscular adipose tissue accumulation is believed to alter glucose disposal leading to insulin resistance. It is well known that muscle accounts for 80% of the total glucose disposal in the body and hence preservation of muscle mass and function is critical for normal glucose metabolism. In addition, the lower basal metabolic rate associated with low lean body mass as well as lower levels of physical activity that are seen in those with reduced muscle mass and function also contributes to the higher risk of obesity, insulin resistance, and diabetes [9]. Aging is also accompanied by a lower muscle mass and a higher fat mass

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and these abnormalities further add to the risk of metabolic disorders including diabetes mellitus [10].

In MrOS study, men aged ≥65 years without type 2 diabetes mellitus but in the highest quartile for insulin resistance had twofold increased odds of losing ≥5% total lean mass over approximately five years [11]. In a recent study, it was seen that waist circumference was negatively associated with muscle strength, quality, and performance in 84 overweight and obese older adults [12].

The typical Asian Indian Phenotype [13] which is also referred to as the "Thin Fat Phenotype" or the "Sarcopenic obesity phenotype" predisposes Indians to a higher risk of cardiometabolic disorders [14] and consists of normal or near normal body weight, excess adiposity, and a lower muscle mass overall. The process of aging in them enhances this risk even further. A lower muscle mass relative to fat mass has been shown to be associated with 2-fold greater risk of type 2 diabetes in several Asian populations [15–17].

It is clear from the above that identification, management, and prevention of sarcopenia in the diabetic patient is also important. Diabetes mellitus is treated with wide variety of available oral antidiabetic agents and insulin. For majority of antidiabetic agents, effect on sarcopenia is unclear. Metformin is being investigated as an adjunct therapy to resistance training in older adults, as it may aid in mobilization of M2 macrophages which could be having anti-inflammatory properties in skeletal muscle [18]. Indirectly, insulin therapy may improve muscle health by reducing blood glucose concentrations which if chronically increased result in glycation end products. Sulfonylureas have been reported to cause muscle atrophy in a very small proportion of users [19]. Possible therapeutic targets could be hormonal interventions such as a transdermal testosterone gel [20] and selective androgen receptor modulators but these are still investigational [21, 22].

Even if pharmacological agents are approved for the treatment of sarcopenia in the future, lifestyle modification will likely remain the primary therapy for the management of diabetes mellitus and sarcopenia [9]. Evidence for the most effective and feasible exercise and dietary interventions in this population is lacking. Epidemiological studies do not report differences in smoking and drinking habits between type 2 diabetes mellitus individuals with and without sarcopenia. A meta-analysis has reported that any engagement in physical activity reduces the likelihood of incident sarcopenia in adults older than 40 years [23].

Resistance training is the most effective strategy for improving both muscle mass and function in sarcopenic individuals [24]. It is effective for improving muscle strength, size, and quality and metabolic health in older adults with type 2 diabetes mellitus [24]. Resistance training may therefore reduce the risk of developing sarcopenia in diabetes mellitus patients.

Protein is important for the maintenance and growth of skeletal muscle [25]. Plant-based protein intake is negatively associated with incidence of type 2 diabetes mellitus. Findings from the Melbourne Collaborative Cohort Study (MCCS) and a meta-analysis showed that individuals in the highest plant protein intake category had reduced, even though modest risk for incident type 2 diabetes mellitus compared with individuals in the lowest intake category [26]. A recent meta-analysis by Morton et al. demonstrated that protein supplementation was effective for improving lean mass in resistance-trained adults, but has very little effect on muscle outcomes in older adults [27]. Omega-3 supplementation alone and in combination with exercise may improve metabolic and muscle health. Omega-3 supplementation can affect muscle directly by increasing muscle protein synthesis [28] and indirectly by decreasing systemic inflammation [29].

The relationship of sarcopenia with diabetes is clearly bidirectional. There is an urgent need to generate sufficient data that clearly defines the problem of sarcopenia among Indians as also from rest of the world especially in the context of diabetes and diabetes risk. The south Asian consensus proposes different criteria to identify sarcopenia among south Asians [30]. Clearly, there is a need for lot more research in this critical area of diabetes which can help understand the association of the two conditions better and help shape effective strategies for prevention and management of diabetes, not only in India but also in rest of the developing and the developed world as well.

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REVIEW ARTICLE

Renal outcomes in Asian patients with type 2 diabetes mellitus treated with SGLT2 inhibitors: a systematic review and meta-analysis of randomized controlled trials

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Abstract

Aim This study investigated the effects of sodium-glucose cotransporter 2 (SGLT2) inhibitors on renal outcomes in Asian patients with type 2 diabetes mellitus (T2DM).

Materials and methods We searched Medline, EMBASE, and the Cochrane Library to identify randomized controlled trials published up to April 2020 that compared SGLT2 inhibitors with placebo or active comparator and reported any renal outcomes in Asian patients with T2DM. Random effects models and inverse variance weighting were used to calculate relative risks with 95% confidence intervals (CIs).

Results We included 14 studies, totaling 3792 patients, in the analysis. In the short term, SGLT2 inhibitors significantly slowed estimated glomerular filtration rate (eGFR) decline (MD: 0.80; 95% CI: 0.66 to 0.94; $p < 0.00001$) and reduced Scr levels (SMD: − 0.17; 95% CI: − 0.23 to − 0.10; $p < 0.00001$) as compared with the control groups. The SGLT2 inhibitor group also had an advantage over the control group in lowering uric acid (UA) (SMD: − 1.2; 95% CI: − 1.30 to − 1.11; $p < 0.00001$). There was no significant difference in urinary albumin creatinine ratio (UACR) reduction between the SGLT2 inhibitor and control groups (MD: − 8.87; 95% CI: − 19.80 to 2.06; $p = 0.11$). However, dapagliflozin does appear to reduce albuminuria ($p = 0.005$). Lastly, SGLT2 inhibitors increased the incidence of adverse events (AEs) related to renal function (OR: 1.90; 95% CI: 1.24 to 2.91; $p = 0.003$), but did not increase the incidence of renal impairment (OR: 0.85; 95% CI: 0.40 to 1.81; $p = 0.68$).

Conclusion The use of SGLT2 inhibitors in Asian patients with T2DM can help delay the decline of eGFR and reduce Scr and UA. Although SGLT2 inhibitors have no overall advantage in reducing albuminuria, dapagliflozin does appear to reduce albuminuria, and while they may increase the occurrence of AEs related to renal function, they do not increase the incidence of renal impairment.

Keywords Type 2 diabetes mellitus · SGLT2 inhibitors · Asian · Renal outcome · Meta-analysis

Introduction

According to the International Diabetes Federation (IDF), the number of patients with diabetes reached 451 million worldwide in 2017. Among them, Asians account for the largest proportion [1]. Diabetic kidney disease (DKD) is one of the most common and serious complications of diabetes [2]. Studies have shown that Asian people with type 2 diabetes (T2DM) have unique clinical presentations and are more likely to have DKD [3].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors reduce the glucose reabsorption capacity of the kidney by inhibiting glucose transporters on the surface of the proximal tubules of the kidney, thereby promoting the excretion of urinary glucose [4]. In recent years, studies from across the globe have shown that SGLT2 inhibitors are beneficial to the cardiovascular and renal outcomes of patients with T2DM [5]. However, although Asians are at high risk of DKD, meta-analyses focused on this population are lacking. Therefore, we performed a systematic review and

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meta-analysis of randomized controlled trials (RCTs) to investigate the effects of SGLT2 inhibitors on renal outcomes in Asian patients with T2DM.

Methods

Study design

This study is a systematic review and meta-analysis that evaluated the effects of SGLT2 inhibitors on the renal outcomes of Asian patients with T2DM as compared with placebo or positive controls. We collected randomized controlled studies related to SGLT2 inhibitors, included studies compared with placebo and active comparator (antidiabetic drugs). Other prospective and non-randomized studies were excluded.

Data sources and searches

We searched Medline, EMBASE, and the Cochrane Central Register of Controlled Trials for RCTs of SGLT2 inhibitors published as full articles and until April 2020. The search terms used for SGLT2 inhibitors were "sodium-dependent glucose transporters 2," "SGLT2 inhibitor," or "SGLT-2 inhibitor." The retrieval process is shown in Fig. 1.

Study selection

We included all studies that reported individual RCT data of any SGLT2 inhibitors versus placebo or active controls in Asian patients with T2DM. We eliminated duplicate

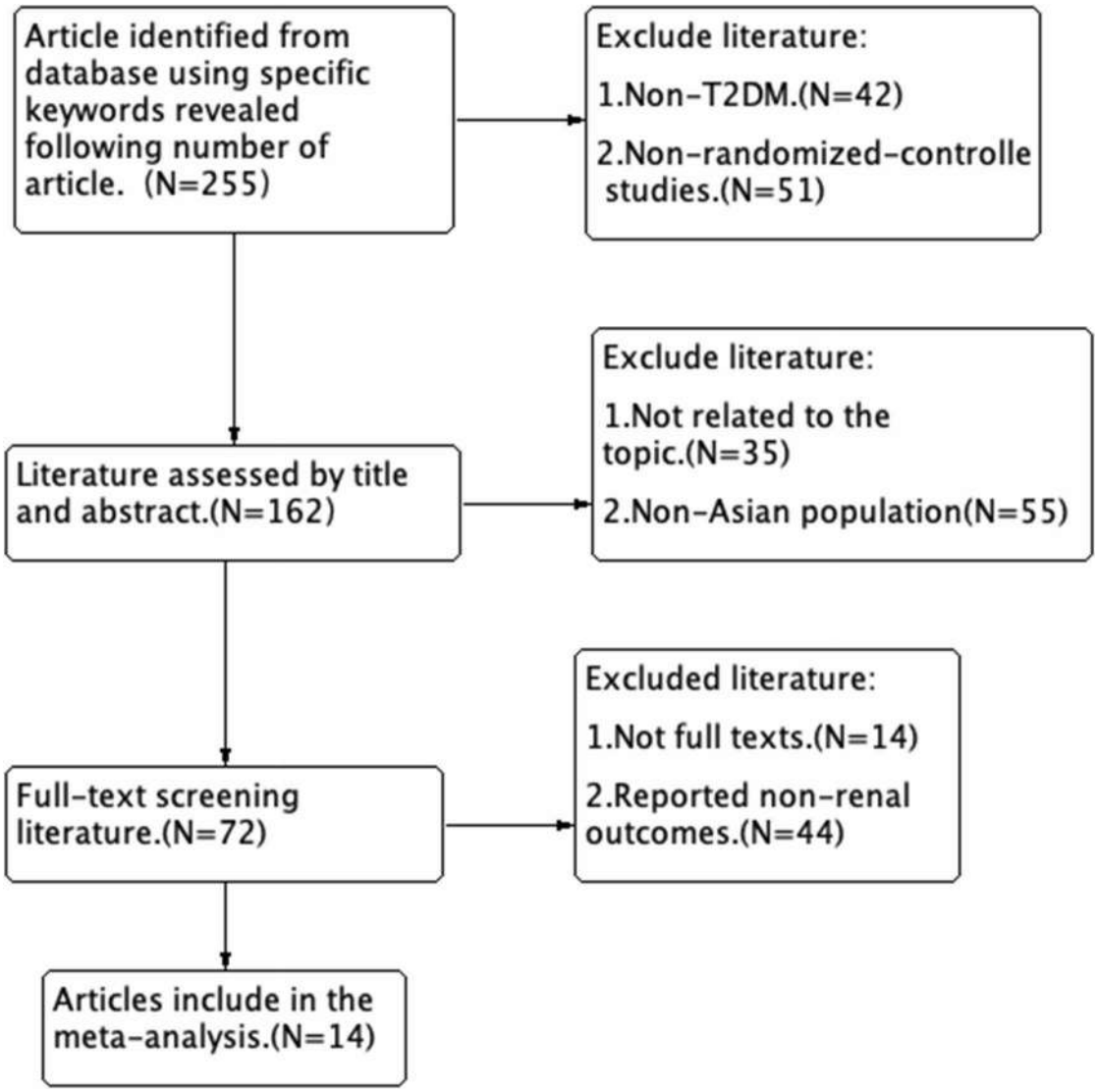

Fig. 1 Identification of eligible studies

Table 1 Baseline characteristics of the studies included in the meta-analysis

Study	Interventions (dose of SGLT2i)	Control		Number of patients SGLT2i	Comp	Duration of intervention, weeks	Quality assessment	Outcome
Lu et al. [16]	Ipragliflozin 50 mg	Placebo	Metformin	87	83	24 weeks	5	eGFR, Scr, UACR
Kaku2014	Tofogliflozin10 mg, 20 mg, 40 mg	Placebo		165	55	24 weeks	5	eGFR, Scr, UA
Kaku2014[2]	Dapagliflozin 5 mg, 10 mg	Placebo		174	87	24 weeks	4	Renal impairment
Han et al. [13]	Ipragliflozin 50 mg	Placebo	Metformin					
Sitagliptin	73	66	24 weeks	5	eGFR, Scr			
Ji et al. [15]	Dapagliflozin 5 mg, 10 mg	Placebo		261	132	24 weeks	5	eGFR, Scr, UA, renal impairment
Ji et al. [14]	Canagliflozin 100 mg, 300 mg	Placebo	Metformin					
Metformin + sulphonylurea	450	226	18 weeks	5	eGFR, Scr			
Inagaki et al. [9]	Canagliflozin 100 mg, 200 mg	Placebo		178	93	24 weeks	5	Scr, UACR
Seino2014	Luseogliflozin 2.5 mg	Placebo		79	79	24 weeks	6	Scr, UA, AE related to renal function
Seino2014[2]	Luseogliflozin 0.5 mg 2.5 mg 5 mg	Placebo	Diet therapy	183	56	12 weeks	4	Scr, UA, AE related to renal function
Seino2014[3]	Luseogliflozin 1 mg, 2.5 mg 5 mg, 10 mg	Placebo		224	58	12 weeks	5	Scr, UA, AE related to renal function
Yang et al. [7]	Dapagliflozin 10 mg	Placebo	Insulin or oral antihyperglycemic	139	133	24 weeks	4	Renal impairment
Yang et al. [19]	Dapagliflozin 5 mg, 10 mg	Placebo	Metformin	299	145	24 weeks	6	AE related to renal function
Terauchi et al. [17]	Tofogliflozin 20 mg	Placebo		140	70	16 weeks	6	UA
Bancha2019	Dapagliflozin 10 mg	Control		28	29	12 weeks	4	eGFR, Scr, UACR, UA

The superscript numbers are used to distinguish between different randomized controlled studies with the same author

eGFR, estimated glomerular filtration rate; *Scr*, serum creatinine; *UACR*, urinary albumin/creatinine ratio; *UA*, uric acid; adverse events (AEs) related to renal function

publications of original RCTs and screened the titles and abstracts of identified studies to identify RCTs that reported at least one of the following renal outcomes: estimated glomerular filtration rate (eGFR), serum creatinine (Scr), urinary albumin creatinine ratio (UACR), uric acid (UA), adverse events (AEs) related to renal function, and renal impairment.

Data extraction

Two reviewers independently extracted the data using a predefined data extraction form. Disagreements were resolved by discussion with a third reviewer. Extracted data included the first author, study characteristics, experimental and control group treatments, and renal outcome(s).

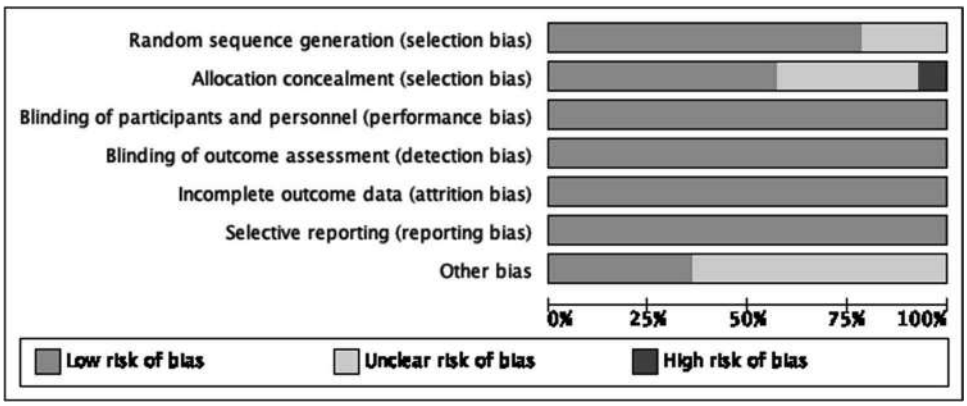

Fig. 2 Risk of bias graph

Quality assessment

The revised Jadad scale [6] was used to evaluate the methodological quality of the included literature.

Data synthesis and analysis

Statistical analyses were conducted with Review Manager (RevMan, version 5.4) software. Measures of treatment effect included mean differences (MDs), standardized mean differences (SMDs), odds ratios (ORs), and 95% confidence intervals (CIs).

Results

Characteristics of the included studies

Fourteen articles were included in the study. All investigated the effects of different SGLT2 doses. We conducted subgroup analyses of the different SGLT2 drugs and treated each drug dose as an independent study. The drugs (doses) included ipragliflozin (50 mg), tofogliflozin (10 mg, 20 mg, and 40 mg), dapagliflozin (5 mg and 10 mg), canagliflozin (100 mg, 200 mg, and 300 mg), and luseogliflozin (0.5 mg, 1 mg, 2.5 mg, 5 mg, and 10 mg). The maximum follow-up time of the RCTs was 24 weeks. Table 1 presents further detail on the characteristics of the included studies.

In the meta-regression, five studies had a baseline eGFR of 60–89 mL/min/1.73 m^2 [7–11], three studies had a baseline eGFR of > 89 mL/min/1.73 m^2 [12–14], and the baseline eGFR of six studies was unknown [15–20].

Assessment of study quality and risk of bias

Each included study reported patients' baseline data and all mentioned "randomization" in the article. The range of the overall Jadad scores was 4–7, with a relatively high correlation between quality and quantity. The risk bias graph is shown in Fig. 2, and the risk bias summary is shown in Fig. 3.

Change in eGFR

The level of eGFR was reported in 6 RCTs (5 placebo comparison and 1 active comparison trials) [10, 11, 13–16]. Their baseline eGFR interval was primarily between 60 and 89 mL/min/1.73 m^2, and all received double-blind treatment within 24 weeks. Analysis of these studies found that SGLT2 inhibitors significantly slowed the decline in eGFR as compared with controls (MD: 0.80; 95% CI: 0.66 to 0.94; $p < 0.00001$; Fig. 4). However, this estimate is heavily weighted in studies using dapagliflozin. Dapagliflozin can delay the decline of eGFR, and the difference was statistically significant (MD: 0.81; 95% CI: 0.67 to 0.95; $p < 0.00001$), while ipragliflozin, tofogliflozin, and canagliflozin had no significant difference compared with the control group. The funnel plot of comparison in eGFR is shown in Fig. 5.

Changes in Scr and UA

The level of Scr was reported in 10 RCTs (9 placebo comparison and 1 active comparison trials) [9–16, 18, 20]. Compared with the control group, SGLT2 inhibitor could reduce the level of Scr (SMD: − 0.17; 95% CI: − 0.23 to − 0.10; $p < 0.00001$; Fig. 6). Dapagliflozin has the largest weight (SMD: − 0.96; 95% CI: − 1.10 to − 0.82; $p < 0.00001$). However, tofogliflozin slightly increased Scr (SMD: 0.32; 95% CI: 0.10 to 0.55; $p = 0.005$); ipragliflozin, canagliflozin, and luseogliflozin had no significant difference compared with the control group. The funnel plot of comparison in Scr is shown in Fig. 7.

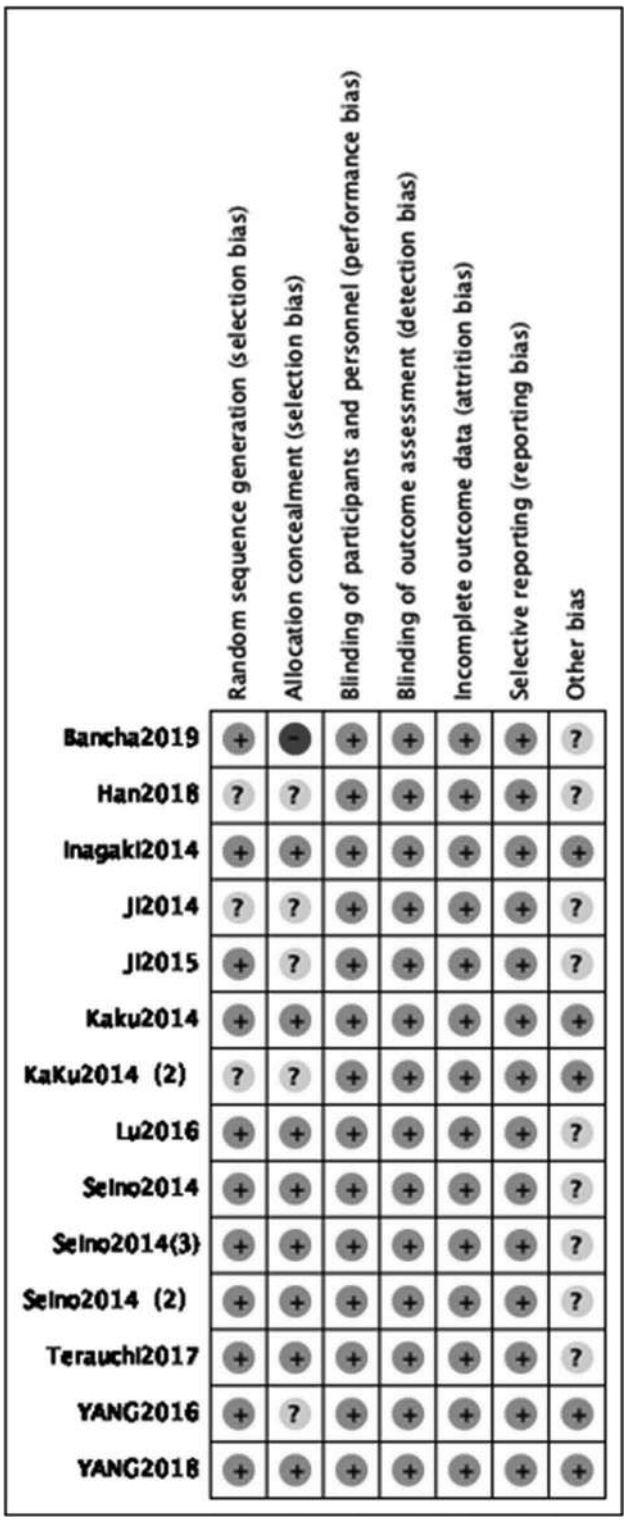

Fig. 3 Risk of bias summary

The level of UA was reported in 8 RCTs (7 placebo comparison and 1 active comparison trials) [7, 10–12, 15, 17, 18, 20]. The paper found that SGLT2 inhibitors significantly reduced the level of UA (SMD: − 1.20; 95% CI: − 1.30 to − 1.11; $p < 0.00001$; Fig. 8). Among them, luseogliflozin (SMD: − 0.55; 95% CI: − 0.68 to − 0.42; $p < 0.00001$), dapagliflozin (SMD: − 3.52; 95% CI: − 3.71 to − 3.33; $p < 0.00001$), and tofogliflozin (SMD: − 0.35; 95% CI: − 0.52 to − 0.17; $p = 0.0001$) to reduce the levels of UA were statistically significant. The funnel plot of comparison in UA is shown in Fig. 9.

Changes in UACR

We used UACR to assess albuminuria. The level of UACR was reported in 3 RCTs (2 placebo comparison and 1 active comparison trials) [9, 11, 16]. There was no significant difference in the reduction of UACR between the SGLT2 inhibitor and control groups (MD: − 8.87; 95% CI: − 19.80 to 2.06; $p = 0.11$; Fig. 10). However, dapagliflozin can reduce the level of UACR; the difference was statistically significant (MD: − 43.20; 95% CI: − 73.68 to − 12.72; $p = 0.005$). Compared with the control group, canagliflozin and ipragliflozin had no significant difference in reducing UACR. The funnel plot of comparison in UACR is shown in Fig. 11.

Effect on AEs related to renal function and renal impairment

AEs related to renal function was reported in 4 RCTs (4 placebo comparison trials) [12, 18–20]. Based on a predefined list of MedDRA preferred terms, AEs related to renal function include presence of albumin in urine, urinary calculus, presence of cells in urine, increased NAG, increased urinary b2 microglobulin, presence of blood in urine, presence of red blood cells in urine, and presence of white blood cells in urine, pollakiuria etc. [18, 19]. All AEs related to renal function were classified as mild and most of these resolved during the study. In the meta-analysis, SGLT2 inhibitors were found to increase the occurrence of AEs related to renal function as compared with the control group (OR: 1.90; 95% CI: 1.24 to 2.91; $p = 0.003$; Fig. 12). Luseogliflozin increased the incidence of AEs related to renal function accounted for the largest weight (OR: 1.90; 95% CI: 1.22 to 2.95; $p = 0.004$), while dapagliflozin had no significant difference in increasing AEs related to renal function compared with the control group (OR: 1.95; 95% CI: 0.35 to 10.74; $p = 0.44$). The majority of AEs had a low incidence and were mild in severity. The funnel plot of comparison in AEs related to renal function is shown in Fig. 13.

The renal impairment was reported in 3 RCTs (3 placebo comparison trials) [7, 8, 15]. Events of renal impairment were observed during within 24-week double-blind treatment period, and all the renal impairment events were

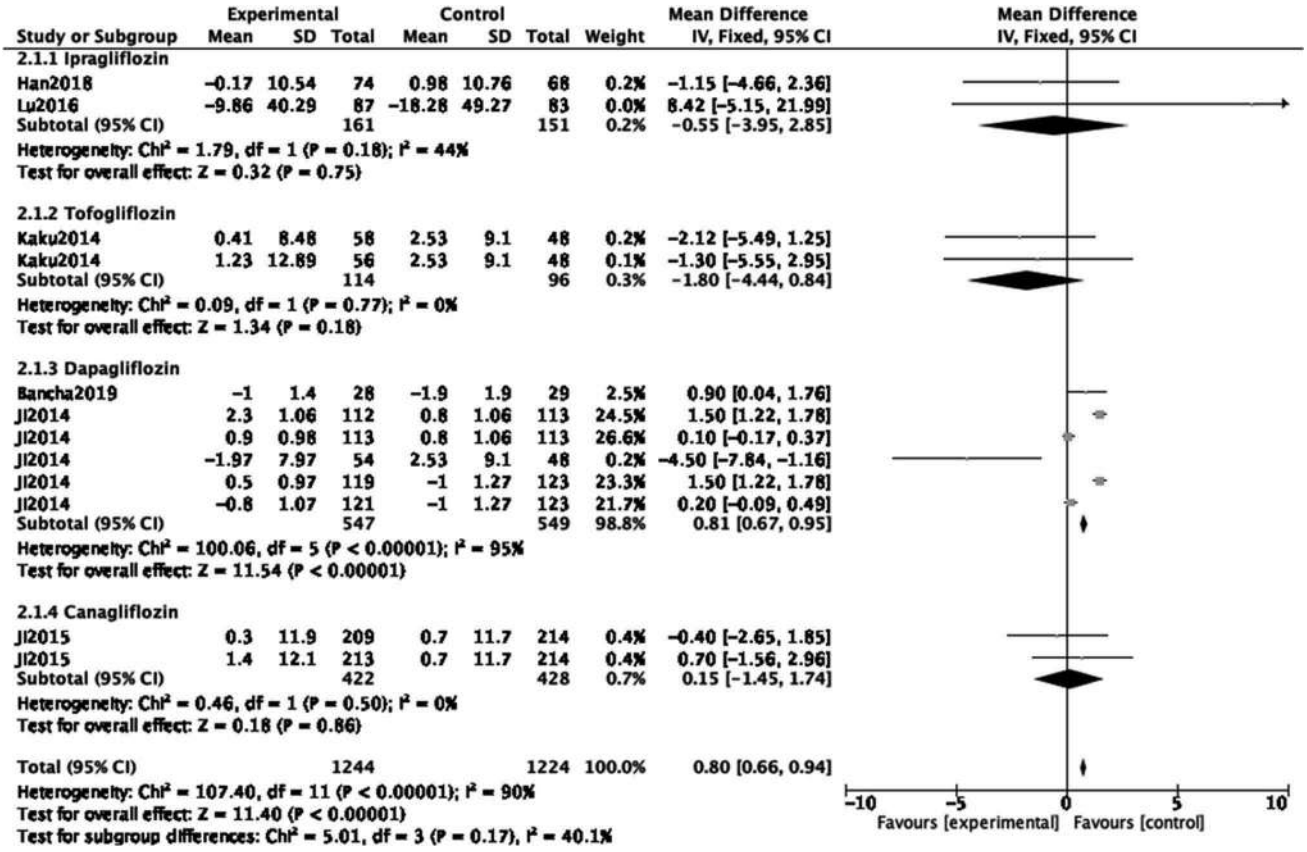

Fig. 4 Forest plot of the % change in the eGFR in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

of mild or moderate intensity, and none were serious or resulted discontinuation. Compared with the control group, the SGLT2 inhibitor group did not increase the risk of renal impairment (OR: 0.85; 95% CI: 0.40 to 1.81; $p = 0.68$; Fig. 14). The funnel plot of comparison in the renal impairment is shown in Fig. 15.

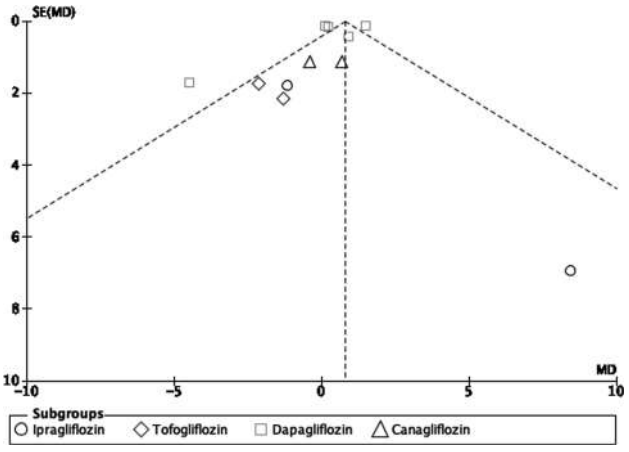

Fig. 5 Funnel plot of comparison in eGFR

Discussion

The diabetes epidemic and its complications pose a major global threat [1]; more than 90% of diabetic patients have T2DM [21]. With the increased incidence of T2DM, the incidence of DKD is also increasing annually [22]. As compared with non-Asian people, Asians have their own characteristics. They have a lower average body mass index, higher body fat content, and more visceral fat [23]. This difference between Asian and non-Asian populations is related to genetic susceptibility and eating habits. Someone found that the Asians diet contains more carbohydrates (e.g., white rice) [24]. Foods with a high glycemic index stimulate the pancreas to secrete a high demand for insulin. When the pancreas has congenital dysfunction, this leads to increased serum glucose levels [25]. A large multinational clinical study found that Asian diabetics are more likely to develop DKD [26]. The incidence of DKD in Asia, America, and the Caribbean is higher than other regions or countries [27]. The differences in the prevalence of DKD are due to many factors, including age, obesity, the course of diabetes, serum

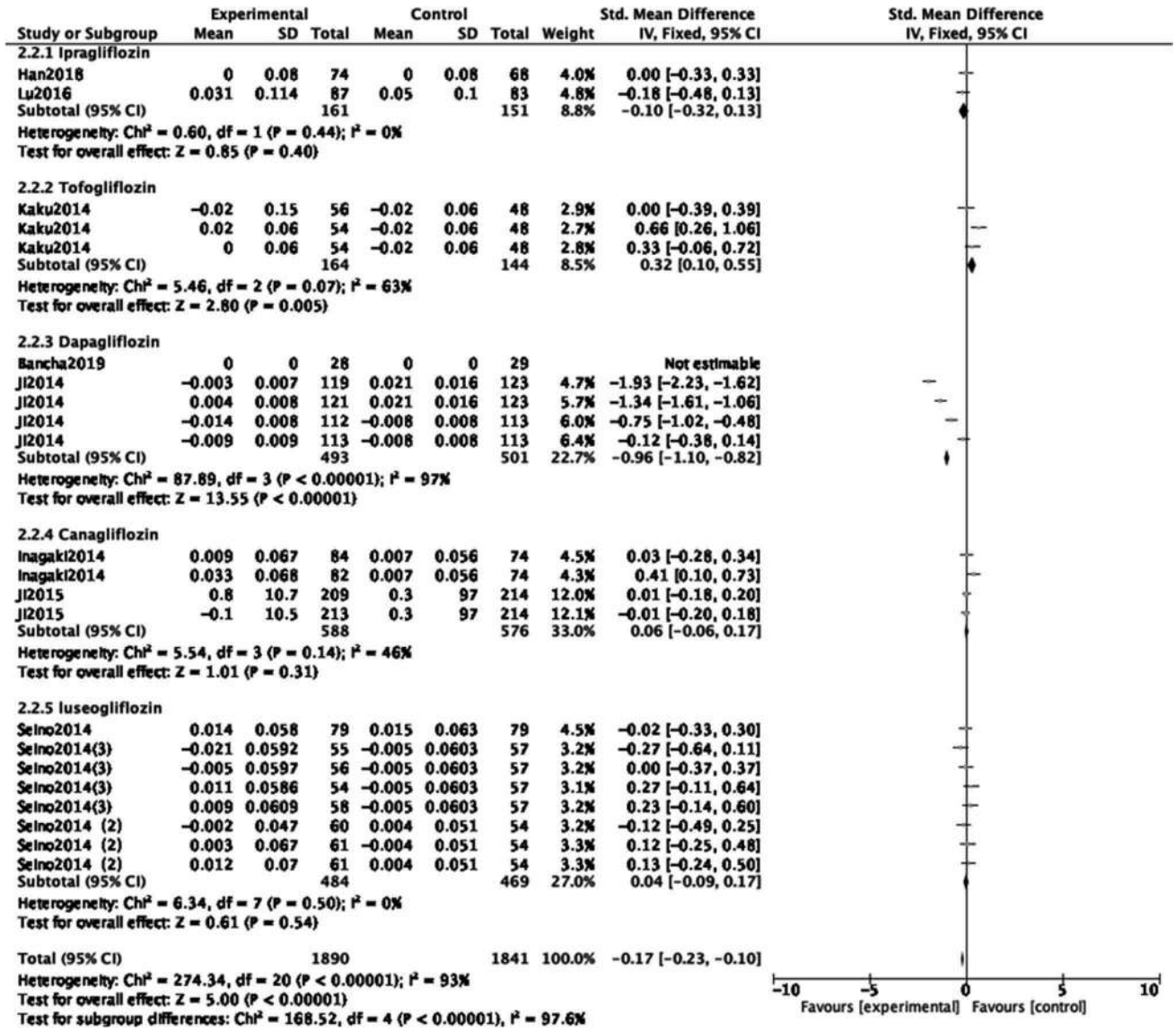

Fig. 6 Forest plot of the % change in Scr in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

glucose, blood pressure, blood lipid levels, criteria used to diagnose DKD, and genetic factors [28].

SGLT2 inhibitors reduce the reabsorption of glucose by the kidney and make excessive glucose excreted from the urine, thereby lower plasma glucose levels [29]. This paper focused on the renal effects of SGLT2 inhibitors in Asian population. Existing studies have found that SGLT2 inhibitors can lower blood lipid level, reduce body weight, decrease systolic blood pressure, and reduce blood UA to slow the progression of DKD [30–33]. In addition, SGLT2 inhibitors have been shown to have beneficial effects on the kidneys. The DAPA-CKD clinical trial enrolled patients with eGFRs between 25 and 75 mL/min/1.73 m^2 and UACRs between 200 and 5000 mg/g. It concluded that dapagliflozin may significantly reduce the risk of renal failure in patients with chronic kidney disease (CKD) [34].

In recent years, four large-scale clinical trials used decline in eGFR, end-stage renal disease, doubling of Scr, or renal death as indicators of renal outcome [35–38]. The EMPA-REG OUTCOME study reported a significant relative risk reduction of 44% in the doubling of Scr and a 55% lower relative risk of the initiation of continuous renal-replacement therapy in the

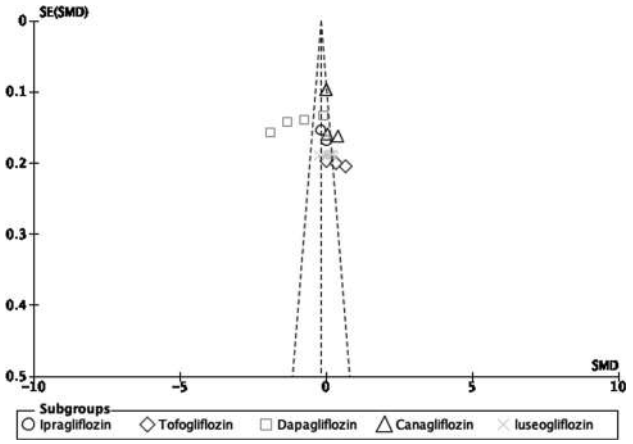

Fig. 7 Funnel plot of comparison in Scr

empagliflozin group as compared with the placebo group [36]. In the CANVAS study, canagliflozin significantly reduced the risk of the renal endpoint outcome [37]. In the DECLARE-TIMI 58 trial, canagliflozin was additionally found to reduce the incidence of adverse renal outcomes in patients with T2DM [35]. Finally, CREDENCE enrolled 4401 participants with a baseline eGFR of 30–90 mL/min/1.73 m^2 and albuminuria (as indicated by a UACR of 300–5000 mg/g). The trial found that during a median follow-up of 2.62 years, the risk of renal failure events in the canagliflozin group was lower than in the placebo group [38].

Although large clinical trials such as EMPA-REG, CANVAS, and DECLARE-TIMI 58 have confirmed the cardiovascular benefits of SGLT2 inhibitors [35–37], due to population differences, the benefits of SGLT2 inhibitors in Asian population need to be further studied. A meta-analysis focusing on Asians found that the use of SGLT2 inhibitors did not reduce the incidence of adverse cardiovascular events, hospitalization heart failure, or cardiovascular death [39]. As a result, we still lack meta-analysis of renal outcomes associated with SGLT2 inhibitors in Asian populations.

Our meta-analysis found that the short-term use of SGLT2 inhibitors in Asians may delay the decline of eGFR and reduce Scr and UA. These findings are

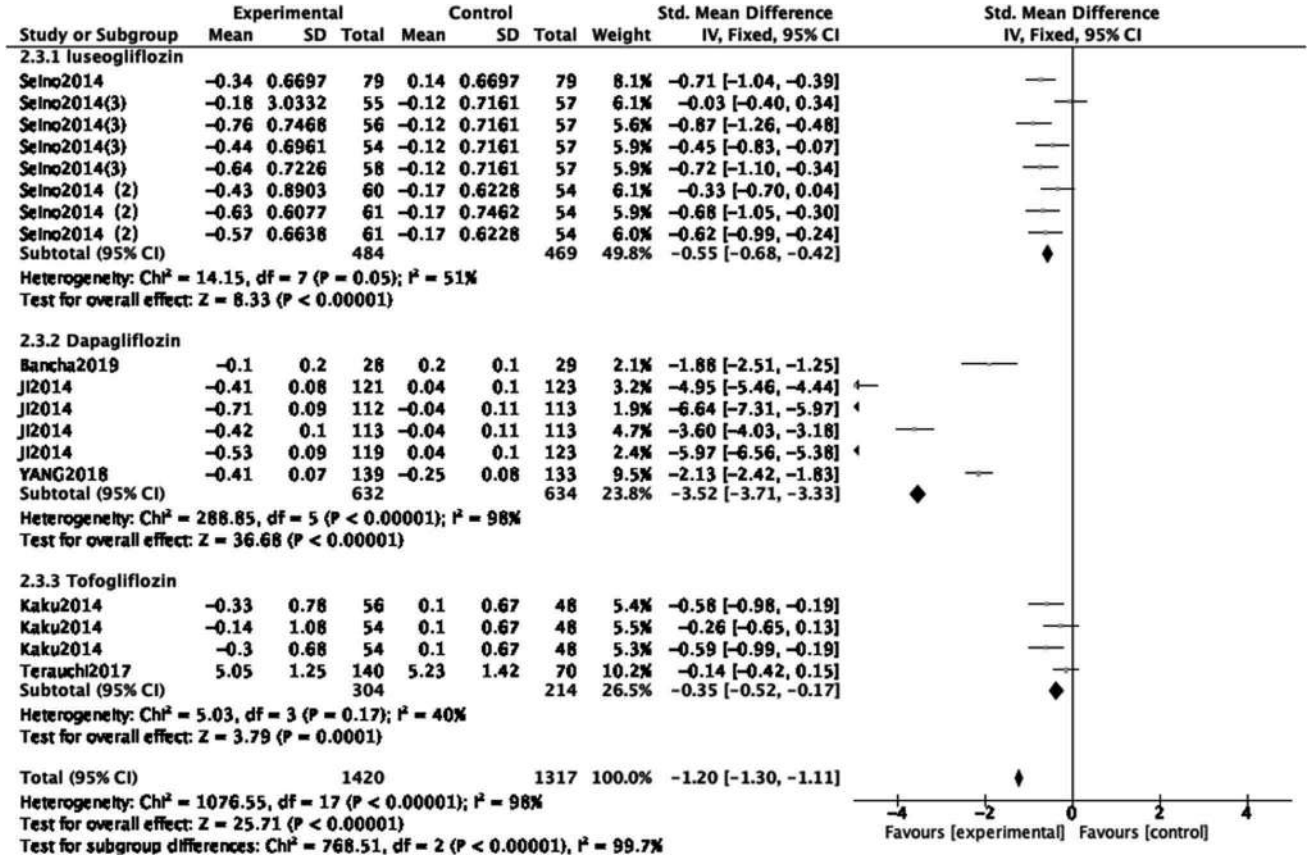

Fig. 8 Forest plot of the % change in uric acid (UA) in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

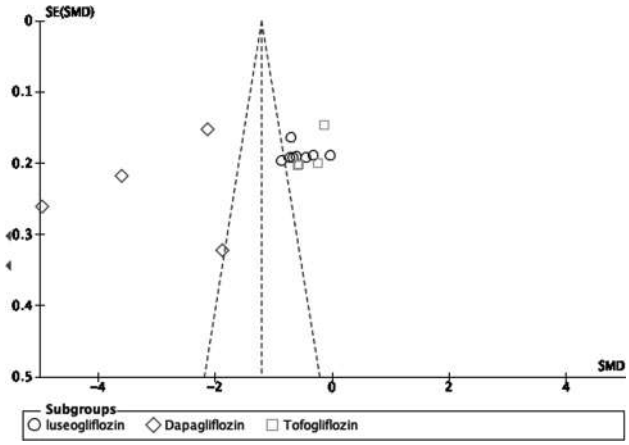

Fig. 9 Funnel plot of comparison in UA

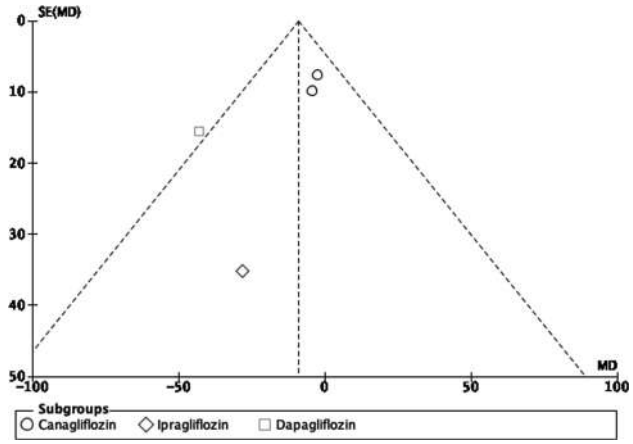

Fig. 11 Funnel plot of comparison in UACR

consistent with a global meta-analysis of renal benefits that indicated the advantages of SGLT2 inhibitors in delaying the decline of eGFR and reducing Scr [40]. The mechanism through which dapagliflozin protects the renal system is unclear and may be related to the reduction of glomerular filtration. Some scientists believe that SGLT2 inhibitors may increase erythrocyte-specific volume, improve the hypoxic state of renal tubules, partially reverse renal tubule injury, and delay the progression of DKD [41]. Glucose reabsorption in the renal tubules is considered to be the most important mechanism for regulating blood glucose homeostasis, which is mainly regulated by the proximal convoluted tubules and the sodium-glucose cotransporter [4]. Among the 6 SGLT superfamilies, SGLT2 plays a leading role in glucose reabsorption. Both T1DM and T2DM models have confirmed that hyperglycemia in vivo stimulates the upregulation of SGLT2 receptors in the proximal tubules, leading to increased sodium ions reabsorption in the proximal tubules. These changes lead to a decrease of NA^+ in the distal tubule fluid, activation of dense plaques, increased GFR, and finally, a cascade reaction [42]. Many animal experiments and clinical trials have established that diabetes leads to inappropriate activation of renal renin–angiotensin–aldosterone system (RAAS), which

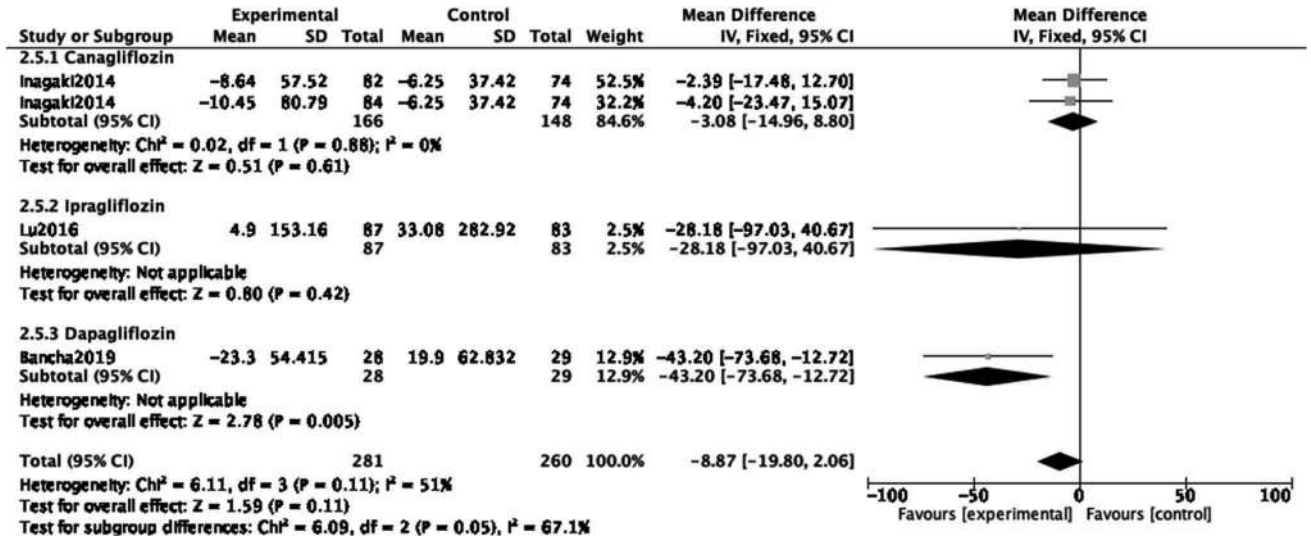

Fig. 10 Forest plot of the % change in the urinary microalbumin/creatinine ratio (UACR) in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

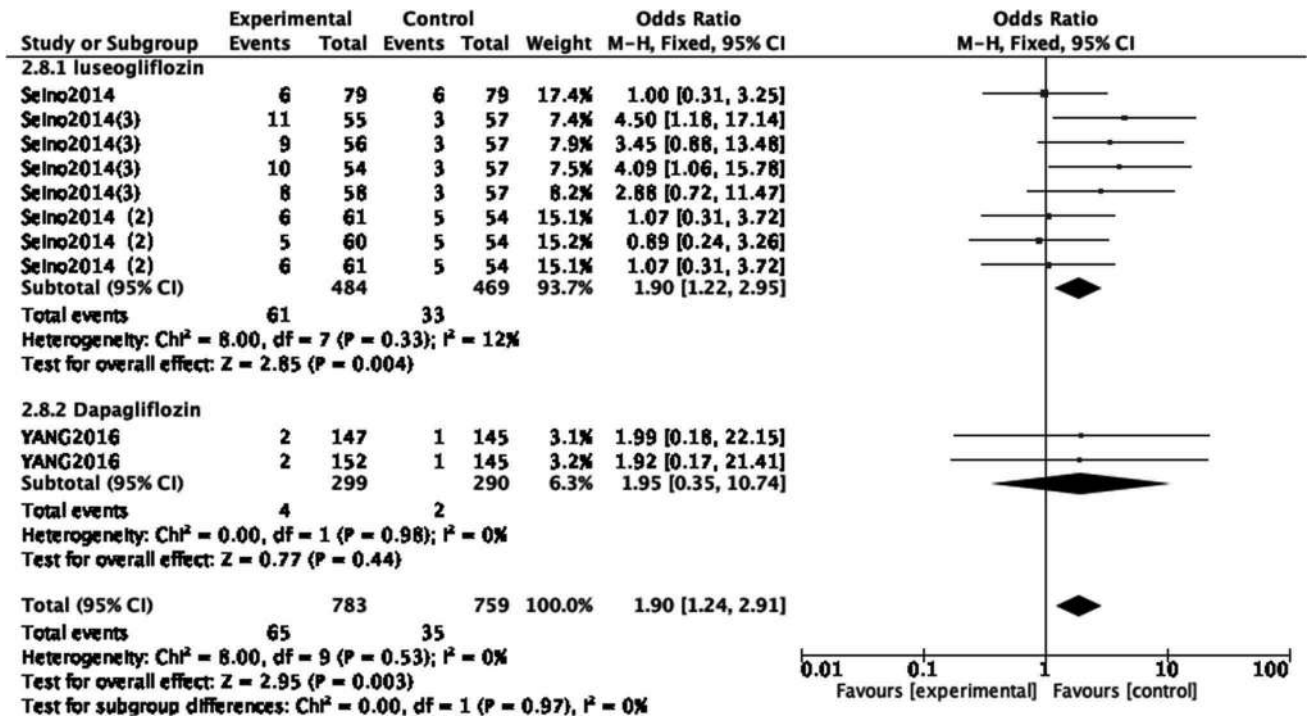

Fig. 12 Forest plot of the % change in AEs related to renal function in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

is an important mechanism for the occurrence of DKD [43]. Increased SGLT2 activity leads to increased reabsorption of dense plaques and decreased NaCl concentration and stimulates the release of renin from accessory cells. High glucose promotes angiotensinogen (AGT) production, which activates RAAS in the kidneys [44]. Studies have shown that the mechanism of UA reduction may be related to the activation of uric acid transporter 9 (glucose induced by GLUT9 and SLC2A9) and other transporters [45]. Although SGLT2 inhibitors reduce UA by increasing UA excretion, past studies did not find an increase in the incidence of kidney stones [46].

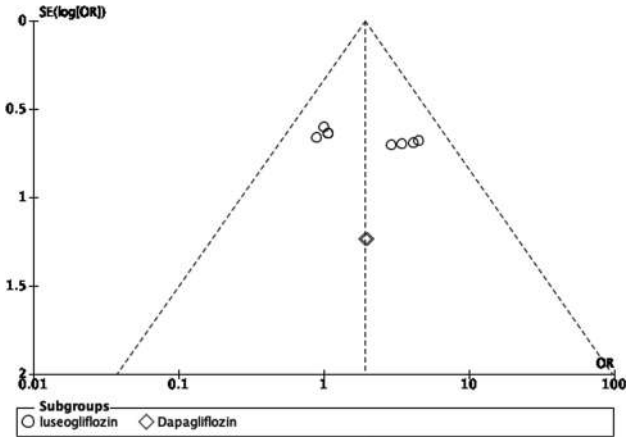

Fig. 13 Funnel plot of comparison in AEs related to renal function

A previous global meta-analysis showed that compared with other traditional therapies or placebos, SGLT2 inhibitors may reduce albuminuria and significantly benefit renal composite endpoint events [47]. They may also improve renal hemodynamics, reduce glomerular ultrafiltration and intrarenal pressure, and reduce albuminuria [41]. These findings of a protective effect of SGLT2 inhibitors on the renal system are consistent with the trends found in our study's results, but we also found differences among our population of Asian patients. Specifically, we determined that the use of SGLT2 inhibitors in Asian patients with T2DM is not associated with reductions in albuminuria. Still, dapagliflozin seems to have some effects on reducing albuminuria, although its mechanism is not yet clear, and more clinical studies are needed to verify this effect. We also found that while SGLT2 inhibitors may increase AEs related to renal function, they did not increase the incidence of renal impairment. Further, the reported AEs were classified as mild-to-moderate and were predominantly resolved during the course of the studies. The mechanism by which

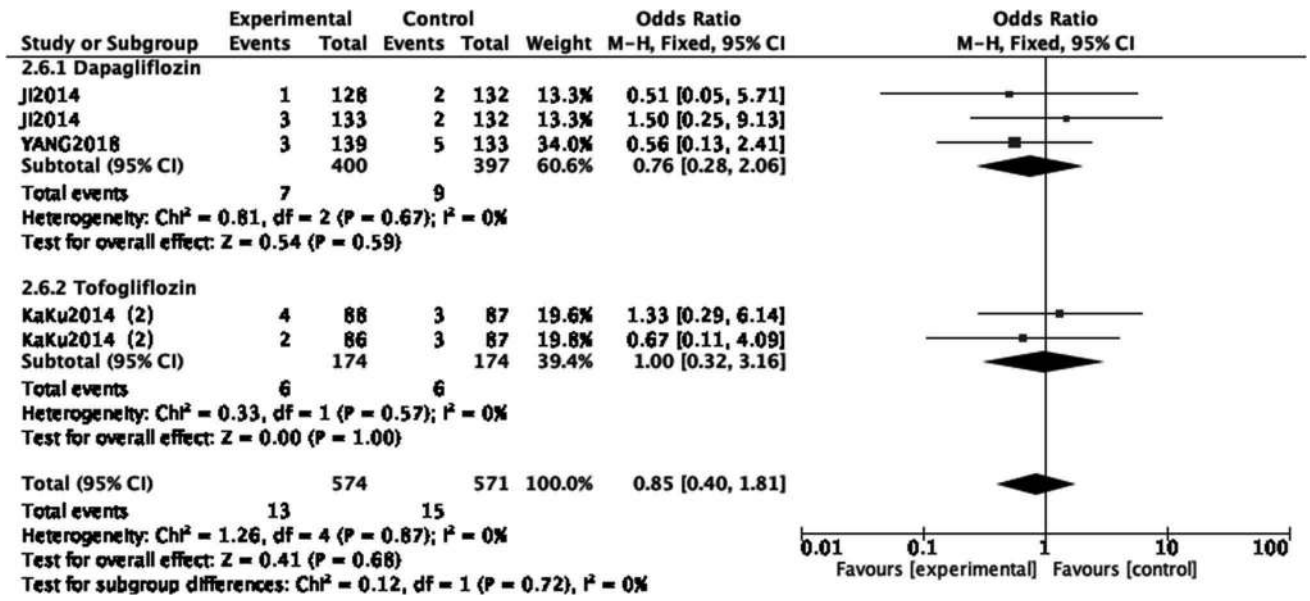

Fig. 14 Forest plot of the % change in renal impairment in the patients randomized to SGLT2 inhibitor therapy versus control therapy. SD standard deviation, CI confidence interval, IV inverse variance

short-term SGLT2 inhibitor use causes transient AEs in Asians is unclear and warrants further study. More RCTs are also needed to study the impact of SGLT2 inhibitors in Asians, apply it to patients with lower levels of eGFR, especially apply it to patients with the CKD.

This study has some limitations. First, the included RCTs only investigated short-term (< 24 weeks) outcomes. Second, the renal outcomes of the included studies were heterogeneous. This may have been related to variation in population baselines, drugs, doses of the same drug, study duration, and definitions of and methods used to collect outcomes.

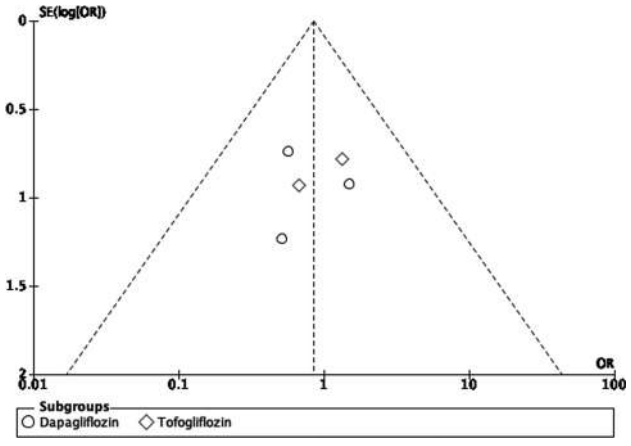

Fig. 15 Funnel plot of comparison in renal impairment

Finally, the included RCTs likely had a publication bias as positive results are more likely to be published. More clinical RCTs focused on renal events are needed, especially in populations with a relatively low GFR and in patients without albuminuria.

In conclusion, the use of SGLT2 inhibitors in Asian patients with T2DM may delay the decline of eGFR and reduce Scr and UA. Although SGLT2 inhibitors have no overall advantage in reducing albuminuria, dapagliflozin does appear to reduce albuminuria. Finally, SGLT2 inhibitors may increase the incidence of AEs related to renal function, but do not increase the incidence of renal impairment. More high-quality RCTs are needed to establish the benefits of SGLT2 inhibitors on the renal system and to better guide clinical medication.

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Declarations

Conflict of interest The authors declare no competing interests.

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REVIEW ARTICLE

Effects of motivational interviewing on HbA1c and depression among cases with type 1 diabetes: a meta-analysis

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Abstract

Objectives The present study aimed to determine the effects of motivational interviewing (MI) on treatment outcome as measured by variations within glycosylated hemoglobin (HbA1c) and depression in cases subject to type 1 diabetes mellitus (T1DM).

Methods Two independent investigators electronically conducted a literature search by exploiting Cochrane Library, EMBASE, PubMed, Google Scholar, and ClinicalTrials.gov from inception to January 2021. Randomized controlled trials and quasi-experimental interventions were involved, in which motivational interviewing was examined to be associated with the treatment outcome in people with T1DM. We calculated standard mean difference (SMD) with 95% confidence intervals (CIs) using a random-effects model by RevMan V 5.4.1 software.

Results Lastly, a total of 9 studies comprising 1322 (680 vs 642) cases were covered, 3 of which involved 332 (166 vs 166) cases for depression meta-analysis. The analysis indicated a 0.11% improvement in glycemic control in populations having accepted a MI intervention in comparison with usual care participants, whereas the effect did not show statistical significance (SMD, − 0.11; 95% CI, − 0.33 to 0.11; $Z = 0.97$, $p = 0.33$, $I^2 = 60\%$). The depression scores between the intervention group and the control showed no significant difference (SMD, − 0.00; 95% CI, − 0.93 to 0.92; $Z = 0.00$, $p = 1.00$, $I^2 = 91\%$).

Conclusions The effect of motivational interviewing in reducing glucose levels and depression is suggested to be limited. But as impacted by the issues of heterogeneity and small number of included studies, caution in interpreting the present findings is advised.

Keywords Type 1 diabetes mellitus · Motivational interviewing · Meta-analysis · Glycosylated hemoglobin · Depression · Systematic review

Introduction

Type 1 diabetes mellitus (T1DM) refers to a metabolic disorder disease, which is characterized by the destruction of insulin-producing beta cells in the pancreas, thereby causing high blood glucose levels in the body [1]. Epidemiological studies demonstrated that the prevalence of T1DM has increased by 2–5% worldwide [2]. The incidence of T1DM increases with age up to a peak of about 10–14 years [3]. Only 21% of adolescents with T1DM satisfied the glycemic goals set forth by the American Diabetes Association (ADA) [4]. Chronic high levels of blood glucose are associated with long-term damage, dysfunction, and functional failure of the heart, kidneys, nerves, and eyes, as well as blood vessels [5]. Nearly 25% of the adult T1DM is in persistent poor glycemic control, so they are at an increased risk of developing microvascular and macrovascular complications [6]. The mentioned comorbidities can severely reduce the quality of life in cases with diabetes, and increase the risk of premature death.

Motivational interviewing (MI) has been extensively applied for managing behavior-related diseases, and researches have established it as an effective approach to

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improve a range of health-related behaviors over the past three decades [7]. MI is defined as a collaborative conversation style that elicits intrinsic motivation and strengthens commitment to behavior change goals. Such an approach referring to a "patient-centered" style of treatment encourages people to explore their ambivalence and find a solution fitting them if they are identified with a negative situation [8]. Though there will not be identical two MI sessions since they are patient-driven, they are likely to include the four core stages (i.e., engaging, focusing, evoking as well as planning) [9].

The successful management of diabetes depends on an individual's ability to abide by a strict daily treatment regimen. T1DM cases require multiple daily decisions about self-management to maintain glycemic control (e.g., dietary choices, physical activity, blood glucose monitoring) [10]. Studies have found that effective patient-provider communication is positively related to treatment adherence, health outcomes, and patient satisfaction [11]. MI refers to a particularly promising communication strategy recommended by the ADA [12]. It is capable of helping cases accept their disease, modify their lifestyle, correct their misconceptions, and tackle down psychological problems. Being diagnosed with T1DM can bring changes to an individual's life and cause emotional responses (e.g., depression, low mood, as well as fear of injection or hypoglycemia) [13]. As revealed from the systematic review, adolescents with T1DM suggesting depressive symptoms are associated with poorer diabetes management [14]. Another study reported a small to moderate association between depression and poor glycemic control [15]. Depression was identified to be more prevalent in T1DM cases relative to the general population, and related to poorer glycemic control and self-care and increased risk of complications [16]. Hence, sticking to good behavior is conducive to glycemic control, which facilitates psychological health.

Existing systematic reviews have focused on the intervention effect of MI in cases with type 2 diabetes mellitus (T2DM) [17–20] or diabetes mellitus (including type 1 and type 2 diabetes) [21], and they did not analyze the MI intervention effect on type 1 and type 2 diabetes respectively. These studies evaluated MI on behavior change outcomes or clinical outcomes or psychosocial health in populations with T2DM. Compared to T2DM, the physical and psychosocial states of population with type 1 diabetes, as well as the problems faced by individuals growing and developing in society, are very different. It is necessary to evaluate the intervention effect of MI among cases with T1DM separately, so as to provide a reference for health education strategy in T1DM. One systematic review [22] evaluated the role of MI on HbA1c in cases with T1DM, but only four articles were included in the final analysis, and they did not perform statistical analysis due to the heterogeneity of the types and the lengths of the interventions. However, to the best of our knowledge, meta-analyses in regard to the effects of MI among cases with T1DM have not been reported. Previous studies have evaluated the effect of MI on glycosylated hemoglobin (HbA1c), psychological and self-care behavioral aspects in cases with T1DM [23–31], but considerable controversies exist concerning the effectiveness of MI in T1DM. We therefore performed this meta-analysis to determine the effects of MI on HbA1c and depression among cases with T1DM.

Materials and methods

The research was conducted and reported according to the framework recommended by the PRISMA Statement [32].

Search strategy

The research team searched the online scientific databases including Cochrane Library, EMBASE, PubMed, Google Scholar, and ClinicalTrials.gov from their inception until the second week of January 2021. Keywords were employed for search which were presented below: "type 1 diabetes mellitus," "type 1 diabetes," "insulin-dependent diabetes mellitus," "juvenile onset diabetes," T1DM, T1D, DM1, IDDM, "motivational interview*," "motivational enhance*," motivational, "patient counsel*," "consult* skills," motivation and interviewing. The search algorithm was tailored to the unique requirements of the respective database. Furthermore, we searched the reference lists of related articles to find any article that might have been missed in our initial search. All analyses were based on published studies and therefore no informed consent and ethical approval were required.

Eligibility criteria

Inclusion criteria were elucidated below according to "PICOS": Population: patient samples diagnosed with T1DM; Intervention: MI consistent techniques including four core phases (e.g., motivational enhancement therapy [MET]/MI); Control: cases without intervention for increasing motivation (e.g., usual care); Outcome: at least reported HbA1c levels; Study design: randomized controlled trials (RCTs) or self-controlled study. Moreover, studies would be eliminated if conducted by an identical research team, or containing insufficient data and poor methodology. Articles were excluded if investigating the impact of MI on other patient populations or other parameters, or focusing on HbA1c assessment in cases with either type 1 or type 2 diabetes without differentiating between them.

Study selection

Duplicate studies and records were eliminated after importing all captured citations into the EndNote X9 software. Two authors screened titles and abstracts independently in order to identify studies satisfying the eligibility criteria. Afterwards, the same two authors retrieved and independently evaluated full texts of potential studies for eligibility. A third reviewer was consulted if there was any disagreement regarding study selection.

Data extraction and quality assessment

We employed a detailed data extraction form to support the data extraction process. Two researchers were assigned to independently extract the basic information and data (e.g., first author, published time, country, sample size, age of participants, duration of diabetes, details of intervention, outcomes of interesting, study design, as well as main findings) from each eligible trial. In addition, any divergence regarding the data extraction was resolved by consulting a third author. Two independent researchers evaluated the methodological quality of the trials included here according to the risk of bias assessment tool released by Cochrane Collaboration for RCTs, and they employed Newcastle–Ottawa Scale (NOS) for a self-control clinical study. A final score > 7 was recognized as high quality.

Outcome measures

The outcomes of interest included glycosylated hemoglobin (HbA1c) levels and the score of depression. The depression score was measured using the scale of Center for Epidemiologic Study-Depression (CES-D) or Well-being Questionnaire (WBQ).

Statistical analysis

In the present meta-analysis, all outcomes were continuous data. Thus, we adopted the mean difference (MD) or standard mean difference (SMD) with a 95% confidence interval (CI) for the relevant estimation. The heterogeneity of included studies was quantitatively estimated using the I^2 statistic, and qualitatively tested using p-value. A fixed-effects model was employed if there was no evidence of heterogeneity ($I^2 < 50\%$, $p > 0.1$). As opposed to the mentioned, a random-effects model was employed. A 2-tailed p-value of less than 0.05 was considered to suggest statistical significance. Subgroup analysis was performed by complying with the considered heterogeneous factors (length of follow-up period and length of intervention). Specific to a single outcome, the funnel plot was generated to qualitatively inspect the publication bias under the accumulated number of included studies over 10. Furthermore, all statistical analyses were conducted with Review Manager (RevMan) software, version 5.4.1.

Results

Study selection and characteristics

After preliminary searching of targeted electronic databases, a total of 308 trials were identified, of which 119 duplicates were removed through EndNote software (Fig. 1). The titles and abstracts of 189 records were carefully screened, and then 28 cites were classified into potential inclusion file. The full texts of 28 potential studies were checked; finally, nine trials [23–31] including 1322 (680 vs 642) participants satisfied the inclusion criteria. Of the nine studies, six studies [24–28, 31] were RCTs, two studies [23, 30] were pseudo-RCT, and one study [29] was a self-controlled trial (Table 1). Ineligible studies were eliminated for the reasons below: not MI as core intervention ($n = 6$), duplicates ($n = 6$), inappropriate data type ($n = 1$), conference abstract ($n = 3$), review ($n = 2$), and qualitative study ($n = 1$). The duration of the MI interventions in the included studies ranged from 2 to 12 months.

Effects of MI on HbA1c level at the end of intervention

A total of 9 studies [23–31] including 1322 (680 vs 642) T1DM cases elucidated HbA1c values before and after intervention. Though the duration of the intervention was different for the respective studies, HbA1c levels were selected at the end of the intervention for a pooled analysis. Therefore, as indicated from the pooled result, the HbA1c was not significantly lower in the MI group than that in the control (SMD, − 0.11; 95% CI, − 0.33 to 0.11; $Z = 0.97$, $P = 0.33$, $I^2 = 60\%$, Fig. 2), demonstrating that the MI interventions were not superior to the usual care on reducing HbA1c levels. Furthermore, the subgroup analysis was conducted with random-effects model according to the length of follow-up period (3 months, 6 months, 9 months, or 12 months) and the length of intervention (≤ 6 months or 12 months).

Effect of MI on HbA1c level at 3 months

From nine eligible trials, four studies [25, 26, 29, 31] involving 510 (262 vs 248) cases reported the HbA1c levels after the interventions were initiated for 3 months. No statistical difference was identified (SMD, − 0.13; 95% CI, − 0.58 to 0.32; $Z = 0.57$, $P = 0.57$, $I^2 = 80\%$, Fig. 2), which indicated the MI intervention groups were not superior to the control on reducing HbA1c at 3 months.

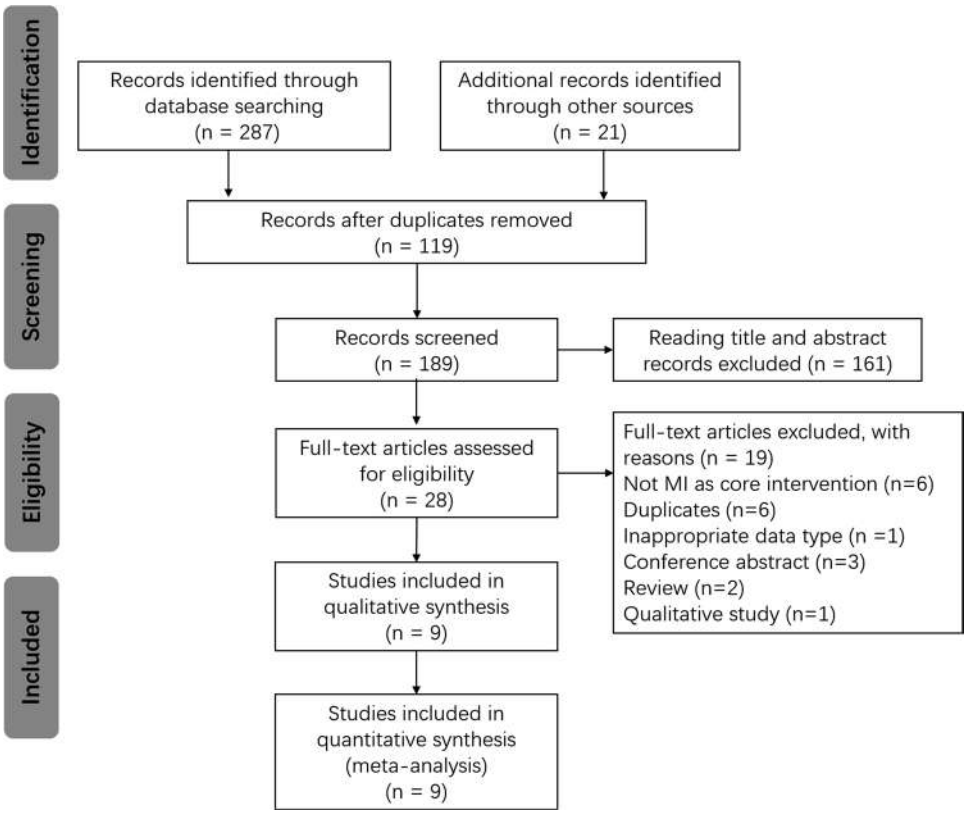

Fig. 1 Flow diagram of the literature selection procedure

Effect of MI on HbA1c level at 6 months

Seven studies [23–26, 29–31] enrolling 612 (304 vs 308) cases evaluated the effect of MI on HbA1c at 6 months between the intervention group and the control. Pooled result revealed that there was no significant difference in the reduction of HbA1c at 6 months (SMD, − 0.23; 95% CI, − 0.54 to 0.09; $Z = 1.42$, $P = 0.16$, $I^2 = 66\%$, Fig. 2).

Effect of MI on HbA1c level at 9 months

Only two studies [25, 31] including 210 (106 vs 104) cases evaluated the effect of MI on HbA1c at 9 months. Pooled result indicated no significant difference in the reduction of HbA1c between the two groups (SMD, 0.24; 95% CI, − 0.16 to 0.63; $Z = 1.18$, $P = 0.24$, $I^2 = 37\%$, Fig. 2).

Effect of MI on HbA1c level at 12 months

Seven studies [23–28, 30] involving 1260 (655 vs 605) T1DM cases reported HbA1c levels after the interventions were initiated for 12 months. However, no significant difference was observed between the MI group and the control (SMD, − 0.02; 95% CI, − 0.16 to 0.12; $Z = 0.29$, $P = 0.77$, $I^2 = 16\%$, Fig. 2).

Effect of the length of intervention time ≤ 6 months on HbA1c level

Five trials [23, 25, 26, 29, 31] involving 524 (256 vs 268) cases, out of nine eligible trials, reported the length of intervention 6 months, 2 months, 6 months, 3.5 months, and 4 months respectively. We pooled the values of HbA1c at the end of the intervention. Meta-analysis suggested no significant difference in terms of HbA1c when MI intervention compared to usual care (SMD, − 0.15; 95% CI, − 0.52 to 0.23; $Z = 0.76$, $P = 0.45$, $I^2 = 71\%$, Fig. 2).

Effect of the length of intervention time is 12 months on HbA1c level

Of the nine reviewed studies, four articles [24, 27, 28, 30] containing 787 (409 vs 378) cases elucidated the effect of MI on HbA1c after 12 months of intervention. For the mentioned reason, according to the result of the random-effects model analysis, HbA1c levels did not significantly decrease as compared with the control (SMD, − 0.09; 95% CI, − 0.39 to 0.21; $Z = 0.58$, $P = 0.56$, $I^2 = 44\%$, Fig. 2).

Table 1 Basic characteristics of all eligible studies

Study: author, year, country	Design, settings	Total sample size (*N*)	Mean age (SD)	Duration of diabetes (years)	Intervention/duration (months)	Pre- and post-treatment measures of HbA1c (incl. months to follow-up from baseline)	Behavioral/psychological measures	Main findings
Channon et al. (2003) UK	Pseudo-RCT; single site	47	15.8	5.1	I: MI = 3 months period using a combination of workshop, training videos, role play, and individual weekly supervision C: Usual care/6 months	I: Baseline: 10.8 ± 1.78, 6mo: 9.7 ± 1.59, 12mo: 10 ± 1.86 C: Baseline: 10.1 ± 1.4, 6mo: 9.9 ± 1.30, 12mo: 9.9 ± 0.99	WBQ, DKN, PMDQ, DSCA, DFBS	Motivational interviewing may be a useful intervention in helping adolescents improve their glycemic control
Channon et al. (2007) UK	RCT; multi-site	66	I = 15.3 (0.97) C = 15.4 (1.19)	I = 9.2 (1.96) C = 9.1 (1.47)	I: MI = averaging 4 sessions of between 20 and 60 min C: Support visits/12 months	I: Baseline: 9.3 ± 2.11, 6mo: 9.0 ± 1.63, 12mo: 8.7 ± 1.84, 24mo: 8.7 ± 1.88 C: Baseline: 9.0 ± 1.56, 6mo: 9.5 ± 1.93, 12mo: 9.2 ± 1.78, 24mo: 9.1 ± 1.51	DQoL, CHLC, HCCQ, DKN, SEDS, WBQ, DFBS, PMDQ	The MI group showed a significantly greater reduction in HbA1c compared to the control group at 12 and 24 months. There were differences in psychosocial variables at 12 months
Ismail et al. (2008) UK	RCT; multi-site	344	36.4 (10.3)	18.5 (9.8)	I: MI = control + 4 individual 50 min MET sessions C: Usual care/2 months	I: Baseline: 9.57 ± 1.03, 3mo:9.29 ± 1.08, 6mo: 9.21 ± 1.36, 9mo: 9.29 ± 1.36, 12mo: 9.30 ± 1.61 C: Baseline: 9.70 ± 1.18,3mo:9.37 ± 1.10, 6mo: 9.35 ± 1.42, 9mo: 9.16 ± 1.17, 12mo: 9.54 ± 1.52	PHQ, HFS, DSCA, DQoL	No significant difference in change in HbA1c was found between groups
Mayer-Davis et al. (2018) USA	RCT; multi-site	258	I = 14.8 (1.1) C = 14.9(1.1)	I = 6.48 (3.76) C = 6.39 (3.71)	I: MI = 4 sessions of between 40 and 60 min, met with each participant three to four times C: Usual care/6 months	I: Baseline: 83 ± 13, 3mo: 81 ± 13, 6mo: 82 ± 15, 12mo: 82 ± 16, 18mo: 84 ± 19 C: Baseline: 80 ± 14, 3mo: 81 ± 15, 6mo: 82 ± 16, 12mo: 82 ± 16, 18mo: 82 ± 17	DQoL, SPSI, DSMP, CES-D	No significant difference in change in HbA1c was found between groups. The CES-D scores were not statistically significant at 18 months
Pulkkinen et al. (2020) Finland	RCT; multi-site	39	I = 14.6 (0.9) C = 14.6(0.8)	I = 8.1 (3.6) C = 7.9 (3.8)	I: MI = SEC + Patients were followed up every 3 months by incorporating the MI principles C: SEC/12 months	I: Baseline: 10.2 ± 1.2, 12mo:9.86 ± 1.70 C: Baseline: 9.9 ± 1.1, 12mo: 9.97 ± 1.99	None	No significant difference in change in HbA1c was found between groups

Table 1 (continued)

Study: author, year, country	Design, settings	Total sample size (*N*)	Mean age (SD)	Duration of diabetes (years)	Intervention/duration (months)	Pre- and post-treatment measures of HbA1c (incl. months to follow-up from baseline)	Behavioral/psychological measures	Main findings
Robling et al. (2012) UK	RCT; multi-site	693	I = 10.4 (2.8) C = 10.7 (2.8)	I = 5.2 (2.8) C = 5.0 (2.7)	I: MI = MI modified routine care 3.5 sessions C: Usual care/12 months	I: Baseline: 9.4 ± 1.7, 12mo: 9.7 ± 1.7 C: Baseline: 9.2 ± 1.8, 12mo: 9.5 ± 1.7	DQoL, HCCQ	No significant difference in change in HbA1c was found between groups
Stanger et al. (2013) USA	Self-controlled study; single site	17	14.8 (1.5)	6.2 (4.5)	I: MI = 14 weekly, 1-h counseling sessions of MI, family-based contingency management/3.5 months	I: Baseline: 11.6 ± 2.5, 3mo: 9.1 ± 0.9, 6mo: 9.8 ± 1.4	SCI	HbA1c was significantly lower at the end of treatment and 3 months post-treatment compared with pretreatment
Viner et al. (2003) UK	Pseudo-RCT; single site	77	I = 13.0 C = 13.3	I = 6.2 C = 5.7	I: MI = 6-weekly sessions in groups of four to five young people C: Usual care/12 months	I: Baseline: 10.2 ± 1.37, 6mo: 8.7 ± 1.37, 12mo: 8.9 ± 1.37 C: Baseline: 10.0 ± 1.34, 6mo: 9.8 ± 1.79, 12mo: 9.9 ± 2.24	SDQ, SEDS	Motivational group intervention is promising in improving HbA1c in adolescents
Wang et al. (2010) USA	RCT; single site	44	I = 15.3 (1.4) C = 15.6 (1.7)	I = 6.7 (3.4) C = 7.6 (4.7)	I: MI = motivational interviewing-based education 4–5 sessions C: structured diabetes education (SDE)/4 months	I: Baseline: 10.9 ± 2.04, 3mo: 11.3 ± 1.53, 6mo: 11.1 ± 1.83, 9mo: 11.7 ± 2.75 C: Baseline: 11.1 ± 1.59,3mo:10.4 ± 1.59, 6mo: 10.2 ± 1.92, 9mo: 10.3 ± 2.40	CES-D, DSCA, EDIC-QOL	SDE is effective at improving metabolic control in adolescents with type 1 diabetes. There were no differences on any of the psychosocial measures

Abbreviations: *C* control group; *CES-D* Centers for Epidemiologic Studies-Depression Scale; *CHLC* Child Health Locus of Control; *DFBS* Diabetes Family Behavior Scale; *DCSA* Diabetes Self-Care Activities; *DKN* Diabetes Knowledge Scale; *DQoL* Diabetes Quality of Life Scale; *DSMP* Diabetes Self-Management Profile; *EDIC-QOL* Epidemiology of Diabetes Interventions and Complications Quality of Life Questionnaire; *HCCQ* Modified Health Care Climate Questionnaire; *HFS* Hypoglycaemia Fear Survey; *I* intervention group; *MET* motivational enhancement therapy; *MI* motivational interviewing; *PHQ* Patient Health Questionnaire; *PMDQ* Personal Models of Diabetes Scale; *RCT* randomized controlled trial; *SCI* Self-Care Inventory; *SDE* structured diabetes education; *SDQ* Strengths and Difficulties Questionnaire; *SEC* standard educational care; *SEDS* Self-Efficacy for Diabetes Scale; *SPSI* Social Problem Solving Inventory; *WBQ* Well-Being Questionnaire

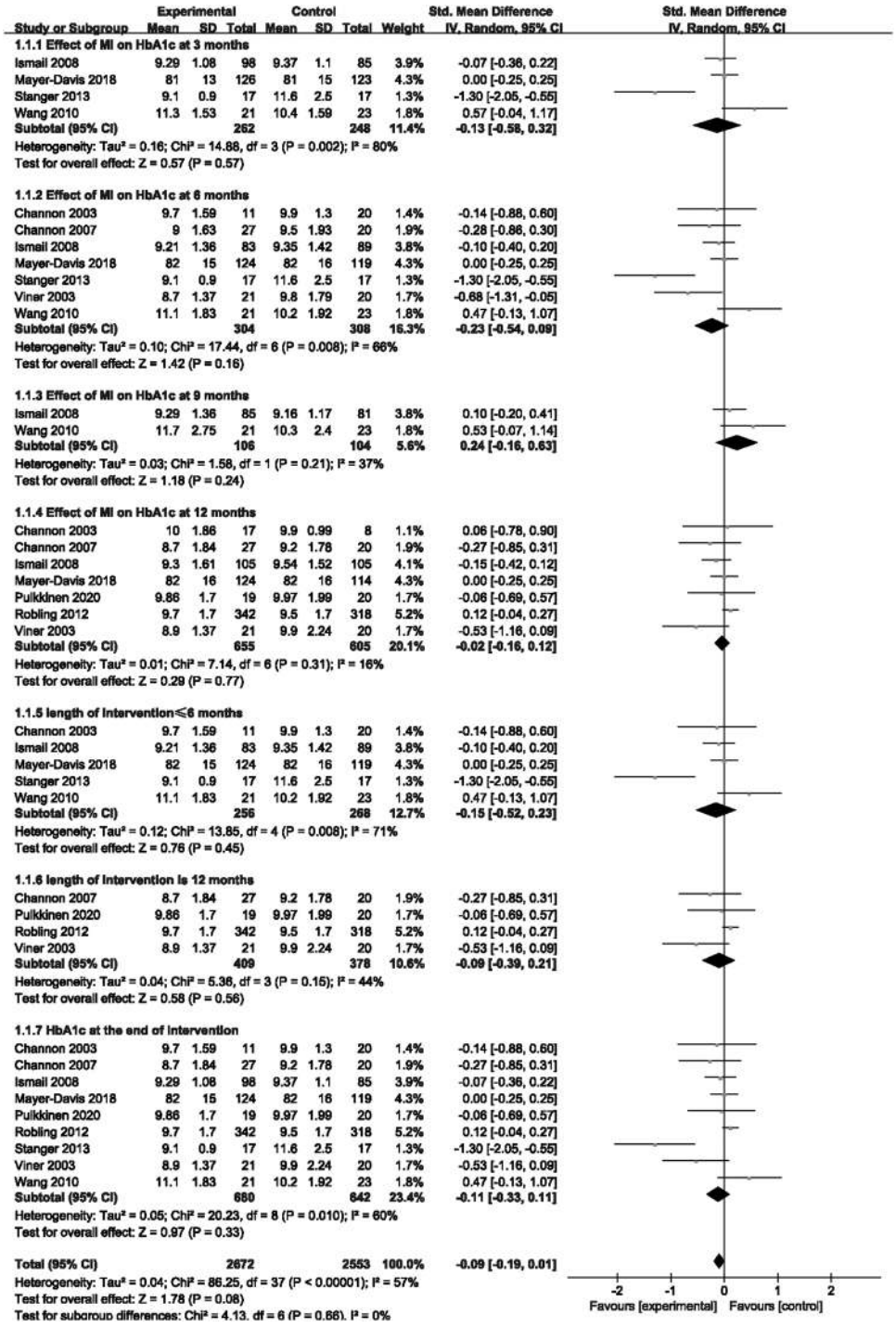

Fig. 2 Subgroup analysis of HbA1c level between MI intervention and the control

Effect of MI on depression

Three of the studies [24, 26, 31] containing 332 (166 vs 166) T1DM cases assessed the changes in depression scores after intervention. According to the pooled result, the significant difference was not identified in this given outcome between MI intervention and usual care (SMD, − 0.00; 95% CI, − 0.93 to 0.92; $Z = 0.00$, $P = 1.00$, $I^2 = 91\%$, Fig. 3), demonstrating that the MI groups were not superior to the controls on improving depression.

Sensitivity analysis and publication bias

We checked the sensitivity of each included study and found that one of the studies [29] was the most sensitive since the I^2 became 15% from 60% and pooled result became − 0.01

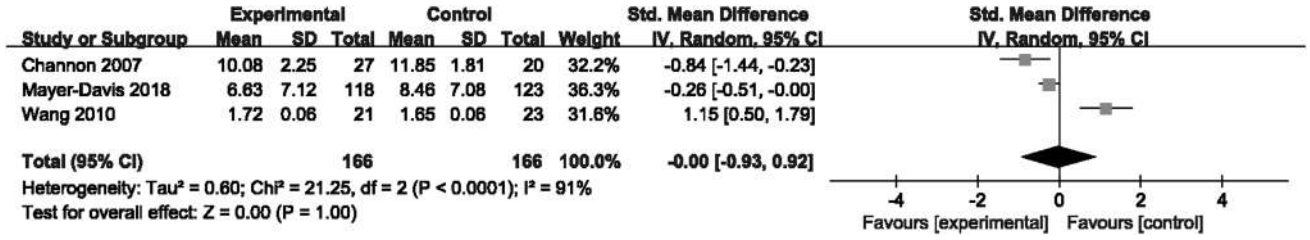

Fig. 3 Meta-analysis of the score of depression between MI intervention and the control

[−0.12, 0.15] from −0.11 [−0.33, 0.11]. In the present meta-analysis, the cumulative number of qualified studies for the respective outcome of interest was all less than ten. However, there were nine studies [23–31] reporting the measurements of HbA1c. Thus, a funnel plot was generated to inspect potential publication bias, which showed that there was a slight publication bias (Fig. 4).

Quality assessment

Four studies [24–26, 28] with adequate sequence generation could be identified; two studies [25, 28] were carried out for allocation concealment; in three studies [24, 26, 31], participants and clinical staff were blinded before the intervention; two studies were non-RCT [23, 30]; only one study [27] illustrated the blinding of outcome assessment. The four studies [24–26, 28] addressed incomplete outcome data. We observed that the baselines were comparable across all included trials. Detailed quality assessment result is shown in Figs. 5 and 6. Only one study [29] was the self-controlled clinical study, and the NOS scale was adopted to evaluate the quality. The final score was six points, demonstrating that it was not a high-quality study.

Discussion

This study aimed to review the evidence for the effect of MI in improving glycemic control and psychology in populations with T1DM, operationalized by variations in HbA1c value and the score of depression. The present meta-analysis evidenced that no effect of MI intervention was exerted on reducing HbA1c and depression symptoms in T1DM cases in comparison with the usual diabetes care.

A key goal of diabetes self-management refers to the control of HbA1c, which captures average glycemia during the prior 6–8 weeks [33]. Elevated HbA1c levels predict the complication risk during subsequent years. T1DM cases with optimal glycemic control suffer markedly less from long-term diabetic complications than those with poor control [34]. The pooled mean difference of 0.11% was achieved in HbA1c change between cases randomized to the MI

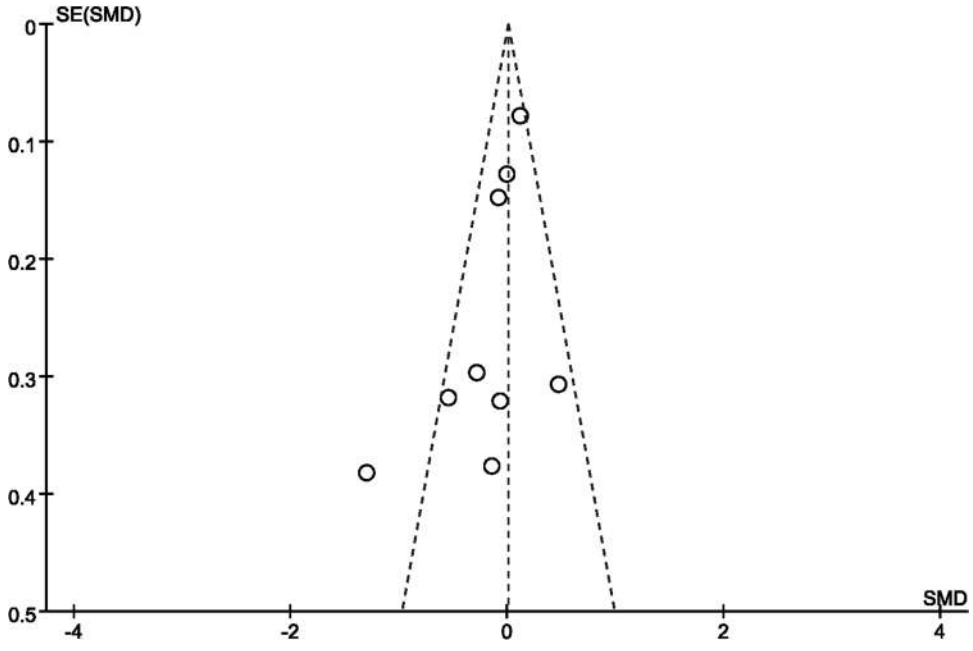

Fig. 4 Funnel plot of the changes of HbA1c levels among the included studies

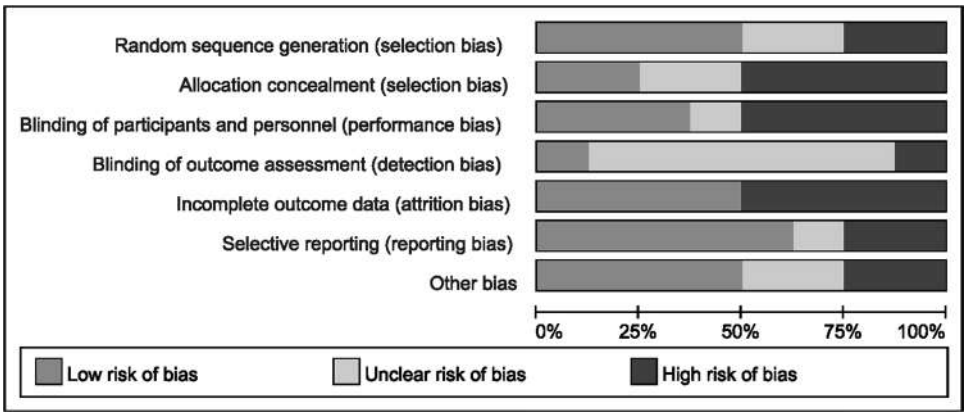

Fig. 5 Risk of bias graph by percentage for eight included studies

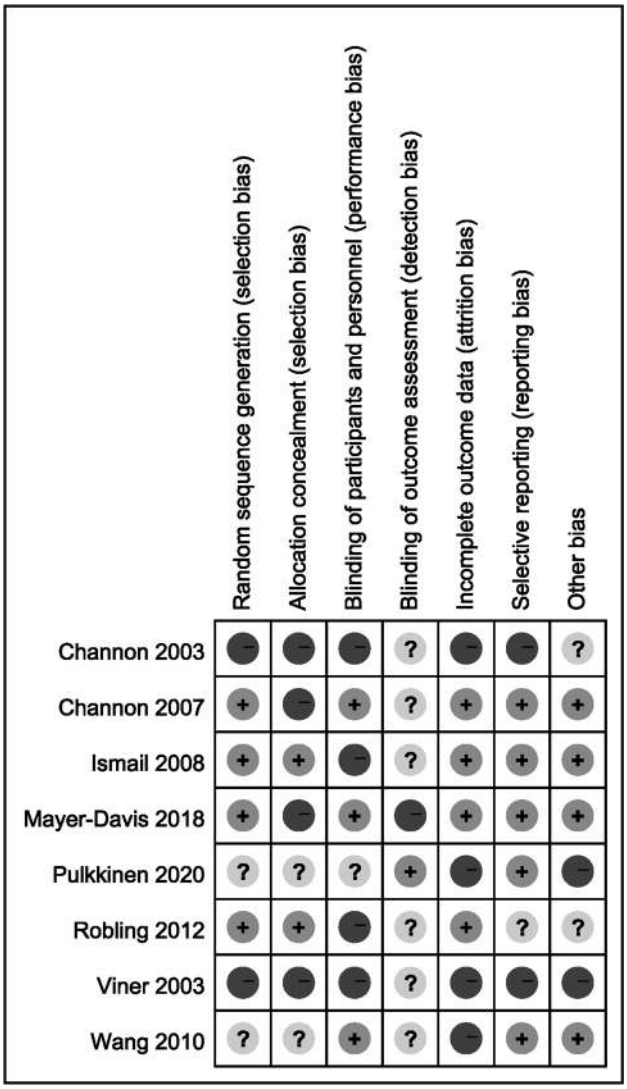

Fig. 6 Risk of bias summary for eight included studies: red, yellow and green solid circle represents high risk of bias, unclear and low respectively

intervention arm or the control, favoring motivational interviewing intervention. Whereas the difference did not show statistical significance, and the total pooled existing moderate heterogeneity ($P = 0.010$, $I^2 = 60\%$). Hence, a random-effects model was adopted for subgroup analysis according to the duration of MI intervention (shorter/ ≤ 6 months or longer/12 months) and the length of follow-up period (3 months, 6 months, 9 months, or 12 months). As indicated from the subgroup analysis result, HbA1c levels did not have a significant improvement in comparison with the control. According to the sensitivity analysis of the included studies, only one article [29] was the most sensitive. This trial was a self-controlled pilot study with a small sample size ($n = 17$), a long intervention duration (3.5 months), and a high frequency (14 weekly, 1-h counseling sessions of MI), as well as a short follow-up time (almost 3 months post-treatment). Three studies [25, 26, 28] showed that larger samples generally produced smaller effect sizes by an exploratory look at the data. It could be explained as the larger trials were more accurate in measuring differences in therapeutic effect between the MI group and the control. Moreover, the smaller sample trails had generally shorter treatment durations as well as higher frequencies of sessions in comparison with the larger sample. Treatment regimens based on a shorter duration with sessions grouped more intensive were previously revealed to generate a larger treatment effect in diabetes [35]. Due to the small number of trials included, any inference of association between treatment effect and study characteristics is tenuous, and the intervention effects may decrease with the time from the treatment completion to follow-up [21]. Moreover, variations in the timing of the follow-up measurements associated with the end of treatment may have introduced heterogeneity. But according to the results of the subgroup analysis based on the follow-up time, the effect of MI on HbA1c was not significantly related to the follow-up time, so time to follow-up was ruled out as a significant source of variance.

Nine studies were MI-based interventions in T1DM cases, whereas the MI interventions remained slightly different. For

instance, MI intervention was conducted by nurses in seven studies, and physicians in the other two studies [27, 29]. However, they all were trained as health psychologists. In five studies, the intervention group received a motivational interviewing intervention; one study adopted motivational enhancement therapy (MET) which is a brief counseling approach (usually one to four sessions) for increasing motivation to change problematic health behaviors by exploring and resolving ambivalence about change; three studies [26, 29, 30] included MI as a component of broader treatment. Fidelity to treatment was discussed in 66.7% ($n=6$) of studies. Only 22.2% ($n=2$) of studies [26, 31] reported the results of objective fidelity measurements according to the scores on the Motivational Interviewing Treatment Integrity (MITI) code [36]. One review reported that trials of MI as a stand-alone treatment were not significantly different than trials that adopted MI with other treatment components [37]. Only one [30] study conducted MI with group MI sessions, and eight studies used individual MI sessions. In one study [31], the control group cases used the structured diabetes education (SDE) which was regarded as usual care. Therefore, the timing and frequency of MI intervention in the included studies were not completely consistent (Table 1). Subsequent studies should focus on the identical MI intervention method. Thus far, unified regulations have not yet been formed for the application of the time and intensity of MI. Eight studies conducted MI with adolescents (mean age, 14.3 years), while only one study [25] enrolled the participant with an average age of 36.4 years. Clinics and methodology homogeneity are key factors to rational pooled results of meta-analysis [38]. As revealed from a comprehensive analysis of the studies, the MI intervention methods were different, especially for the one study [29], so methodological heterogeneity might exist in the trial.

For depression scores, the total pooled existing significant heterogeneity ($P<0.001$, $I^2=91\%$), and the result suggested that no significant improvement in depressive symptoms in the MI arm compared to the control. Two studies [26, 31] used the CES-D to measure the cases' depression syndrome. While the WBQ was used to assess depressed mood in another study [24]. Moreover, the duration of MI intervention and frequency of session are related to the treatment effect. The long-term effectiveness of psychological treatments in diabetes is being researched; the average follow-up is 7 months [39]. The therapeutic process in psychological treatments is gradual and accumulative [40]. Thus, a coherence remains lacked for the optimal combination of intervention components that result in consistent benefit for glycemic control and psychosocial well-being for adolescents with T1DM [26].

As indicated from a study, young generations with diabetes reported significantly more depression in comparison with peers [41]. In the general population, late adolescence and young adulthood are suggested to be a time of particular vulnerability to the development of depression [42]. A meta-analysis displayed a combined prevalence of self-reported depressive symptoms of 30% in young generations with T1DM [15]. Diabetic cases with psychosocial problems often exhibit negative coping strategies that adversely affect their future, thereby causing increased diabetes fatalism (e.g., perceptions of powerlessness and despair, decreased medication adherence), and reduced self-care ability [17]. Thus, this situation could more adversely affect the individual's physical and psychological health. Young adults with diabetes have significant psychosocial needs regardless of whether diabetes poses an increased risk for emotional maladjustment.

The conclusions remain limited without serious attention paid to conducting highly rigorous studies following the MI principles. Likewise, subsequent studies should consider measurement process variables to clarify the mechanisms of change in MI interventions, as well as consider other aspects of quality assurance having not been measured by our rating system [37]. The present meta-analysis did not include sufficient trials to determine secondary moderators and was likely underpowered to detect small moderating effects. In addition, RCTs evaluating the effect of MI on HbA1c and depression are sparse. The present meta-analysis included studies of a range of quality (e.g., those containing small samples, using pre-post-design) due to the emerging nature of this field. As such, the clinical implications of the present findings are uncertain pending further research. Besides, subsequent meta-analyses might consider calculating effect sizes associated with target behavior (e.g., medication adherence, dietary intake) to provide insights into MI's effectiveness in T1DM populations.

In brief, though MI was developed more than 30 years ago, the evidence of its benefits in T1DM cases still seems somewhat limited, whereas the delivery and focus of MI in helping individuals manage their diabetes may be required to be revisited. Behavioral strategies may be particularly helpful for individuals with higher HbA1c, who may be experiencing a greater degree of ambivalence or even stagnation in managing their diabetes. The unique contribution of MI intervention is likely to be more effectively evaluated by behavioral or other intermediate outcomes. MI interventions should concentrate more on the behavioral components of change may be more effective. Besides taking a more behavioral approach to diabetes management, subsequent studies may also seek to determine whether the frequency of sessions is related to treatment effectiveness. When considering the limitations of the meta-analysis as well as the clinical importance of optimizing the treatment process for diabetes, the employed of MI-based intervention should not be discouraged. More detailed analyses can and should be undertaken as more studies are conducted in this field.

Author contribution YC and YT designed the study; YC, XS, and FZ screened and selected the eligible studies; XS and XH abstracted the basic information; YC and YT analyzed the data and written the manuscript. All authors reviewed the whole manuscript and agreed to the submission.

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Declarations

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Sarcopenia in patients with type 2 diabetes mellitus: a case–control study in Maracaibo city, Venezuela

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Abstract

Background/purpose of the study Several studies implicate skeletal muscle as a physio-pathological target among the metabolic disturbances of diabetes, or with the mechanisms shared by sarcopenia and type 2 diabetes mellitus (T2DM). The present study aimed to determine the relationship between sarcopenia and T2DM in outpatients of the Zulian Diabetes Institute, Maracaibo, Venezuela.

Methods A case–control study was performed on patients diagnosed with T2DM that went to the Zulian Diabetes Institute between January and June 2019. After obtaining a final sample of 131 patients with T2DM, control subjects were selected in a 1:1 ratio according to sex. The European Working Group on Sarcopenia in Older People (EWGSOP) criteria were followed to diagnose sarcopenia, so muscle mass, muscle strength, and muscle performance were determined.

Results A total of 261 patients were evaluated (131 with T2DM and 131 controls), with no differences in mean age (with T2DM 63.6 ± 9.3 vs. controls 63.6 ± 9.4 years old; $p = 0.99$). The prevalence of sarcopenia in control subjects was 9.9% and in subjects with diabetes 25.2%; $p < 0.01$; decreased muscle strength was the component with the most significant difference within the two groups (controls 16.8% vs. T2DM 49.6%; $p < 0.01$). In the multivariate analysis, subjects with worse metabolic control had a greater risk of sarcopenia (OR, 3.31; 95%CI, 1.10–9.97; $p = 0.03$).

Conclusion The prevalence of sarcopenia was higher in subjects with T2DM, especially in those with worse metabolic control and normal weight status.

Keywords Type 2 diabetes mellitus · Sarcopenia · Aging · Muscle function · Glycemic control

Introduction

Aging is defined as a series of morphological, psychological, functional, and biochemical modifications caused by the passing of time on living beings. It is a universal, sequential, cumulative, irreversible, not pathological process of deterioration of an organism. As time passes, the organism's ability to adapt to all kinds of changes is progressively lost until it becomes incapable of facing its environment's stress. As a result, the risk of dying increases [1].

Additionally, it is considered a multifactorial process characterized by multiple changes involving body composition, even if the observed variations in body mass, fat tissue, muscle, and bone throughout life are significantly influenced by sex, ethnicity, and physical activity [2]. From an epidemiological perspective, the aging of the world population is happening in a heterogeneous fashion. By 1999, 10% of the population was 60 years old or older, varying between 19% in developed countries and 5% in developing countries. Projections of the United Nations estimate that these numbers will double by 2050 [3].

The variation in body mass throughout life has been evaluated in the last decade, and there is consensus that it increases with age, and then it decreases or remains stable in old age. Even though the age at which body mass starts to fall varies between studies, it has been evidenced that this

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decrease is not massive, and it does not exceed 0.4% of body mass per year [4]. Nevertheless, even if the variation in body mass in older people is not huge, there are several transformations in different components of body composition that can mask certain illnesses, even in cases with no significant increase or decrease in weight [5].

In this sense, the concept of sarcopenia emerged (from the Greek *sarx* = flesh and *penia* = poverty). It is a syndrome characterized by the gradual and generalized loss of skeletal muscle mass and strength, with a risk of presenting adverse results such as physical disability, low quality of life, and mortality [6]. It emerged recently and is linked to the development of modern geriatrics. In the late 70s, Nathan Shock described the progressive physiological deterioration caused by the passing of decades in several body functions, with the loss of muscle mass and the functional loss it may ensue being especially remarkable. However, it was not until the late 80s when the American scientist Irwin H. Rosenberg proposed a name for this phenomenon as a first step to recognize its importance [7, 8].

Sarcopenia may emerge or worsen when it coincides with other body mass or metabolic disturbances, such as protein-calorie malnutrition, or obesity with sarcopenia, also called "sarcopenic obesity," which accumulates problems associated with both entities, being favored or worsened by inadequate diets and sedentary lifestyles. Several studies have also proposed the existence of additional factors that involve the skeletal muscle as a physio-pathological target within the metabolic disturbances of diabetes or with the mechanisms shared by sarcopenia and type 2 diabetes mellitus (T2DM) [9].

Around the world and especially in Latin America, there are very few studies that evaluate the relationship between these two disorders and the influence of treatment and level of control of T2DM over the functioning of the musculoskeletal system and muscle mass. Because of this reason, and due to the lack of local studies that determine the impact of sarcopenia in older adults, especially when there is also T2DM, as well as the community impact of patients with poor metabolic control and greater risk of comorbidities and death, it is the objective of this study to determine the relationship between sarcopenia and T2DM in outpatients from the Zulian Diabetes Institute, Maracaibo, Venezuela.

Materials and methods

Study design and sampling

An observational, analytic study with a non-experimental design was performed on patients diagnosed with T2DM that went to the Zulian Diabetes Institute, located in Maracaibo city, Zulia state, Venezuela, between January and June 2019. The sampling was performed via a probabilistic intentional method. The following formula is used to calculate the sample size since the expected relative frequency of exposure in controls and the expected association odd ratios in our population were not available:

$$n = \frac{N * Z_a^2 p * q}{d^2 * (N-1) + Z_a^2 * p * q}$$

where,

N	Size of the assessed population in that period (1,000)
Z_a^2	1.96^2 (if confidence is 95%)
p	expected sample proportion (in this case, 50% = 0.5)
q	1 – p (in this case, 1–0.5 = 0.5)
d	precision (in this case, 8%)

Obtaining the final sample was 131 patients with T2DM. Control subjects were selected in a 1:1 ratio matched for sex and age from the outpatient internal medicine service of the same hospital during this time. Patients with chronic illnesses or sequelae (different than T2DM) and those taking drugs that could modify body composition were excluded from the study, both in the diabetic and control groups. Examples of these are chronic obstructive pulmonary disease, cancer, thyroid disorders, hepatopathy, chronic kidney disease in dialysis, sequelae from stroke, dementia or severe cognitive deficit, psychosis, terminal illnesses, arthropathy, chronic corticosteroid use, use of anabolic steroids, and chemotherapy. Other exclusion criteria were hospitalization in the previous month, body mass index (BMI) < 18.5 or > 35 kg/m^2, patients with pacemakers or coronary stents, amputation of any limbs, use of a particular nutritional regime in the last month, and patients who opposed participating in the study.

Patient evaluation

Afterward, the designed test was applied to each patient. It involved their identification information: age, sex, place of origin, educational status, current consumption of tobacco and alcohol, regular physical activity, nutritional state, personal history of hypertension, fractures, osteoporosis, time of evolution of T2DM, presence of complications (retinopathy, nephropathy, neuropathy, diabetic foot), and used diabetic treatment. The mini-nutritional assessment-short form (MNA®-SF) was used to evaluate the nutritional status [10]. Their weight and height were also quantified to determine BMI with the formula (weight/height^2). Height was measured with a measuring rod, and weight was measured with a clinical Health-o-meter® scale, with a 5-g precision, without shoes.

For the assessment of sarcopenia, the diagnostic criteria proposed by EWGSOP were used [11]. These consist in the demonstration of muscle mass, muscle strength, and muscle performance loss. Skeletelal muscle mass was measured with a bioelectric impedance analysis (BIA), using a Tanita® BC-420-S digital scale (Tanita Corporation, Japan), using the Janssen predictive equation: SMM = [(Height2/R × 0.401) + (sex × 3.825) + (age x –0.071)] + 5.102, where R is impedance in ohms, sex (male = 1; female = 0), and age, in years. Afterward, the muscle mass index (MMI) was determined, dividing the SMM by height (expressed in Kg/m2). SMM loss was determined if the MMI was lower than 8.31 kg/m^2 in males and 6.68 kg/m^2 in females [12].

On the other hand, muscle strength was measured via handgrip strength, with a JAMAR® hydraulic dynamometer (Lafayette Instrument Company, USA). The cutoff points recommended by the EWGSOP were used to define a decrease in handgrip strength (Table 1). A gait speed test in 6 m was used for physical performance, considering it abnormal if the speed was ≤ 0.8 m/seg.

Most of the questionnaires and measuring tools in this study included quantitative scales with variability and test–retest reliability validation among observers. Furthermore, patients with specific clinical conditions that could modify the presence of sarcopenia were excluded from the study. As a result, the internal validity of the results increased.

After a 12-h nocturnal fast, peripheral blood samples were drawn to determine the serum levels of glycemia (enzymatic-colorimetric glucose oxidase kit; Sigma, USA), using the cutoff points suggested by the American Diabetes Association (≤ 130 mg/dl) to define metabolic control [13].

Statistical analysis

Once the information was collected, a spreadsheet for data tabulation was designed, to facilitate its organization. Results were expressed as mean ± SD, others in absolute numbers and percentages. A Student's *t*-test (2 groups) was used to determine differences among continuous variables, and Pearson chi-squared test was used to evaluate association. A multiple logistic regression model was performed for sarcopenia (dependent variable), adjusted by age group, glycemic control, mini-nutritional assessment, and BMI categories. The alpha level was set at 0.05. All the analyses were performed using the statistical program SPSS version 20 for Windows (Chicago, IL).

Table 1 Cutoff points used to define dynapenia, according to handgrip strength, adjusted for BMI and sex

Men		Women	
BMI (kg/m^2)	Handgrip strength (Kg)	BMI (kg/m^2)	Handgrip strength (Kg)
≤ 24	≤ 29	≤ 23	≤ 17
24.1–26	≤ 30	23.1–26	≤ 17.3
26.1–28	≤ 30	26.1–29	≤ 18
≤ 28	≤ 32	≤ 29	≤ 21

Results

A total of 262 patients were evaluated; there were no differences in mean age (non-diabetics 63.6 ± 9.4 years old vs. T2DM 63.6 ± 9.4; $p = 0.99$) or in distribution according to sex (diabetic women 61.8% vs. non-diabetic women 62.6%; $p = 0.90$). Most of the subjects came from urban areas (97.3%; $n = 255$), only had primary education (57.6%; $n = 151$), and had a normal nutritional state (88.9%; $n = 233$). The frequency of tobacco consumption was 8.4%, alcohol consumption was 10.3%, and regular physical activity was 18.3%. Personal history of hypertension was present in 53.8% of cases. The rest of the general characteristics of the sample are shown in Table 2.

The average time with T2DM was 16.3 ± 9.6 years. The most frequent complication was diabetic nephropathy with 6.1% ($n = 8$), followed by neuropathy with 3.8% ($n = 5$). Oral antidiabetic agents were the most used pharmacotherapy (49.6%, $n = 65$), and 54.2% had no metabolic control (basal glycemia: 130 mg/dl or greater) (Table 3).

Table 4 shows anthropometric parameters according to the presence of T2DM, observing a similar mean BMI between cases and controls ($p = 0.23$). However, diabetic subjects had a higher percentage of decreased muscle mass (non-diabetics 12.2% vs. T2DM 29.8%, $p < 0.01$), a higher percentage of decreased muscle strength (non-diabetics 16.8% vs. T2DM 49.6%; $p < 0.01$), a higher percentage of decreased muscle performance (non-diabetics 15.3% vs. T2DM 32.1%; $p < 0.01$), and a higher prevalence of sarcopenia (non-diabetics 9.9% vs. T2DM 25.2%; $p < 0.01$).

The factors associated with sarcopenia were age, nutritional and anthropometric state, observing in subjects with sarcopenia a higher mean age, percentage of risk of malnutrition, and normal weight, both in healthy subjects and diabetics. Additionally, in subjects with diabetes and sarcopenia, there was a higher frequency of worse metabolic control. In the multivariate analysis, subjects with worse metabolic control had a greater risk of sarcopenia (OR, 3.31; 95%CI, 1.10–9.97; $p = 0.03$) and those with overweight (OR, 0.72; 95%CI, 0.19–0.27; 0.001) and obesity (OR, 0.33; 95%CI, 0.004–0.276; 0.002) had a lower risk of sarcopenia compared to normal weight subjects (Table 5).

Table 2 General characteristics of the studied sample

	No T2DM		T2DM		Total		*p*
	n	%	*n*	%	*n*	%	
Age (years) mean ± SD	63.6 ± 9.4		63.6 ± 9.3		63.6 ± 9.4		0.99*
Sex							0.90**
Female	82	62.6	81	61.8	163	62.2	
Male	49	37.4	50	38.2	99	37.8	
Origin							0.70**
Rural	3	2.3	4	3.1	7	2.7	
Urban	128	97.7	127	96.9	255	97.3	
Education level							0.02**
Illiterate	5	3.8	3	2.3	8	3.1	
Primary education	72	55.0	79	60.3	151	57.6	
Secondary education	40	30.5	47	35.9	87	33.2	
College education	14	10.7	2	1.5	16	6.1	
Smoking habit							9,5 × 10–5**
No	109	83.2	131	100	240	91.6	
Yes	22	16.8	0	0.0	22	8.4	
Alcohol habit							0.001**
No	109	83.2	126	96.2	235	89.7	
Yes	22	16.8	5	3.8	27	10.3	
Regular physical activity							0.52**
No	105	80.2	109	83.2	214	81.7	
Yes	26	19.8	22	16.8	48	18.3	
PH hypertension							1,3 × 10–6**
No	80	61.1	41	31.3	121	46.2	
Yes	51	38.9	90	68.7	141	53.8	
PH fractures							0.16**
No	131	100	129	98.5	260	99.2	
Yes	0	0,0	2	1.5	2	0.8	
PH osteoporosis							0.32**
No	131	100	130	99.2	261	99.6	
Yes	0	0,0	1	0.8	1	0.4	
Mini-nutritional assessment							0.30
Normal nutritional state	119	90.8	114	87	233	88.9	
Risk of malnutrition	11	8.4	17	13	28	10.7	
Malnutrition	1	0.8	0	0.0	1	0.4	
Total	131	50	131	50	262	100	

PH personal history, *SD* standard deviation, *T2DM* type 2 diabetes mellitus

*Student's *t*-test

**Chi-squared test

Discussion

Sarcopenia is a disorder that frequently accompanies diabetes mellitus. In fact, it is believed that similar mechanisms are implicated both in the development of sarcopenia and in the pathogenesis of T2DM, such as insulin resistance, chronic inflammation, and mitochondrial dysfunction [14]. This study evaluates the existing association between both illnesses in outpatients of INZUDIABETES, one of the main attention centers for diabetic patients in Maracaibo city, and a referral center for the Zulia state, Venezuela.

The observed prevalence of sarcopenia in healthy subjects is similar to the one reported by Gao et al. [15], who found a percentage of 9.8% of older adult subjects affected by this condition in rural and urban communities in China. However, our results show that when comparing with diabetic patients, there was a considerable increase in the percentage of sarcopenic patients, even higher than the one shown by Trierweiler et al. [16], who evidenced a

Table 3 Clinical characteristics of the patients with diabetes

	n	%
Time with disease (years) mean ± SD	16.3 ± 9.6	
Complications		
Neuropathy	5	3,8
Nephropathy	8	6.1
Diabetic foot	2	1,5
Retinopathy	2	1,5
Type of treatment		
None	5	3.8
Oral antidiabetics	65	49.6
Insulinotherapy	61	46.6
Glycemic control (mg/dl)		
< 130	60	45.8
130 or higher	71	54.2
Total	131	100

sarcopenia prevalence of 15.6% in diabetic subjects evaluated in an outpatient endocrinology and metabolism service of the Hospital de Clinicas da Universidade Federal do Paraná. Meanwhile, in the Asian region, Wang et al. [17] reported a percentage of 14.8% of older adults in the city of Zhenjiang in China, using the Asian criteria to define sarcopenia, and Kim et al. [18], at the Korean Sarcopenic Obesity Study, found a prevalence of 15.7% in patients from the Diabetes Center of Korea University Guro Hospital.

With regard to the individual components of the skeletal muscle assessment, our findings show an increase in the frequency of subjects with a decrease in muscle mass, physical performance, and muscle strength, with the latter being the one with the most remarkable differences between diabetic subjects and controls, like it was evidenced by Vergara et al. [19], who despite not seeing statistical differences in the prevalence of sarcopenia between healthy and ill subjects did find differences in the prevalence of dynapenia in subjects from three health centers located in a rural area south of Castilla-La Mancha. Furthermore, in the report carried out in Brazil, muscle strength was the skeletal muscle parameter that showed the most remarkable decrease in subjects with diabetes, indirectly suggesting a decrease in muscle quality [16].

It is essential to mention that the differences between the prevalence of sarcopenia observed in our study and those from previous reports may be associated with the duration of the illness. For example, in the study from China, time with T2DM was approximately 6 years lower than in our study [17]. Furthermore, there was a low percentage of regular physical activity in our study, a high frequency of hypertension, and overall, poor glycemic control, even if most subjects were receiving some type of pharmacotherapy. These aspects could also influence the differences in the prevalence

Table 4 Anthropometric characteristics of the studied sample

	No T2DM		T2DM		Total		*p*
	n	%	*n*	%	*n*	%	
BMI (kg/m^2) **mean ± SD**	25.6 ± 3.5		26.1 ± 4.2		25.8 ± 3.9		0.23*
BMI classification							0.09**
Normal	60	45.8	56	42.7	116	44.3	
Overweight	55	42.0	46	35.1	101	38.5	
Obese	16	12.2	29	22.1	45	17.2	
Muscle mass							0.0005**
Normal	115	87.8	92	70.2	207	79.0	
Decreased	16	12.2	39	29.8	55	21.0	
Muscle strength							1.7×10^{-6}**
Normal	109	83.2	66	50.4	175	66.8	
Decreased	22	16.8	65	49.6	87	33.2	
Physical performance							0.001**
Normal	111	84.7	89	67.9	200	76.3	
Decreased	20	15.3	42	32.1	62	23.7	
Sarcopenia							0.001**
No	118	90.1	98	74.8	216	82.4	
Yes	13	9.9	33	25.2	46	17.6	
Total	131	50	131	50	262	100	

SD standard deviation, *T2DM* type 2 diabetes mellitus

*Student's *t*-test

**Chi-squared test

Table 5 Factors associated with sarcopenia in the studied sample

		No Sarcopenia		Sarcopenia		*p*	OR (95%CI); *p****
		n	%	*n*	%		
No T2DM	Age	62.2±8.4		76.0±8.2		1.2×10^{-7}*	
	Glycemic control (mg/dl)					-	
	< 130	118	100	13	100		
	130 or higher	0	0	0	0		
	Mini-nutritional assessment					0.006**	
	Normal nutritional state	109	92.4	10	76.9		
	Risk of malnutrition	9	7.6	2	15.4		
	Malnutrition	0	0	1	7.7		
	BMI categories						
	Normal weight	50	42.4	10	76.9	0.05**	
	Overweight	52	44.1	3	23.1		
	Obesity	16	13.6	0	0		
T2DM	Age	62.3±9.0		67.4±9.5		0.007*	1.37 (0.50–3.79); 0.55
	Glycemic control (mg/dl)					0.004**	
	< 130	52	100	8	24.2		1
	130 or higher	46	0	25	75.8		3.31 (1.10–9.97); 0.03
	Time with diabetes					0.12**	
	< 5 years	10	10.2	0	0		
	5–10 years	28	28.6	13	39.4		
	> 10 years	60	61.2	20	60.6		
	Mini-nutritional assessment					5.7×10^{-5}**	
	Normal nutritional state	92	93.9	22	66.7		1
	Risk of malnutrition	6	6.1	11	33.3		2.12 (0.56–8.08); 0.27
	Malnutrition	0	0	0	0		-
	BMI categories					1.02×10^{-7}**	
	Normal weight	27	27.6	29	87.9		1
	Overweight	43	43.9	3	9.1		0.72 (0.19–0.27); 0,001
	Obesity	28	28.6	1	3.0		0.33 (0.004–0.276); 0,002

T2DM type 2 diabetes mellitus, *BMI* body mass index

*Student's *t*-test

**Chi-squared test

***Adjusted model for: T2DM status, age groups (< 65 to ≥ 65 years), mini-nutritional assessment, glycemic control, BMI categories

shown according to the investigation group. Nevertheless, it is vital to highlight the low frequency of fractures and chronic complications of T2DM compared to other reports [16], which may be caused by under-reporting due to lack of knowledge, since these variables were asked to the patient, representing a limitation in our study.

From the nutritional perspective, like expected, there was a higher prevalence of obesity in diabetics, like in the cross-sectional analysis of Dejo and Palacios in a Peruvian public hospital where physical performance and muscle strength were assessed in older adults [20]. When evaluating the nutritional state according to the MNA in our study, the low frequency of malnutrition stands out, but the proportion of subjects at risk of malnutrition is higher than the one shown in the Spanish report [19].

Regarding the associated factors, both in people with diabetes and healthy subjects, the mean age and the percentage of subjects at risk of malnutrition were more notable in those with sarcopenia, which suggests the influence of aging and nutritional state in this disease [21]. For this reason, the identification of these groups must be fundamental in primary care settings to establish early therapeutic strategies. On the other hand, in patients with T2DM and sarcopenia, a worse metabolic control from fasted samples was observed, which matches with the results from Kalyani et al. [22], who observed in the Baltimore Longitudinal Study of Aging data that hyperglycemia (measured via HbA1c) is associated with lower muscle strength over time. Furthermore, Akpinar et al. [23] evidenced in patients in a hospital in Turkey that those with not-complicated diabetes (short evolution) did not have

a loss of strength and muscle mass related to age. Also, He et al. [24] also observed in a case–control study in a Chinese population a worse metabolic control, nutritional state, and kidney function in patients with sarcopenia and T2DM. Other studies have shown that poor glucose regulation, insulin fluctuation, and insufficient insulin secretion have been related to sarcopenia [25–27]. The relationship with nutritional state is closely related to anthropometric status, specifically the presence of normal or even underweight. However, it is important to consider that specific groups (e.g., T2DM obese and sarcopenic subject) have a small sample size in our study. Therefore, the results cannot be fully generalized.

Within this study's limitations is the use of a formula to calculate the sample size that is not specific for case–control studies. This was a result of the lack of local records that provide data on frequency and odds ratio, which are necessary for this type of formula. Other limitations were the inability to match controls according to nutritional status; the lack of determination of additional biochemical parameters related to diabetes, like HbA1C, to determine metabolic control; and other factors that can allow establishing possible underlying mechanisms to the relationship of these two illnesses (kidney function, albumin levels, and bone mineral density).

Conclusions

The prevalence of sarcopenia in control subjects from the Zulian Diabetes Institute from Maracaibo city was 9.9% and jumped to 25.2% in patients with T2DM, being decreased muscle strength the most frequent diagnostic criteria and the one that had the most remarkable increase between controls and diabetic subjects. Patients with sarcopenia and T2DM had a higher frequency of subjects with inadequate metabolic control, and a normal weight status. For them, it is necessary to systematically assess muscle function and the presence of sarcopenia in patients with T2DM, especially in older adults and in subjects at risk of undernutrition. It is also vital to recommend regular physical exercise and healthy diet habits in the general population, especially in people with diabetes. Future studies should focus on how the coexistence of these diseases impacts the quality of life of patients and their self-monitoring and care, taking into consideration a greater number of patients for a longitudinal evaluation.

Declarations

Informed consent Each patient selected to be included in the study received a comprehensive explanation of the study, and their authorization was requested via a written informed consent before they participated in the study.

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ORIGINAL ARTICLE

Increased of fasting active glucagon-like peptide-1 is associated with insulin resistance in patients with hypertriglyceridemia

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Abstract

Background Glucagon-like peptide-1 (GLP-1) is an incretin hormone that facilitates insulin secretion and preserves β cell function. hypertriglyceridemia plays an important role in the pathogenesis of insulin resistance and T2DM. The purpose of this study was to measure fasting active GLP-1 levels in hypertriglyceridemia subjects and analyse the relationship between GLP-1 and insulin resistance.

Methods We recruited 146 subjects including 38 diabetes patients with hypertriglyceridemia, 33 diabetes patients without hypertriglyceridemia, 35 hypertriglyceridemia subjects, and 40 healthy subjects as the normal control group. Serum fasting active GLP-1 was tested with ELISA in the four groups, and associations with insulin resistance were analysed.

Results Serum fasting active GLP-1 levels were significantly increased in hypertriglyceridemia subjects with or without T2DM compared with healthy controls, particularly hypertriglyceridemia patients with T2DM ($p < 0.01$). GLP-1 levels positively correlated with triglyceride (TG) levels, fasting insulin (FINS) levels, and HOMA-IR ($p < 0.01$). Furthermore, multiple stepwise regression showed that TG levels and HOMA-IR were independently associated with fasting active GLP-1 levels ($p < 0.01$).

Conclusions Hypertriglyceridemia was associated with elevated fasting active GLP-1 levels, and a significant association was noted between GLP-1 and HOMA-IR. This finding provides evidence that the increase in GLP-1 may play a compensatory role in the pathogenesis of insulin resistance induced by hypertriglyceridemia.

Keywords Glucagon-like peptide-1 · hypertriglyceridemia · Diabetes mellitus · Insulin resistance

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is increasing rapidly worldwide. As the largest developing country, China has the largest number of diabetic patients in the world. More than 90% of diabetic patients suffer from T2DM. It is estimated that the prevalence rate of T2DM was 9.32% among the Chinese population aged 18 to 79 in 2014 [1]. Insulin resistance is an important mechanism in the pathogenesis of T2DM [2]. Hyperinsulinemia is a physiological response that maintains glucose homeostasis, which may play a role in β cell compensation for insulin resistance.

Hypertriglyceridemia, accompanied by increased free fatty acids, is the most common dislipidemia in patients with insulin resistance and T2DM [3]. The level of fatty acids may more easily increase in the insulin resistance-related state when insulinemia is insufficient to suppress lipolysis [4]. Chronic elevated fasting or stimulated fatty acids play an essential role in the development of hyperinsulinemia. Continuous lipid infusion can also induce liver insulin resistance and β cell dysfunction in subjects who are predisposed to T2DM [5]. People with high concentrations of fasting plasma free fatty acids have a higher risk of developing insulin resistance and T2DM [6].

Glucagon-like peptide-1 (GLP-1) is a major member of the incretin hormone family and is secreted from ileal and colonic mucosa enteroendocrine L cells. Active GLP-1 has

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a half-life of only a few minutes as it is degraded by dipeptidyl peptidase-IV (DPP-IV). The bioactive forms of GLP-1 (7–37) and (7–36 amide) interact with appropriate receptors and play a variety of roles in the human body [7]. GLP-1 stimulates endogenous insulin secretion in response to oral glucose or feeding and inhibits glucagon secretion, hepatic glucose production, and gastric emptying [8]. Furthermore, GLP-1 seems to preserve pancreatic β cell function [9]. Among type 2 diabetic patients, a reduced incretin effect has been described [10]. Impaired secretion of GLP-1 has been regarded as one of the potential mechanisms underlying this defect [11]. However, in recent years, the secretion patterns of GLP-1 in type 2 diabetes and type 2 diabetes-related conditions have remained controversial. A previous study reported that plasma GLP-1 levels were increased in obese subjects [12]. Calanna et al. and Naucket et al. [13, 14] also reported that GLP-1 levels were higher in patients with T2DM than in healthy controls.

In addition to carbohydrates, free fatty acids (FFAs) and other lipids [15] have been shown to stimulate the secretion of GLP-1 by activating G protein-coupled receptors (GPCRs). FFAs stimulate GLP-1 secretion from L cells in a dose-dependent manner [16]. Animal studies have found that a high-fat diet can increase serum GLP-1 levels by stimulating the GPR40 pathway [17, 18]. A human study also showed that 7-day overfeeding resulted in an increase in fasting serum GLP-1 [19]. Different hyperlipidaemia conditions may lead to changes in β cell function and insulin sensitivity and may further lead to the divergence of GLP-1 secretion levels. However, to date, few studies have focused on the effect of hypertriglyceridemia on GLP-1 secretion.

In the present research, we aimed to investigate fasting GLP-1 levels in hypertriglyceridemia subjects and hypertriglyceridemia patients with diabetes and to further explore the correlation between blood lipids and GLP-1 levels. Additionally, the relationship between GLP-1 levels and HOMA-IR was evaluated. Our study may provide new insight into the effect of triglyceride (TG) on GLP-1 secretion, which may be a new potential pathogenesis of insulin resistance and diabetes in patients with hypertriglyceridemia.

Materials and methods

Study subjects

The subjects recruited for this study were randomly selected from the Physical Examination Centre and Endocrine Department of Yantai Yuhuangding Hospital from February 2018 to February 2019. All subjects signed written informed consent before commencement of the study. T2DM was diagnosed according to the diagnostic criteria of the America Diabetes Association (ADA) Guideline [20]: FPG ≥ 7.0 mmol/l (125 mg/dl) and/or 2hPG ≥ 11.1 mmol/l (200 mg/dl) during the 75 g oral glucose tolerance test (OGTT). Hypertriglyceridemia was defined according to the National Cholesterol Education Program-Adult Treatment Panel III [21] as serum TG levels ≥ 1.7 mmol/l.

A total of 400 subjects participated in this study. The exclusion criteria were dependent on self-reported history and the data from our measurements. Subjects were excluded if they had liver function disorders, renal diseases, thyroid disease, excessive drinking history (ethanol intake of ≥ 40 g/d), familial hyperlipidaemia, myocardial infarction, or stroke. None of the subjects were treated with GLP-1 analogues, DPP-4 inhibitors, insulin, or any medication that was known to influence lipid metabolism or body weight. Patients with malignant disease, inflammatory bowel disease, previous bowel surgery, and documented malabsorption were also excluded. Finally, 146 subjects were considered eligible for this study; 38 patients were diagnosed with T2DM with hypertriglyceridemia (HTG + T2DM) group, and 33 patients were diagnosed with T2DM without hypertriglyceridemia (T2DM) group. In addition, 35 subjects were included in the hypertriglyceridemia (HTG) group, and 40 healthy subjects were chosen as normal control (NC) group.

Anthropometric and laboratory measurements

The height and weight of the subjects were measured in light clothing and without shoes. BMI was calculated by dividing weight (kg) by the square of height (m). Blood pressure was measured two times at 5-min intervals, and then the mean value was calculated. The fasting plasma glucose (FPG), concentration was determined using the glucose oxidase method. The levels of serum TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using the peroxidase method with Beckman AU5800 Clinical Chemistry Analyzer (Beckman, Indianapolis, USA). Hemoglobin A1c (HbA1c) was measured using high-pressure liquid chromatography. Fasting insulin (FINS) was measured using a radioimmunoassay kit (Northern Bioengineering Institute, Beijing, China). HOMA-IR was calculated as FINS (μM/mL) × FPG (mmol/L) / 22.5.

The blood used for GLP-1 determinations was collected in ice-cooled Vacutainer EDTA-plasma tubes containing an appropriate amount of DPP-IV inhibitor (Millipore, Billerica, MA). After centrifugation at 4 °C, samples were stored at – 80 °C before GLP-1 measurement. The concentrations (pM) of active GLP-1 were determined by a commercial enzyme-linked immunoassay (ELISA) kit from Millipore (Billerica, MA, EZGLP1T-35 K) according to the manufacturer's directions. The kit detects the active forms of GLP-1, including GLP-1 (7–37) and GLP-1 (7–36).

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, Illinois). Values were presented as the mean ± SD. Differences in the proportions of variables were determined by chi-squared analysis. Comparisons between different groups of normally distributed parameters were made using one-way analysis of variance (ANOVA). Pearson's correlation analysis was performed between serum fasting active GLP-1 levels and clinical characteristics. Partial correlation was used to determine the associations after adjusting for the effects of age, sex, and blood pressure. Stepwise multiple linear regressions using GLP-1 as the dependent variable and other variables as the independent variable were performed to identify independent factors affecting GLP-1 levels. $p < 0.05$ were considered statistically significant.

Results

Anthropometric and clinical characteristics as well as laboratory measurements of the subjects are shown in Table 1. Serum TG, TC, and LDL-C levels in the HTG, HTG + T2DM, and T2DM groups were significantly higher than those in the NC group, while HDL-C levels were significantly lower in the HTG + T2DM group than in the NC group. FPG, HbA1c, FINS, and HOMA-IR were significantly higher in the HTG + T2DM and T2DM groups than in the NC group. Systolic blood pressure (SBP) in the HTG + T2DM group was significantly higher than that in the NC group. There was no significant difference in other clinical factors, including age, sex, BMI, and diastolic blood pressure (DBP).

Compared with the NC group, the levels of fasting active GLP-1 in the HTG and HTG + T2DM groups were markedly increased by 32.3% and 37.9%, respectively ($p = 0.000$). Fasting active GLP-1 levels were also significantly higher in the HTG and HTG + T2DM groups than in the T2DM group ($p = 0.04$ and $p = 0.007$, respectively). There were no significant differences in fasting active GLP-1 levels between the NC and T2DM groups (Table 1).

A correlation analysis was performed between fasting active GLP-1 and the clinical characteristics in all the subjects. As shown in Fig. 1, fasting active GLP-1 levels were positively associated with TG, FINS levels, and HOMA-IR and negatively associated with HDL-C levels in all subjects. No correlation was observed between fasting active GLP-1 levels and other clinical characteristics, including BMI, FPG, HbA1c, and LDL-C. After controlling for age and sex, fasting active GLP-1 levels were also positively correlated with TG and FINS levels and HOMA-IR and negatively correlated with HDL-C levels. When also controlling for SBP and DBP, the correlations between fasting active GLP-1 levels and these variables (TG levels, FINS levels, HDL-C levels, and HOMA-IR) remained (Table 2).

To determine the factors that influenced serum fasting GLP-1 levels, we performed multiple stepwise linear regression analysis (Table 3). The following independent variables were considered for the model: BMI, SBP, DBP, TC, TGs, HDL-C, LDL-C, FPG, HbA1c, and HOMA-IR. Only the variables that had a $p < 0.05$ were considered in

Table 1 Clinical and laboratory characteristics of all subjects in the four groups

Characteristics	NC ($n = 40$)	T2DM ($n = 33$)	HTG ($n = 35$)	HTG + T2DM ($n = 38$)	p value
Age (year)	55.38 ± 6.73	56.79 ± 6.33	56.66 ± 6.89	58.16 ± 7.56	0.364
Sex(F/M)	20/20	20/13	17/18	21/17	0.740
BMI (kg/m^2)	25.79 ± 1.95	25.99 ± 2.13	26.07 ± 2.03	26.67 ± 2.04	0.247
SBP (mmHg)	129.08 ± 12.77	135.12 ± 15.74	132.03 ± 18.30	140.08 ± 13.02**	0.012
DBP (mmHg)	78.10 ± 9.05	80.88 ± 11.10	79.17 ± 9.49	81.21 ± 7.43	0.442
TG (mmol/l)	0.90 ± 0.26	1.18 ± 0.27**	2.12 ± 0.47**	2.43 ± 0.61**	0.000
TC (mmol/l)	4.18 ± 0.54	4.78 ± 0.70**	5.06 ± 0.69**	4.63 ± 0.76**	0.000
HDL-C (mmol/l)	1.34 ± 0.28	1.36 ± 0.25	1.23 ± 0.23	1.16 ± 0.19**	0.002
LDL-C (mmol/l)	2.34 ± 0.46	2.94 ± 0.56**	3.00 ± 0.66**	2.66 ± 0.65*	0.000
FPG (mmol/L)	5.35 ± 0.30	8.58 ± 1.54**	5.40 ± 0.44	8.26 ± 1.29**	0.000
HbA1c (%)	5.68 ± 0.23	7.47 ± 0.98**	5.63 ± 0.26	7.39 ± 0.95**	0.000
FINS (mU/L)	5.99 ± 2.07	7.99 ± 3.87**	7.13 ± 2.79	9.54 ± 4.44**	0.000
HOMA-IR	1.43 ± 0.52	2.99 ± 1.39*	1.73 ± 0.74	3.48 ± 1.64**	0.000
Fasting active GLP-1 (pM)	4.12 ± 1.40	4.67 ± 1.37	5.45 ± 1.93**	5.68 ± 1.44**	0.000

BMI body mass index; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *TG* triglycerides; *TC* total cholesterol; *HDL-C* high-density lipoprotein cholesterol; *LDL-C* low-density lipoprotein cholesterol; *FPG* fasting plasma glucose; *HbA1c* glycosylated haemoglobin; *FINS* fasting insulin. *GLP-1*, glucagon-like peptide-1.* $P < 0.05$ and ** $P < 0.01$ vs. NC group, respectively

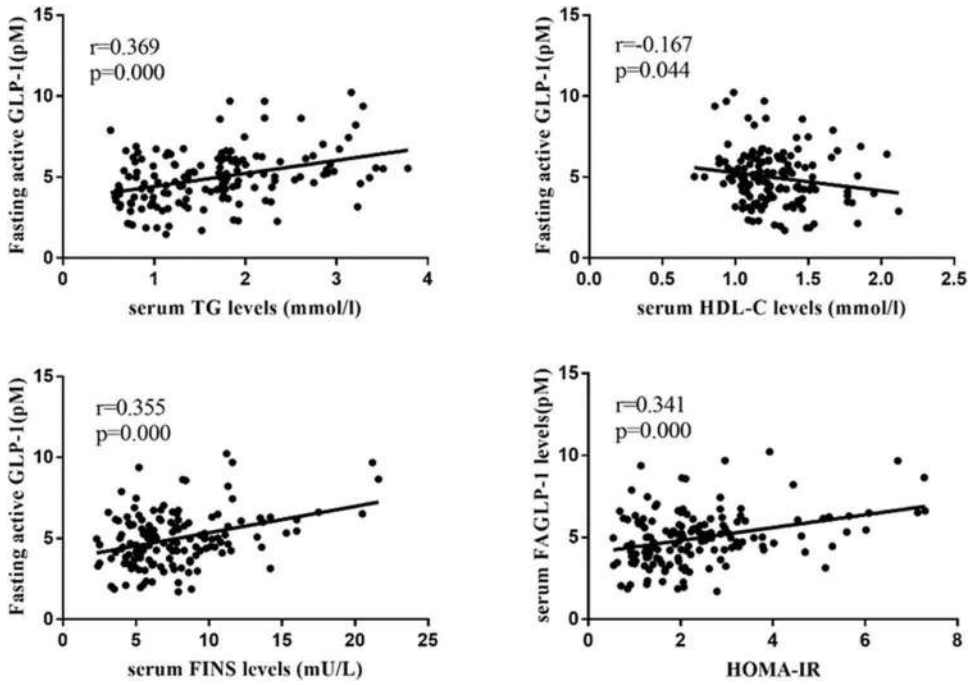

Fig. 1 Plasma Fasting active GLP-1 concentrations correlated positively with TG, FINS, HOMA-IR, and negative with HDL-C

Table 2 Correlation analysis of Fasting active GLP-1 and clinical laboratory characteristics

Variables	GLP-1		GLP-1 (age and sex adjusted)		GLP-1 (age, sex and BP adjusted)	
	r	*p* value	Partial r	*p* value	Partial r	*p* value
BMI (kg/m^2)	0.116	0.164	0.144	0.175	0.097	0.252
TG (mmol/l)	0.369	0.000*	0.369	0.000*	0.354	0.000*
TC (mmol/l)	0.165	0.046*	0.147	0.080	0.117	0.167
HDL-C (mmol/l)	−0.167	0.044*	−0.175	0.036*	−0.187	0.026*
LDL-C (mmol/l)	0.131	0.115	0.113	0.177	0.088	0.300
FPG (mmol/L)	0.123	0.138	0.112	0.181	0.070	0.470
HbA1c (%)	0.100	0.231	0.088	0.293	0.055	0.513
FINS (mU/L)	0.355	0.000*	0.365	0.000*	0.352	0.000*
HOMA-IR	0.341	0.000*	0.342	0.000*	0.318	0.000*

r = Pearson's correlation coefficient; *$P < 0.05$ were considered statistically significant

Table 3 Stepwise multiple linear regression analysis of variables associated with Fasting active GLP-1 levels

Independent Variable	B	Beta	95% CI	P
TG (mmol/l)	0.654	0.306	0.330–0.978	0.000*
HOMA-IR	0.309	0.268	0.134–0.484	0.001*

Abbreviation: B (unstandardized coefficients), 95% confidence interval (CI) of B, Beta (standardized coefficients). *$P < 0.05$ were considered statistically significant

the final fitted model. The results revealed that TG and HOMA-IR were independently associated with fasting active GLP-1 levels ($p < 0.05$).

All the subjects were categorized into four groups (G1, G2, G3, and G4) according to fasting active GLP-1 levels. SBP in G3 and G4 was significantly higher than that in G1 ($p < 0.05$ and $p < 0.01$, respectively). TGs in G3 and G4 were significantly increased compared with those in G1 ($p < 0.01$). TC in G4 was significantly higher than that in G1 ($p < 0.05$). There was a linear increase in FINS and HOMA-IR with increasing fasting active GLP-1 levels. More importantly, FINS and HOMA-IR increased significantly in G4 (GLP-1 6.0–10.23 pM) compared with G1 and G2 ($p < 0.01$ and $p < 0.05$, respectively). No significant differences existed concerning age, sex, the levels of BMI, DBP, TC, LDL-C, HDL-C, FPG, or HbA1c (Table 4).

Table 4 Clinical characteristics of different Fasting active GLP-1 categories

Characteristics	G1 GLP-1 1.71–3.79 pM	G2 GLP-1 3.86–4.96 pM	G3 GLP-1 4.97–5.99 pM	G4 GLP-1 6.0–10.23 pM	*p* value
Age (year)	55.11 ± 6.80	57.61 ± 6.40	57.05 ± 7.84	57.69 ± 6.98	0.359
Sex(F/M)	16/21	21/15	19/18	22/14	0.422
BMI (kg/m^2)	25.84 ± 1.85	26.37 ± 1.98	25.88 ± 2.06	26.45 ± 2.09	0.443
SBP (mmHg)	128.05 ± 11.97	134.69 ± 16.11	136.08 ± 16.25*	137.33 ± 15.93**	0.046
DBP (mmHg)	79.03 ± 9.19	79.47 ± 7.52	81.29 ± 10.51	79.36 ± 9.82	0.722
TG (mmol/l)	1.26 ± 0.62	1.55 ± 0.62	1.87 ± 0.79**	1.93 ± 0.87**	0.000
TC(mmol/l)	4.48 ± 0.71	4.58 ± 0.59	4.61 ± 0.89	4.90 ± 0.69*	0.095
HDL-C (mmol/l)	1.31 ± 0.25	1.29 ± 0.25	1.21 ± 0.23	1.28 ± 0.27	0.311
LDL-C (mmol/l)	2.63 ± 0.62	2.63 ± 0.53	2.76 ± 0.75	2.86 ± 0.63	0.334
FPG (mmol/L)	6.33 ± 1.57	6.96 ± 1.75	7.08 ± 1.88	7.04 ± 2.05	0.250
HbA1c (%)	6.27 ± 1.02	6.51 ± 1.03	6.61 ± 1.19	6.68 ± 1.24	0.429
FINS (mU/L)	6.51 ± 3.42	7.33 ± 2.71	7.63 ± 3.04	9.34 ± 3.91**	0.010
HOMA-IR	1.87 ± 1.24	2.31 ± 1.23	2.41 ± 1.19	3.19 ± 2.24**	0.007

BMI body mass index; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *TG* triglycerides; *TC* total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C* low-density lipoprotein cholesterol; *FPG* fasting plasma glucose; *HbA1c* glycosylated haemoglobin; *FINS* fasting insulin; *GLP-1* glucagon-like peptide-1. *$P < 0.05$ and **$P < 0.01$ vs. G1, respectively

Discussion

Fasting active GLP-1 levels in the hypertriglyceridemia and hypertriglyceridemia with T2DM groups were significantly higher than those in the T2DM group and normal control group. Several studies reported that GLP-1 levels in patients with insulin resistance and diabetes were increased [22, 23]. Rydgren et al. also found increased plasma total GLP-1 in non-obese diabetic mice when compared to normal control mice [24]. In addition, another important finding of our study is that TGs are independently associated with fasting active GLP-1 levels. The same results were found in patients with MetS conditions [25] and in overweight/obese subjects [19]. Our findings, along with other published research results, tend to suggest an effect of TG on serum fasting GLP-1 levels in diabetes patients complicated with hypertriglyceridemia and subjects with hypertriglyceridemia, and the main effect may be that hypertriglyceridemia can promote the secretion of fasting GLP-1.

We hypothesize that part of the effects of hypertriglyceridemia on fasting GLP-1 levels can be explained by the following mechanisms. T2DM patients with hypertriglyceridemia usually have elevated circulating FFAs, which are the main degradation products of TGs. Long-term exposure to FFAs induced insulin resistance and impaired β cell function [26]. Research in vitro showed that increasing GLP-1 production promoted β cell function and survival in response to β cell injury from FFAs [27]. Kappe et al. studies also found that the FFA palmitate stimulated GLP-1 secretion but significantly increased DNA fragmentation in GLU Tag cells [28]. Pancreatic levels of GLP-1 were also increased after rats were administered STZ [29]. Another study revealed that GLP-1 release was higher from T2DM patient islets than from non-diabetic islets [30]. In our study, GLP-1 secretion was increased in diabetic patients with hypertriglyceridemia, consistent with the studies listed above. Therefore, we infer that the compensatory secretion of active GLP-1 protects β cells from apoptosis induced by high levels of FFAs. This might be one important reason for the elevated levels of GLP-1 in patients with hypertriglyceridemia. The secretion of GLP-1 also depends on the nutritional components of the diet, especially carbohydrates and fat [31]. The long-term consumption of a high-fat and high-carbohydrate diet may be another reason for the elevated levels of GLP-1. More studies are needed to elucidate the possible mechanism of elevated fasting GLP-1 levels.

Fasting active GLP-1 levels were positively correlated with TC levels and negatively correlated with HDL-C levels. The hypertriglyceridemia group showed higher TC and lower HDL-C levels, which could explain why GLP-1 was related to TC and HDL-C. It has been reported that fasting plasma GLP-1 levels are significantly correlated with blood pressure in healthy individuals [32]. After controlling for SBP and DBP, there was still a significant correlation between GLP-1 and HDL-C. Similar to our research results, a previous study also observed a negative correlation between fasting GLP-1 levels and HDL-C levels in overweight/obese subjects [19]. These findings indicated that cholesterol metabolism may also affect GLP-1 secretion. Since TGs have a greater effect on GLP-1 than HDL-C, multiple linear regression results showed no significant correlation between HDL-C and GLP-1 levels.

In the present study, we also found a significant correlation between serum GLP-1 levels and HOMA-IR in the fasting state. The subjects with higher fasting GLP-1 levels had higher insulin resistance (HOMA-IR and insulin increased). GLP-1 was not related to glucose as a parameter of the insulin resistance index, indicating that there is a direct correlation between fasting active GLP-1 and fasting insulin. Similarly, a recent large study of T2DM also suggested that T2DM patients with hyperinsulinemia have higher basal GLP-1 than healthy subjects [33]. In addition, a study in obese nondiabetic patients also observed that basal GLP-1 levels were positively correlated with basal insulin levels [34]. Furthermore, the results from healthy subjects with insulin resistance showed an increase in fasting active GLP-1 levels but a decrease in the incretin effect [35, 36]. Hyperinsulinemia was accompanied by increased levels of fasting active GLP-1 in our results, suggesting that increased fasting active GLP-1 secretion in hypertriglyceridemia subjects could possibly contribute to hyperinsulinemia to compensate for insulin resistance. Additional research is needed to confirm this hypothesis.

This research has some potential limitations. First, the sample size is relatively small. Second, our study only measured active GLP-1 levels under fasting conditions, which may not reflect the change in GLP-1 levels over time. Finally, the cross-sectional design of this study cannot definitively allow us to distinguish the causes and consequences between the two variables.

As a result, data from this study showed that hypertriglyceridemia promotes fasting active GLP-1 secretion, and the potential reason for the GLP-1 increase may be to protect islet cells from apoptosis induced by high levels of FFAs. GLP-1 was positively associated with insulin resistance, representing the stimulus for insulin hypersecretion under fasting conditions, which may be a compensatory secretion process. These findings provide evidence that the increase in fasting active GLP-1 may play an important role in the pathophysiological process of insulin resistance induced by hypertriglyceridemia.

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Declarations

This study was approved by the local ethics committee at Yantai Yuhuangding Hospital and all participants provided the written informed consents. This research conforms to the latest revision of the Declaration of Helsinki.

Ethics approval The study complied with the principles of the Declaration of Helsinki, and the protocol was approved by the Medical Ethics Committee of Yantai Yuhuangding Hospital, Shandong, China. The purposes and procedures of the study were explained to patients before blood sampling.

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

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ORIGINAL ARTICLE

Effects of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline on diabetes prediction among the Chinese Han population

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Abstract

Background Fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline are each associated with the risk of diabetes. The aim of this study was to explore the effects of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline on diabetes prediction in the Chinese Han population.

Methods Our study consisted of 490 subjects with glucose metabolism dysfunction (GMD) and 770 healthy subjects. Spearman's correlation analysis was used to assess the relationship of clinical characteristics with prediabetes. Receiver operation characteristic (ROC) curve analysis was used to identify the diagnostics value in diagnosing prediabetes.

Results Our study indicated that fasting proinsulin/fasting insulin, vitamin D3, and waistline were positively associated with prediabetes. Fasting proinsulin/fasting insulin (OR = 1.73, $p = 0.002$), vitamin D3 (OR = 1.02, $p < 0.001$), and waistline (OR = 1.04, $p < 0.001$) were significantly related to an increased risk of prediabetes. Stratified analyses results showed that fasting proinsulin/fasting insulin (odds ratio [OR] = 2.51, $p = 0.001$), vitamin D3 (OR = 1.02, $p = 0.043$), and waistline (OR = 1.03, $p = 0.006$) had a strong association with prediabetes in men, while only vitamin D3 (OR = 1.03, $p < 0.001$) and waistline (OR = 1.05, $p < 0.001$) were strongly related to prediabetes risk in women. ROC curve analysis results revealed that the area under the curve (0.936, $p < 0.001$) and sensitivity (100%) in combination of the fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline provided a better diagnostic value of than either parameter alone.

Conclusion The fasting proinsulin/fasting insulin, vitamin D3, and waistline showed a strong association with an increased risk of prediabetes. The combination of fasting proinsulin/ fasting insulin, proinsulin/true insulin, vitamin D3, and waistline provides a helpful diagnostic indicator for diagnosing prediabetes in the Chinese Han population.

Keywords Diabetes · Proinsulin · Insulin · Vitamin D3 · Waistline

Introduction

Diabetes is serious metabolic disorder characterized by pancreatic β cell dysfunction and insulin resistance [1]. There are approximately 382 million diabetic people, with type 2 diabetes (T2D) comprising about 90% of the cases [2, 3]. The incidence of type 1 diabetes (T1D) has increased by 2–5% worldwide, and T1D prevalence in the USA is about 1 per 300 up to the age of 18 years [3, 4]. China has the world's largest diabetes epidemic. It is estimated that the overall prevalence of total diabetes in China is 10.9% and that of prediabetes is 35.7% [5]. Diabetes has contributed to the large burden of mortality and disability worldwide. The risk factors for the development of diabetes include diet, lifestyle, genetic, and environmental factors. Early screening of high-risk groups is necessary to prevent or delay the development of diabetes.

Proinsulin is the precursor form of insulin synthesized by pancreatic β cells. High concentrations of proinsulin are observed in glucose intolerant individuals [6]. Fasting proinsulin levels have a strong relationship with insulin resistance

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and T2D [7, 8]. The proinsulin/insulin ratio is an additional measure of β cell function [9]. An increase in the proinsulin/insulin ratio represents a primary islet β cell dysfunction response that occurs earlier than hyperglycemia [10]. In non-diabetic individuals, a disproportionate elevation of proinsulin/insulin could predict the development of T2D [6, 11]. Vitamin D3 is an essential vitamin that maintains mineral homeostasis and regulates bone metabolism [12]. Vitamin D3 plays an important role in immune response, insulin resistance, renine-angiotensin system, and endothelial function [13–15]. Increasing observational studies show that vitamin D status is linked to T2D [16–20], and an increased intake of vitamin D contributes to the prevention of diabetes and metabolic disorder. Obesity is another powerful risk factor for T2D [21]. Waistline is used to assess fat distribution, and an increased waistline has a high correlation with T2D [22–24]. However, there are no prospective studies investigating the effects of proinsulin/insulin, vitamin D3, or waistline as predictors for diabetes.

In this study, we aimed to assess the association of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline with glycometabolism in the Chinese Han population. We also investigated the diagnostic value of these clinical indicators, alone and in combination, in diagnosing prediabetes among the Chinese Han population.

Materials and methods

Between March 2019 and July 2019, 1685 study subjects were recruited from Hainan Provincial People's Hospital, Hainan Province, China. Based on the World Health Organization (WHO) diagnostic criteria for diabetes and the American Diabetes Association (ADA) medical diagnosis and treatment standards for diabetes, exclusion criteria included people who had diabetes, malignant tumors, severe liver and kidney diseases, and metabolic bone diseases. Characteristics of participants were collected from their medical records, including age, sex, body mass index (BMI), waistline, triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), uric acid (UA), vitamin D3, fasting proinsulin, fasting insulin, fasting proinsulin/fasting insulin, proinsulin, insulin, and proinsulin/insulin.

All the statistical analyses were performed using the SPSS 21.0 software (SPSS, Chicago, IL, USA). Study subjects were divided into two groups based on levels of fasting plasma glucose (FPG). The variables were presented as mean ± standard deviation. The chi-square test was used to compare differences in gender between the two groups, and Student's *t* test was performed to determine differences in age and clinical characteristics between the two groups. Spearman's correlation analysis was conducted to explore the relationship between clinical characteristics and FPG. Correlations stratified by gender were also evaluated. In addition, the impacts of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline on prediabetes risk were determined by using univariate and multivariate regression models that were adjusted for age, BMI, waistline, TG, TC, LDL, UA, vitamin D3, fasting proinsulin, fasting insulin, proinsulin, insulin, fasting proinsulin/fasting insulin and proinsulin/insulin. The associations between the clinical indicators and prediabetes in men and women were checked using a univariate regression model that was adjusted for age, BMI, waistline, TG, TC, LDL, UA, vitamin D3, fasting proinsulin, fasting insulin, proinsulin, insulin, fasting proinsulin/fasting insulin, and proinsulin/insulin. Finally, the cut-off points for fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline were calculated using receiver operation characteristic curve (ROC) analysis. The area under the ROC curve (AUC), sensitivity, and specificity in fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline, plus the combination of these factors, were calculated to obtain their diagnostic value in predicting prediabetes. Youden's index was calculated by sensitivity and specificity. All the tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Characteristics of study subjects

The characteristics of study subjects with and without glucose metabolism dysfunction (GMD) are shown in Table 1. A total of 490 cases (mean age 55.42 ± 11.19 years) and 1195 controls (mean age 45.22 ± 13.26 years) were enrolled from Hainan Provincial People's Hospital. There were no differences in the sex distribution and HDL level between the two groups ($p = 0.189$). The following factors were significantly different between the GMD and control groups: age, BMI, waistline, TG, TC, LDL, HDL, UA, vitamin D3, fasting proinsulin, fasting insulin, fasting proinsulin/fasting insulin, proinsulin, insulin, and proinsulin/insulin ($p < 0.001$).

Correlation analysis between clinical characteristics and prediabetes

Spearman's correlation analysis was used to show the relationship between clinical characteristics and prediabetes (Table 2). The results reveal that fasting proinsulin ($r = 0.264$, $p < 0.001$), fasting insulin ($r = 0.239$, $p < 0.001$), fasting proinsulin/fasting insulin ($r = 0.053$, $p = 0.030$), proinsulin ($r = 0.155$, $p < 0.001$), insulin ($r = 0.121$, $p < 0.001$), vitamin D3 ($r = 0.154$, $p < 0.001$), waistline

Table 1 Characteristics of study subjects based on levels of FPG

Variables	Cases (FPG ≥ 5.6 mmol/L)	Controls (FPG < 5.6 mmol/L)	*p* value
Age (years)	55.42 ± 11.19	45.22 ± 13.26	**< 0.001** [a]
Sex (number)			0.189 [b]
Man	191	425	
Woman	299	770	
BMI (kg/m^2)	24.60 ± 3.41	23.41 ± 3.68	**< 0.001** [a]
Waistline (cm)	84.13 ± 11.55	80.01 ± 10.07	**< 0.001** [a]
TG	2.26 ± 2.54	1.78 ± 1.51	**< 0.001** [a]
TC	5.65 ± 1.21	5.33 ± 1.04	**< 0.001** [a]
LDL	3.08 ± 0.90	2.90 ± 0.81	**< 0.001** [a]
HDL	1.48 ± 0.46	1.51 ± 0.40	0.129 [a]
UA	371.73 ± 88.55	351.12 ± 89.25	**< 0.001** [a]
Vitamin D3 (μg)	39.42 ± 10.61	36.97 ± 10.79	**< 0.001** [a]
Fasting proinsulin (mU/l)	19.28 ± 19.19	11.75 ± 9.08	**< 0.001** [a]
Fasting insulin (mU/l)	83.51 ± 73.07	59.86 ± 36.16	**< 0.001** [a]
Fasting proinsulin/fasting insulin	0.23 ± 0.15	0.18 ± 0.06	**< 0.001** [a]
Proinsulin (mU/l)	71.83 ± 57.38	58.37 ± 51.35	**< 0.001** [a]
Insulin (mU/l)	563.01 ± 492.17	469.33 ± 421.40	**< 0.001** [a]
Proinsulin/insulin	0.13 ± 0.09	0.12 ± 0.08	**< 0.001** [a]

FPG fasting plasma glucose, *BMI* body mass index, *TG* triglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *UA* uric acid

The p^a values were obtained by Student's *t* test. The p^b values were calculated by $\chi 2$ test. The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

Table 2 Association analysis between clinical characteristics and prediabetes

Clinical characteristics	*r*	*p*
Fasting proinsulin	0.264	**< 0.001**
Fasting insulin	0.239	**< 0.001**
Fasting proinsulin/fasting insulin	0.053	**0.030**
Proinsulin	0.155	**< 0.001**
Insulin	0.121	**< 0.001**
Proinsulin/insulin	0.036	0.143
Vitamin D3	0.154	**< 0.001**
Waistline	0.247	**< 0.001**
BMI	0.225	**< 0.001**

BMI body mass index

The *r* and *p* values were calculated by Spearman's correlation analysis. The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

Table 3 The Spearman's correlation between clinical characteristics and prediabetes stratified by gender

Clinical characteristics	Men		Women	
	r	*p*	*r*	*p*
Fasting proinsulin	0.247	**< 0.001**	0.267	**< 0.001**
Fasting insulin	0.167	**< 0.001**	0.282	**< 0.001**
Fasting proinsulin/fasting insulin	0.139	**0.001**	− 0.019	0.546
Proinsulin	0.106	**0.009**	0.177	**< 0.001**
Insulin	0.048	0.242	0.166	**< 0.001**
Proinsulin/insulin	0.062	0.132	0.004	0.884
Vitamin D3	0.107	**0.008**	0.174	**< 0.001**
Waistline	0.161	**< 0.001**	0.301	**< 0.001**
BMI	0.135	**0.001**	0.272	**< 0.001**

BMI body mass index

The *r* and *p* value were calculated by Spearman's correlation analysis. The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

($r = 0.247$, $p < 0.001$), and BMI ($r = 0.225$, $p < 0.001$) were positively associated with prediabetes.

Gender-based stratification revealed a correlation of clinical characteristics with prediabetes (Table 3). The following clinical characteristics were significantly associated with prediabetes in men: fasting proinsulin ($r = 0.247$, $p < 0.001$), fasting insulin ($r = 0.167$, $p < 0.001$), fasting proinsulin/fasting insulin ($r = 0.139$, $p = 0.001$), proinsulin ($r = 0.106$, $p = 0.009$), vitamin D3 ($r = 0.107$, $p = 0.008$), waistline ($r = 0.161$, $p < 0.001$), and BMI ($r = 0.135$, $p = 0.001$). In women, fasting proinsulin ($r = 0.267$, $p < 0.001$), fasting insulin ($r = 0.282$, $p < 0.001$), proinsulin

($r=0.177$, $p<0.001$), insulin ($r=0.166$, $p<0.001$), vitamin D3 ($r=0.174$, $p<0.001$), waistline ($r=0.301$, $p<0.001$), and BMI ($r=0.272$, $p<0.001$) were positively related to prediabetes.

Relationship of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline with prediabetes risk

The impacts of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline on prediabetes risk were detected by using univariate and multivariate regression models with adjustments for age, BMI, waistline, TG, TC, LDL, UA, vitamin D3, fasting proinsulin, fasting insulin, proinsulin, insulin, fasting proinsulin/fasting insulin, and proinsulin/insulin. As shown in Table 4, fasting proinsulin/fasting insulin (OR = 1.73, 95% CI = 1.22–2.44, $p=0.002$), vitamin D3 (OR = 1.02, 95% CI = 1.01–1.03, $p<0.001$), and waistline (OR = 1.04, 95% CI = 1.03–1.05, $p<0.001$) were significantly related to an increased risk of prediabetes according to univariate regression analysis results. Multivariate regression analysis results revealed that fasting proinsulin/fasting insulin (OR = 1.69, 95% CI = 1.18–2.41, $p=0.004$), vitamin D3 (OR = 1.01, 95% CI = 1.00–1.02, $p=0.007$), and waistline (OR = 1.04, 95% CI = 1.03–1.05, $p<0.001$) also significantly enhanced prediabetes susceptibility. These data suggest that fasting proinsulin/fasting insulin, vitamin D3, and waistline may be used as risk factors for prediabetes.

We also evaluated the risk factor for prediabetes stratified by gender. Fasting proinsulin/fasting insulin (OR = 2.51, 95% CI = 1.48–4.24, $p=0.001$), vitamin D3 (OR = 1.02, 95% CI = 1.00–1.03, $p=0.043$), and waistline (OR = 1.03, 95% CI = 1.01–1.05, $p=0.006$) had a strong association with prediabetes in men, while in women, vitamin D3 (OR = 1.03, 95% CI = 1.00–1.03, $p<0.001$), and waistline (OR = 1.05, 95% CI = 1.01–1.05, $p<0.001$) were strongly related to the risk of prediabetes (Table 5).

Effects of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline on diagnosing prediabetes

The diagnostic value of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline and their combination in prediabetes diagnosing were analyzed using an ROC curve. As shown in Table 6 and Figs. 1, 2, 3, 4, and 5, the AUC of the combination of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline was 0.936 ($p<0.001$, 95% CI = 0.778–0.909), and the sensitivity, specificity, and Youden's index were 100%, 85.4%, and 0.854%, respectively.

Interestingly, the AUC and sensitivity in the combination of the indicators were larger than the diagnostic value of each indicator alone.

The diagnostic value data assessed using gender stratification analyses are summarized in Table 7. In men, the combination of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline was the best model to diagnose prediabetes (AUC = 0.669, $p<0.001$, sensitivity = 74.0%, sensitivity = 47.9%, Youden's index = 0.218). In women, the diagnostic value of waistline was better than other factors in diagnosing prediabetes

Table 4 The effects of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline on prediabetes by logistic regression analysis

Mode	Clinical factors	*B*	S.E	Wald	*P*	OR	95% CI
Univariate analysis	Fasting proinsulin/fasting insulin	0.546	0.175	9.687	**0.002**	1.73	1.22–2.44
	Proinsulin/insulin	0.253	0.209	1.468	0.226	1.29	0.86–1.94
	Vitamin D3	0.021	0.005	17.57	**< 0.001**	1.02	1.01–1.03
	Waistline	0.039	0.006	47.56	**< 0.001**	1.04	1.03–1.05
Multivariate analysis	Fasting proinsulin/fasting insulin	0.525	0.182	8.360	**0.004**	1.69	1.18–2.41
	Proinsulin/insulin	0.096	0.102	0.880	0.348	1.10	0.90–1.34
	Vitamin D3	0.014	0.005	7.219	**0.007**	1.01	1.00–1.02
	Waistline	0.037	0.006	40.065	**< 0.001**	1.04	1.03–1.05

OR odds ratio, *CI* confidence interval, *S.E* standard error

The *p* values were calculated by univariate logistic regression analysis after adjustment for age, body mass index, waistline, triglyceride, total cholesterol, low-density lipoprotein, uric acid, vitamin D3, fasting proinsulin, fasting insulin, proinsulin, insulin, fasting proinsulin/fasting insulin, and proinsulin/insulin. The $p<0.05$ indicates statistical significance. Numbers in bold mean statistical significance

Table 5 The risk factors for prediabetes stratified by gender

Characteristics	*B*	S.E	Wald	*p*	OR	95% CI
Men						
Fasting proinsulin/fasting insulin	0.919	0.268	11.750	**0.001**	2.51	1.48–4.24
Proinsulin/insulin	0.232	0.244	0.903	0.342	1.26	0.78–2.03
Vitamin D3	0.015	0.007	4.081	**0.043**	1.02	1.00–1.03
Waistline	0.027	0.010	7.672	**0.006**	1.03	1.01–1.05
Women						
Fasting proinsulin/fasting insulin	0.262	0.218	1.441	0.230	1.30	0.85–1.99
Proinsulin/insulin	0.200	0.401	0.247	0.619	1.22	0.56–2.68
Vitamin D3	0.031	0.008	14.084	**< 0.001**	1.03	1.01–1.05
Waistline	0.052	0.008	44.268	**< 0.001**	1.05	1.04–1.07

OR odds ratio, *CI* confidence interval, *S.E* standard error

The *p* values were calculated by univariate logistic regression analysis after adjustment for age, body mass index, waistline, triglyceride, total cholesterol, low-density lipoprotein, uric acid, vitamin D3, fasting proinsulin, fasting insulin, proinsulin, insulin, fasting proinsulin/fasting insulin, and proinsulin/insulin. The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

Table 6 The analysis of the impact of indicators on the diagnosis of prediabetes

Indicators	Cutoff	AUC (95% CI)	*p*	Sensitivity (%)	Specificity (%)	Youden index
Fasting proinsulin/fasting insulin	0.5	0.522 (0.491–0.553)	0.150	9.7	94.8	0.045
Proinsulin/insulin	0.5	0.509 (0.478–0.540)	0.559	5.6	96.3	0.019
Vitamin D3	37.5 μg	0.572 (0.542–0.602)	**< 0.001**	53.9	57.6	0.115
Waistline	80.5 cm	0.624 (0.596–0.653)	**< 0.001**	68.2	51.9	0.201
Fasting proinsulin/fasting insulin + proinsulin/insulin + vitamin D3 + waistline	–	0.936 (0.778–0.909)	**< 0.001**	100	85.4	0.854

AUC area under a curve

The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

(AUC = 0.659, $p < 0.001$, sensitivity = 65.2%, sensitivity = 59.7%, Youden's index = 0.249).

Discussion

In this study, we evaluated the link of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline with prediabetes among the Chinese Han population. Our study showed that fasting proinsulin/fasting insulin, vitamin D3, and waistline were positively associated with prediabetes in men. For women, there was significant association between vitamin D3, waistline, and prediabetes. The logistic regression analysis indicated that fasting proinsulin/fasting insulin, vitamin D3, and waistline could increase the risk of prediabetes in men. Furthermore, vitamin D3 and waistline showed an enhanced risk of prediabetes in women. In addition, it was observed that the combination of fasting proinsulin/fasting insulin, proinsulin/ true insulin, vitamin D3, and waistline had a larger diagnostic value than these indicators alone in diagnosing prediabetes. These results suggest that fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline can be used as diagnostic indicators for prediabetes.

Insulin is secreted by islet β cells as a regulator of blood glucose, which promotes glucose uptake via activating a series of signaling cascades [25]. Insulin resistance and islet β cell dysfunction are the two well-known characteristics of diabetes [26]. In the present study, fasting proinsulin, fasting insulin, and fasting proinsulin/fasting insulin were positively associated with glycometabolism. On the other hand, vitamin D3 and obesity were previously shown to be closely associated with insulin resistance [25, 27]. Vitamin D not only increases the production of some anti-inflammatory cytokines, but also directly increases insulin

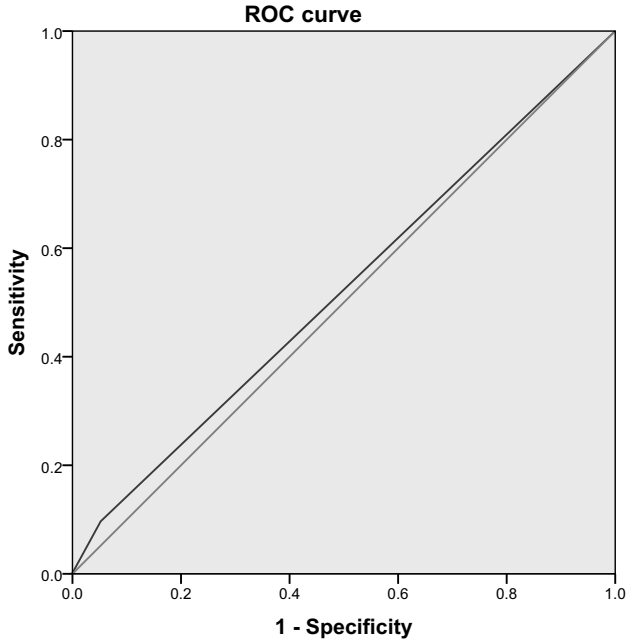

Fig. 1 ROC analysis of fasting proinsulin/fasting insulin for diagnosing prediabetes

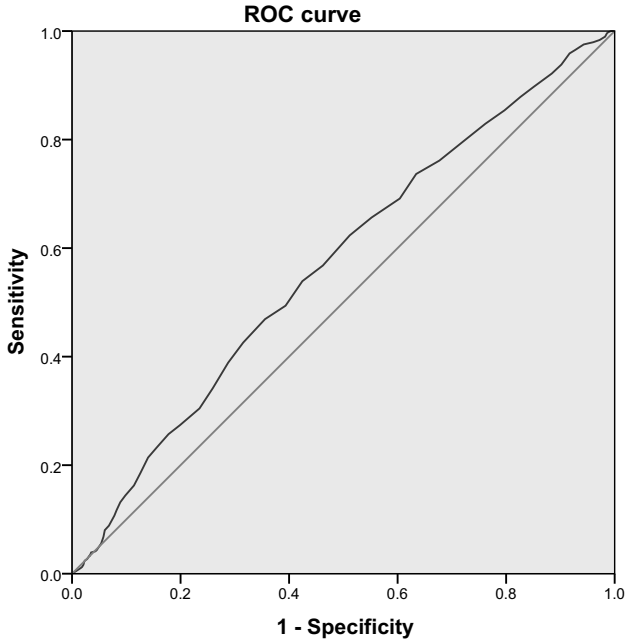

Fig. 3 ROC analysis of vitamin D3 for diagnosing prediabetes

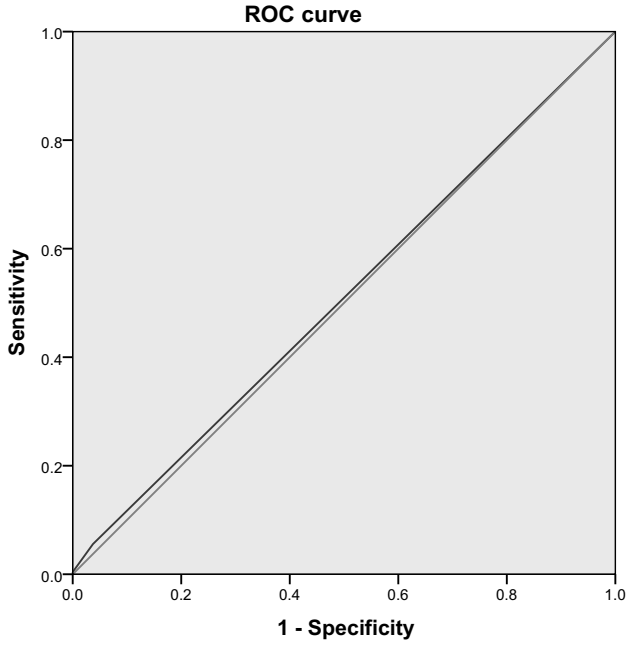

Fig. 2 ROC analysis of proinsulin/insulin for diagnosing prediabetes

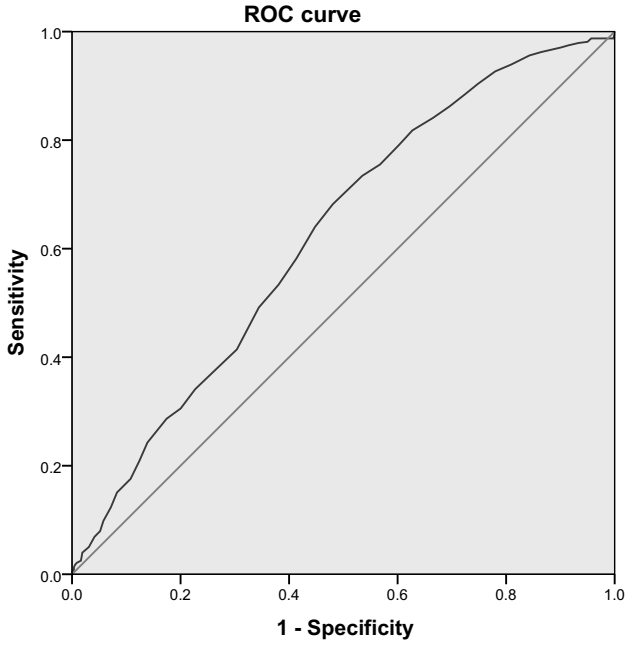

Fig. 4 ROC analysis of waistline for diagnosing prediabetes

sensitivity and secretion. Numerous studies have shown an association between vitamin D3 levels metabolic syndrome and diabetes [28, 29]. Obesity, or excess fat mass, increases the risk of diabetes and many other diseases [30]. Waistline is an important indicator of obesity, and increased proinsulin levels were observed in obese adolescents with normal blood glucose [31]. Consistent with previous studies, we found a significant linkage of waistline with glycometabolism indicators [32, 33].

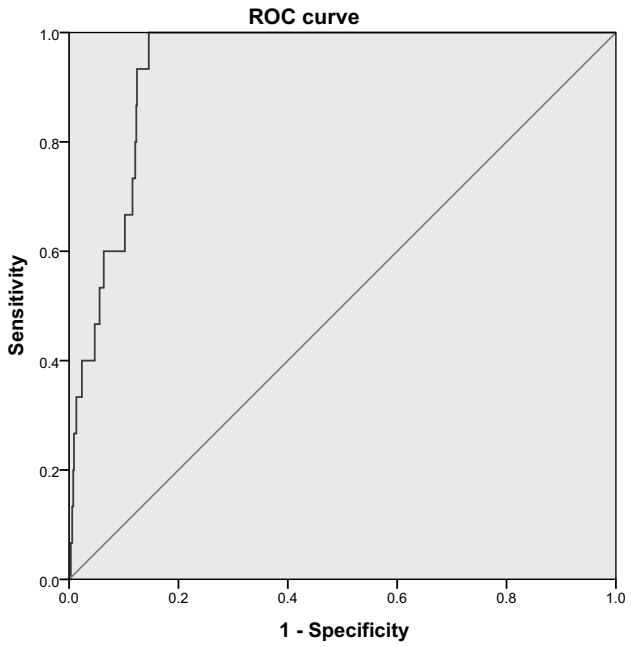

Fig. 5 ROC analysisof the combination of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline for diagnosing prediabetes

The impacts of fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline were investigated in relation to potential roles in diagnosing prediabetes. Based on the ROC curve analysis, the combination of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline has a better diagnostic value than each of those indicators alone in prediabetes diagnosing. This finding suggests that the combination of fasting proinsulin/fasting insulin, proinsulin/insulin, vitamin D3, and waistline could be used as a diagnostic biomarker for prediabetes among the Chinese Han population.

However, we recognize that the study has a number of limitations. First, the overall number of study subjects was relatively small. Second, the ethnicity of the study population was relatively limited. Third, we did not perform stratification analysis due to the limited information we collected. Hence, further well-designed and large population studies should be conducted.

Conclusion

In conclusion, our results indicate that fasting proinsulin/fasting insulin, vitamin D3, and waistline were associated with prediabetes, and which may be used as a potential risk factor for prediabetes. The diagnostic value of the combination including fasting proinsulin/fasting insulin, proinsulin/true insulin, vitamin D3, and waistline was better than these parameters alone in diagnosing prediabetes. Our study provides useful diagnostic indicators for diagnosing prediabetes in the Chinese Han population.

Table 7 The effect of indicators on the diagnosis of prediabetes stratified by gender

	AUC (95% CI)	*p*	Sensitivity (%)	Specificity (%)	Youden index
Men					
Fasting proinsulin/fasting insulin	0.543 (0.492–0.594)	0.089	15.4	93.1	0.086
Proinsulin/insulin	0.519 (0.468–0.570)	0.460	10.5	93.3	0.038
Vitamin D3	0.562 (0.514–0.611)	**0.014**	65.4	47.4	0.128
Waistline	0.565 (0.516–0.613)	**0.012**	83.8	26.3	0.101
Fasting proinsulin/fasting insulin + proinsulin/insulin + vitamin D3 + waistline	0.669 (0.633–0.705)	**< 0.001**	74.0	47.9	0.218
Women					
Fasting proinsulin/fasting insulin	0.509 (0.470–0.548)	0.658	6.0	95.7	0.018
Proinsulin/insulin	0.502 (0.462–0.541)	0.929	2.4	97.9	0.004
Vitamin D3	0.572 (0.534–0.611)	**< 0.001**	51.3	60.9	0.123
Waistline	0.659 (0.624–0.695)	**< 0.001**	65.2	59.7	0.249
Fasting proinsulin/fasting insulin + proinsulin/insulin + vitamin D3 + waistline	0.636 (0.607–0.665)	**< 0.001**	79.9	48.7	0.286

AUC area under a curve

The $p < 0.05$ indicates statistical significance. Numbers in bold mean statistical significance

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Declarations

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

Ethical approval Before the start of the study, all individuals provided written informed consents in compliance with the World Medical Association ethics policy. The study protocol was approved by the Ethics Committee of the Hainan Provincial People's Hospital.

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ORIGINAL ARTICLE

Genetic polymorphisms in ABCA1 (rs2230806 and rs1800977) and LIPC (rs2070895) genes and their association with the risk of type 2 diabetes: a case control study

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Abstract

Background and aim Adenosine triphosphate-binding cassette transporter A1 (*ABCA1*) and hepatic lipase (*LIPC*) genes both play an important role in lipid metabolism. *ABCA1* and *LIPC* gene polymorphism has been reported with conflicting results as genetic risk factor for T2DM in different populations. Due to lack of conclusive data from India, present study was conducted to assess the association of *ABCA1* and *LIPC* gene polymorphisms with T2DM risk in the North Indian population.

Methods Two SNPs (G656A and C69T) in *ABCA1* gene and one SNP (G-250A) in *LIPC* gene were genotyped in total of 270 subjects using PCR–RFLP genotyping method.

Results The polymorphism at position G656A in *ABCA1* and at position G-250A in LIPC gene was significantly associated with development of T2DM ($p=0.016$, $p=0.001$). Significant association could not be observed between C69T variant of ABCA1 gene and T2DM. Regression analysis also showed that *ABCA1* variant G656A and *LIPC* variant G-250A are associated with T2DM risk in adjusted model for clinical/demographic variables ($p=0.000$ and $p=0.034$). The levels of total Chl and LDL-C were significantly higher in T2DM patients having GA + AA genotype of *ABCA1* gene at SNP G656A ($p=0.012$ and $p=0.021$). However, BMI, HOMA-IR, TG, and insulin levels were significantly higher in T2DM patients having GA + AA genotype of LIPC gene at SNP G-250A ($p=0.011$, 0.048, 0.017, 0.045 respectively).

Conclusion It can be concluded that A allele of *ABCA1* variant G656A and *LIPC* variant G-250A might serve as risk factors in the development of T2DM in the North Indian population.

Keywords Type 2 diabetes mellitus · Adenosine triphosphate transporter A1 · Hepatic lipase · Gene polymorphism

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous group of disorders caused by interaction of environmental factors and predisposing genotypes [1, 2]. It is characterized by persistent hyperglycemia; insulin resistance in peripheral tissues like muscles, fat, and liver coupled with effects of aging, obesity, and reduced exercise; and dysregulated insulin secretion by pancreatic beta cells. As per Diabetes Atlas (2019), there will be 745 million people with diabetes worldwide by 2045 [3]. Meta-analysis studies established that polymorphism in cytokine gene RETN serves as a major risk factor for T2DM [4].

ABCA1 gene encodes for a 247 KDa protein consisting of membrane spanning domains and nucleotide binding domain which is a gatekeeper of the reverse cholesterol transport [5]. *ABCA1* plays an important role in insulin secretion by pancreatic β-cells as it is highly expressed in

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pancreatic β-cells and absence of *ABCA1* protein in pancreatic β-cell resulted in accumulation of cellular cholesterol which causes a marked reduction in insulin secretion in vivo and impairment in glucose tolerance, so *ABCA1* gene is linked to diabetes risk [6]. Studies have suggested that cellular cholesterol metabolism in β-cells plays an important role in regulating insulin secretion by β-cells [7–9]. Several studies reported that mutations in *ABCA1* gene leads to non-functional membrane transporter causing a reduced efflux of cholesterol from cells, but with conflicting results [10–12]. Therefore, to clarify the possibility of associations between ABCA1 gene polymorphism and risk of T2DM, two variants G656A and C69T of the *ABCA1* gene which are present in the two major extracellular rings of the *ABCA1* protein and play a significant role in apolipoprotein A-1 (APO-I) and cholesterol efflux were selected. Mutations in these SNPs may affect the level of high-density lipoprotein (HDL-C) and ultimately lead to disease variation [11]. Based on review of literature, most of the studies on ABCA1 gene were conducted on 3 SNPs, viz., R219K (rs2230806), C69T (rs1800977), and R230C (rs9282541) in different populations to determine their genetic associations with T2DM, but studies are not available in Indian population on these SNPs. The SNP R230C (rs9282541) was not studied in the Indian population as it is a very rare variant present in the exon 7 of ABCA1 gene, and furthermore, the meta-analysis using data from four studies of this SNP did not report significant association between R230C polymorphism and risk of T2DM [12].

Human hepatic lipase gene (*LIPC*) is located on chromosome 15q21 and spans over 30 kb of DNA. It is comprised of nine exons and eight introns and encodes a 449 amino acid residue hepatic lipase (HL) [13]. HL regulates the metabolism of low-density lipoproteins (LDL), intermediate-density lipoprotein, and HDL-C particles. It also catalyzes the hydrolysis of triglycerides (TG) and phospholipids [14]. A mutation in SNP G-250A in the promoter region of the LIPC gene has been reported to be involved in modifications of plasma lipid levels [15]. Approximately 20 to 30% of the individual variation in HL activity is due to the presence of common polymorphisms (G-250A and C-514 T) in the promoter region of the *LIPC* gene [16, 17]. Studies are available on the G-250A and C-514 T polymorphism; however, most of them were correlating the polymorphisms with coronary artery disease (CAD) risk and includes patients with prehistory of CAD [17–21]. Many studies have also investigated the effect of SNP G-250A of *LIPC* gene on the risk of T2DM; however, the effect of G-250A in the Indian population has not been investigated yet.

As information regarding C-514 T polymorphism and T2DM risk was not available in the literature when the current study was performed (August 2017–June 2020), so study was not performed on SNP C-514 T.

Therefore, the present study was undertaken to investigate the role of SNPs G656A (rs2230806) and C69T (rs1800977) of *ABCA1* gene and SNP G-250A (rs2070895) of *LIPC* gene on the risk of T2DM in the North Indian population.

Materials and methods

Enrollment of subjects

The present case control study was conducted during August 2017 to July 2020, and a total of 270 unrelated Indian subjects over 45 years of age, including 168 T2DM patients and 102 healthy individuals, were recruited from different hospitals from the State of Haryana. Diabetes was diagnosed according to the Indian Council of Medical Research (ICMR) guidelines, which include fasting blood glucose (FBG) ≥ 126 mg/dl and glycosylated hemoglobin (HbA1c) ≥ 6.5%. Patients with type 1 diabetes mellitus, gestational diabetes and maturity-onset diabetes of the young, cardiovascular disease, and any other major disease were excluded from this study. Before participation, the purpose of this study was precisely explained, and an informed written consent was obtained from all the members who participated in this study. Ethical clearance for the collection of blood samples was given by ethical committee of Kurukshetra University, Kurukshetra, and all the samples were collected by trained medical professional under the supervision of doctor approved by Medical Council of India (MCI) as per ICMR guidelines.

Blood sampling and biochemical analysis

Data regarding age, sex, and age of onset of diabetes mellitus were collected from self-reported questionnaires and as provided by the doctor. Weight, height, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured at the time of enrolment, and body mass index (BMI) was calculated by dividing weight in kilograms (kg) by the height in meters square (m^2). HbA1c and FBG concentrations were determined from blood collected in ethylenediaminetetraacetic acid (EDTA)-coated vials. Serological tests, including total cholesterol (total Chl), triglyceride (TG), and HDL-cholesterol (HDL-C) levels, were performed by means of standard enzymatic method, and plasma insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA). Homeostasis model assessment for insulin resistance (HOMA-IR) score was calculated using the formula: $\text{fasting insulin (IU/ml)} \times \text{glucose (mg/dl)}/405$. Homa-β score was calculated using the formula: $\text{fasting insulin (IU/ml)} \times 360/\text{fasting glucose (mg/dl)} - 63$.

DNA extraction and genotyping in ABCA1 and LIPC genes

Genomic DNA was extracted from peripheral blood leucocytes using a QIAamp DNA Blood Mini Kit (Qiagen, New Delhi, India) according to the manufacturer's instructions. DNA samples were tested for quality and concentration using an ultraviolet spectrophotometer and stored at − 20 °C for further analysis.

Genotyping of *ABCA1* for SNPs G656A and C69T and hepatic lipase gene (*LIPC*) for SNP *G-250A* was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP). The primer sequences and restriction enzyme used for different SNPs are listed in Table 1. PCR reactions were performed in a total volume of 25 µL containing 12.5 µl commercially available PCR mastermix (GoTaq® Green Master Mix, Promega, India), 0.5 µl (15 pmol/µl) each of forward and reverse primers, 40–60 ng genomic DNA, and 9.5 µl sterile nuclease free water. The PCR involved initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50–60 °C for 30 s, extension at 72 °C for 45 s, and final extension at 72 °C for 10 min. 1.5% and 2–3% agarose gels were run to study the PCR-amplified products and restriction-digested products respectively. G to A mutation in SNP G656A creates a restriction site for enzyme *Eco*NI. AA homozygous mutants displayed two bands of 185 bp and 124 bp, while wild-type GG homozygotes display : a single band of 309 bp. For SNP C69T, C to T transition mutation creates a restriction site for *Alw26*I (*Bsm*AI) restriction enzyme, TT homozygotes showed 310 bp band, and the wild-type CC homozygotes yield a 345 bp fragment. For SNP *G-250A* of *LIPC* gene, G to A inversion mutation creates a restriction site for *Dra*I restriction enzyme. Mutated DNA has AA genotype displayed bands of 302 bp and 109 bp whereas wild-type GG homozygotes showed single band of 411 bp.

Statistical analysis

Hardy–Weinberg analysis was performed to compare the genotype frequencies using the Chi square test (χ^2). Student's *t*-test was used to analyze the associations of genotypes with clinical variables and compare the clinical parameters between two groups. Multiple logistic regression analysis was applied to study the association between continuous and categorical variables with T2DM. All the statistical analysis was performed using the SPSS software. To determine the extent of association and pairwise measure of linkage disequilibrium (LD) of the (G656A) and (C69T), Lewontin's coefficient D' and r^2 were calculated. Haplotype analysis was performed using the SHEsis online software. *p* value less than 0.05 was considered statistically significant.

Results

Clinical profiling

The clinical and demographic profiles of the healthy control subjects (n=102) and T2DM patients (n=168) cases has already been published in our previous communications as the samples under reference were common in these studies and the data is reproduced here for the sake of clarity [22–25]. The values of FBG, HbA1c, BMI, total-Chl, LDL-C, fasting insulin, and HOMA-IR were significantly higher in the T2DM group than in the control group with a *p*-value of less than 0.05, while HDL-C levels and HOMA-β were significantly lowered in T2DM patients. However, there was no significant difference between the two groups involving TG and blood pressures (Table 2).

The genotype and allele frequencies of SNPs G656A and C69T of ABCA1 gene

The genotypic frequency of SNP *G656A* was significantly different between the T2DM group and the control group with a $p = 0.010$. Frequency of A allele is significantly higher in T2DM patients as compared to the healthy controls ((47.3% vs. 36.7%), $p = 0.016$) (Table 3). However, the genotype distribution of SNP *G656A* ($p = 0.0029$, $\chi^2 = 8.86$) was not as per HWE among T2DM cases as assessed by the Chi square test.

The genotypic and allelic frequencies of SNP *C69T* between the control and case groups did not show statistically significant differences ($p = 0.344$ and $p = 0.148$

Table 1 The sequences of the forward and reverse primers for different SNPs and restriction enzymes

GENE	SNP	Primer sequence	Product length	Restriction enzyme
ABCA1	G656A	F-5- AAAGACTTCAAG GACCCAGCTT-3 R-5- CCTCACATTCCGAAAGCATTA-3	309 bp	***Eco*NI**
	C69T	F-5- CAGCGCTTCCCGCGCGTCTTA-3 R-5- CCACTCACTCTCGTCCGCAATTAC-3	345 bp	*Alw*26I (*Bsm*AI)
LIPC	G-250A	F-5- GGCAAGGGCATCTTTGCTTC-3 R-5- GGTCGATTTACAGAAGTGCTTC-3	411 bp	*Dra*I

SNP, single nucleotide polymorphism

Table 2 Comparison of demographic data and clinical and biochemical characteristics of studied subjects

Parameters	T2DM Cases	Control Subjects	*p*-value
Sex (M/F)	100/68	57/45	
Age (Yrs)	57.58 ± 8.8	57.16 ± 9.49	NS
BMI (Kg/m^2)	25.45 ± 4.02	24.50 ± 4.29	NS
SBP (mmHg)	133.22 ± 16.52	134 ± 12.6	NS
DBP (mmHg)	88.02 ± 13.79	85.84 ± 9.74	NS
FBG (mg/dl)	183.08 ± 63.15	103.1 ± 10.24	**< 0.000***
HbA1C %	7.50 ± 1.18	5.47 ± 0.45	**< 0.000***
TG (mg/dl)	162.71 ± 37.23	154.0 ± 45.2	NS
HDL-C (mg/dl)	49.46 ± 11.98	55.39 ± 9.05	**< 0.000***
LDL-C (mg/dl)	106.96 ± 41.26	93.81 ± 25.30	**0.001***
Total CHL (mg/dl)	188.80 ± 42.74	179.95 ± 26.88	**0.037***
Insulin (IU/ml)	6.72 ± 5.44	4.96 ± 3.49	**< 0.000***
HOMA-IR index	3.15 ± 3.56	1.28 ± 0.96	**< 0.000***
HOMA- β	24.17 ± 20.24	47.38 ± 37.92	**< 0.000***

Data shown as mean ± standard deviation

*$p < 0.05$ statistically significant

NS, not significant; *BMI*, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *FBG*, fasting blood glucose; *HbA1c%*, glycated hemoglobin; *TG*, triacylglycerides; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein; *HOMA-IR*, homeostasis model assessment for insulin resistance; *HOMA-β*, homeostasis model assessment for assessing beta cell function

respectively) (Table 3). In addition, no significant differences were observed under any genetic models, viz., additive, dominant, recessive, and heterozygous genetic models. LD between two loci (G656A and C69T) could not be observed, as assessed through D' and r^2 in both control and T2DM subjects ($D' = 0.042$, $r^2 = 0.002$ and $D' = 0.058$, $r^2 = 0.003$ respectively) (data not tabulated). Four haplotypes were derived from the two SNPs, and no haplotype was found to be significantly associated with the increased risk of T2DM (Table 3).

The genotype and allele frequencies of SNP G-250A of LIPC gene

Distribution of genotypes and frequency of A allele differ significantly between controls and T2DM cases ($p = 0.001$ and $p < 0.005$ respectively). There was a significant association of SNP *G-250A* and T2DM (Table 4) in additive models ($p = 0.031$, $p = 0.001$), dominant model ($p < 0.001$), and heterozygous model ($p = 0.005$), but recessive model does not show significant association.

Genotypic comparison of ABCA1 and LIPC gene variants with clinical parameters

A significant association of genotypes of SNP *G656A* with serum LDL-C and total-Chl was observed as T2DM cases with GA + AA genotype had higher total-Chl ($p = 0.021$) and LDL-C ($p = 0.009$) concentrations compared to GG genotype. In the control group, significant association of biochemical parameters between GG and GA + AA genotypes was not observed (Table 5). Significant association of genotypes of SNP *C69T* polymorphism with clinical parameters in both T2DM and control group could not be observed (Table 6).

The levels of BMI, TG, HOMA-IR, and insulin were significantly higher in GA + AA genotypes as compared to GG genotype in T2DM cases with *p* value of 0.011, 0.017, 0.048, and 0.045 respectively whereas levels of HDL-C decreased in GA + AA genotypes in T2DM cases, yet it was statistically significant with *p* value of 0.019, while no significant difference is observed in GG vs GA + AA genotypes with the clinical parameters in the control group (Table 7).

Regression analysis showed significant association of SNP G656A of *ABCA1* gene and G-250A of *LIPC* gene with T2DM risk in unadjusted model ($p = 0.000$ and $p = 0.006$) and in adjusted model for clinical and demographic variables ($p = 0.000$ and $p = 0.034$) (Table 8).

Discussion

Earlier studies evaluated the relationship between plasma HDL-C levels and G656A polymorphism of *ABCA1* gene in patients having prehistory of CAD/underwent coronary angiography/cerebral infraction [26–32], and a few studies are available involving G656A polymorphism related to T2DM patients [33, 34]. G656A is a non-synonymous polymorphism located in exon 7 of ABCA1 gene, and substitution of G to A (AGG → AAG) causes substitution of lysine in place of arginine (R219K) in the transmembrane domain 1 (TMD1) of ABCA1 transporter protein and influences the protein structure and stability [6]. ABCA1 transporter is highly expressed in the mice pancreatic β-cells, and lack of ABCA1 transporter in beta cells decreases insulin secretion and cellular cholesterol accumulation, leading to a progressive impairment in glucose tolerance. Rosiglitazone poses a therapeutic effect in the mice with T2DM through ABCA1 upregulation and improving beta cell function [6], and G656A polymorphism was reported to be associated with response to rosiglitazone antidiabetic drug which is an insulin sensitizer [35]. Significant association between G656A polymorphism and T2DM has been observed ($p = 0.010$), and genotypic results are in consistent with the findings of Haghvirdizadeh et al. in the Malaysian population [10]; in

Table 3 Allelic frequency and genetic distribution of SNPs (G656A and C69T) of *ABCA1* gene in T2DM and control subjects

SNP	*T2DM Cases*	*Control subjects*	χ^2	OR (CI 95%)	α	*p*-value
(G656A) genotypes						
GG	37 (0.22)	40 (39.2%)				
GA	103 (0.613)	49 (0.48)				
AA	28 (0.166)	13 (0.127)	9.20			**0.010***
Allelic (G vs A)			5.76	1.54 (1.08–2.20)	0.05	**0.016***
Additive						
(GG vs GA)			8.36	2.27 (1.29–3.98)		**0.003***
(GG vs AA)			4.43	2.32 (1.05–5.15)		**0.035***
Dominant (GG vs GA + AA)			9.20	2.28 (1.33–3.91)		**0.002***
Recessive (AA vs GG + GA)			0.76	0.73 (0.35–1.43)		0.384
Heterozygous (GA vs GG + AA)			4.54	0.58 (0.35–0.95)		**0.033***
(C69T) genotypes CC	81 (0.482)	40 (0.392)				
CT	66 (0.392)	46 (0.45)				
TT	21 (0.125)	16 (0.156)	2.12			0.344
Alleic (C vs T)			2.09	0.76 (0.53–1.10)	0.05	0.148
Additive						
(CC vs CT)			1.60	0.70 (0.41–1.20)	0.05	0.205
(CC vs TT)			1.28	0.64 (0.30–1.37)		0.257
Dominant (CC vs CA + TT)			2.08	0.69 (0.42–1.42)		0.149
Recessive (AA vs CT + CA)			0.54	1.30 (0.64–2.62)		0.462
Heterozygous (CT vs CC + TT)			0.88	1.26 (0.77–2.08)		0.348
Haplotype analysis						
AC	59.48 (0.177)	39.20 (0.192)	0.19	0.904 (0.57–1.41)	0.012	0.658
AT	33.52 (0.100)	26.80 (0.131)	1.27	0.733 (0.42–1.25)		0.258
GC	168.52 (0.502)	87.80 (0.430)	2.57	1.332 (0.93–1.89)		0.108
GT	74.48 (0.222)	50.20 (0.246)	0.42	0.872 (0.57–1.31)		0.513

α, threshold for significance after applying Bonferroni's correction where required; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval

*Statistically significant findings

addition, significant association between *G656A* genotype and LDL-C ($p = 0.009$) and total-Chl levels ($p = 0.021$) was also observed in our study.

C69T polymorphism is present close to the regulatory elements of the proximal promoter region of ABCA1 gene, and C69T variant plays a regulatory role in the transcription

Table 4 Genotypic and allelic frequency of G-250A polymorphism in LIPC gene

SNP	T2DM Cases	Control subjects	χ^2		OR (CI 95%)	α	*p*-value
(G-250A) genotypes							
GG	80 (0.476)	71 (0.696)					
GA	71 (0.422)	26 (0.254)					
AA	17 (0.101)	5 (0.49)	12.57			0.05	**0.001***
Allelic (G vs A)			32.47		3.21 (2.12–4.85)	0.05	**0.001***
Additive							
(GG vs GA)				10.14	2.42 (1.39–4.20)	0.05	**0.001***
(GG vs AA)			4.60		3.01 (1.05–8.59)		**0.031***
Dominant (GG vs GA + AA)			12.45		2.51 (1.49–4.23)	0.05	**0.001***
Recessive (AA vs GG + GA)				2.31	0.45 (0.16–1.28)		0.128
Heterozygous (GA vs GG + AA)			7.76		2.13 (1.24–3.67)		**0.005***

SNP, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval

*$p < 0.05$ statistically significant

Table 5 Statistical analysis of clinical parameters in association with genotype distribution of SNP G656 of ABCA1 gene

Parameters	T2DM Cases			Control Subjects		
	GG	GA + AA	*p*-value	GG	GA + AA	*p*-value
BMI (Kg/m^2)	24.48 ± 3.53	25.72 ± 4.12	0.071	25.02 ± 4.47	24.16 ± 4.16	0.332
SBP (mm Hg)	136.78 ± 13.73	132.22 ± 17.13	0.094	132.62 ± 10.96	134.09 ± 13.58	0.532
DBP (mm Hg)	92.48 ± 14.57	86.77 ± 13.36	**0.033***	84.90 ± 7.97	86.45 ± 10.75	0.406
FBG (mg/dl)	182.05 ± 76.31	183.38 ± 59.24	0.922	104.43 ± 9.22	102.37 ± 10.96	0.309
HbA1c %	7.37 ± 1.28	7.54 ± 1.15	0.467	5.45 ± 0.37	5.48 ± 0.49	0.726
TG (mg/dl)	157.24 ± 42.67	164.25 ± 35.57	0.362	148.85 ± 36.18	156.88 ± 50.24	0.350
HDL-C (mg/dl)	47.15 ± 11.88	50.11 ± 11.97	0.183	56.79 ± 8.87	54.49 ± 9.13	0.209
LDL-C (mg/dl)	99.09 ± 28.21	108.97 ± 44.14	**0.009***	92.79 ± 22.05	94.47 ± 27.34	0.733
Total ChL (mg/dl)	177.69 ± 28.22	191.94 ± 45.77	**0.021***	179.35 ± 23.95	180.34 ± 28.80	0.851
HOMA- IR index	3.23 ± 3.4	3.38 ± 3.94	0.822	1.25 ± 1.09	1.18 ± 0.85	0.768
Insulin (IU/ml)	7.05 ± 4.90	7.17 ± 6.39	0.902	4.71 ± 3.93	4.55 ± 3.02	0.827
HOMA-β	29.59 ± 25.95	24.70 ± 21.60	0.296	36.78 ± 29.23	46.22 ± 40.97	0.178

Data shown as mean ± standard deviation

*$p < 0.05$ statistically significant

BMI, body mass index; *FBP*, fasting blood glucose; *HbA1c%*, glycated hemoglobin; *TG*, triacylglycerides; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein; *HOMA-IR*, homeostasis model assessment for insulin resistance; *HOMA-β*, homeostasis model assessment for assessing beta cell function

Table 6 Statistical analysis of clinical parameters in association with genotype distribution of SNP C69T of ABCA1 gene

Parameters	T2DM Cases			Control subjects		
	CC	CT + TT	*p*-value	CC	CT + TT	*p*-value
BMI (Kg/m^2)	25.14 ± 4.07	25.74 ± 3.98	0.335	25.63 ± 4.60	23.91 ± 3.92	0.053
SBP (mm/Hg)	132.62 ± 17.51	133.78 ± 15.61	0.651	133.5 ± 12.24	133.66 ± 12.79	0.949
DBP (mm/Hg)	87.74 ± 14.19	88.29 ± 13.49	0.797	85 ± 9.22	86.355 ± 10.12	0.488
FBG (mg/dl)	188.03 ± 67.56	194 ± 58.76	0.543	105.08 ± 9.26	102.4 ± 10.91	0.186
HbA1c %	7.53 ± 1.19	7.47 ± 1.17	0.742	5.52 ± 0.48	5.45 ± 0.42	0.452
TG (mg/dl)	161.79 ± 33.44	163.56 ± 40.61	0.757	155.81 ± 39.34	152.82 ± 49.15	0.735
HDL-C (mg/dl)	49.25 ± 11.09	49.66 ± 12.81	0.824	54.95 ± 8.05	55.55 ± 9.83	0.737
LDL-C (mg/dl)	103.15 ± 40.25	110.66 ± 41.96	0.268	88.75 ± 17.93	97.13 ± 28.73	0.072
Total ChL (mg/dl)	184.76 ± 43.16	192.56 ± 42.49	0.239	174.87 ± 18.49	183.25 ± 30.82	0.089
HOMA- IR index	3.73 ± 4.46	2.78 ± 2.72	0.100	1.32 ± 1.05	1.18 ± 0.84	0.479
Insulin (IU/ml)	7.58 ± 5.84	7.02 ± 6.53	0.558	5.01 ± 3.83	4.36 ± 3.10	0.370
HOMA-β	26.50 ± 22.22	26.05 ± 23.79	0.899	42.91 ± 31.28	44.39 ± 40.48	0.836

Data shown as mean ± standard deviation

*$p < 0.05$ statistically significant

BMI, body mass index; *FBP*, fasting blood glucose; *HbA1c%*, glycated hemoglobin; *TG*, triacylglycerides; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein; *HOMA-IR*, homeostasis model assessment for insulin resistance; *HOMA-β*, homeostasis model assessment for assessing beta cell function

activity of ABCA1 and consequent lipid translocation, alone or in combination with other polymorphisms in the same gene or other related genes [35]. A possible association between C69T variant and susceptibility to T2DM has been reported in different populations. Ergen et al. [34] in Turkish T2DM patients observed that the T allele and TT genotype of the C69T polymorphism in *ABCA1* gene was associated with a reduced risk of T2DM and may serve as protective factor against T2DM; however, significant association between genotype and lipid level could not be observed [35], and similar results were obtained in Saudi population [36]. Haghvirdizadeh et al. reported that *C69T* polymorphism in *ABCA1* gene may increase the T2DM risk in Malaysian population; however, the clinical and biochemical characteristics of this polymorphism did not reveal any significant differences between the case and control groups [10]. Yao et al. and Hasan et al. did not report significant association between *C69T* polymorphism and T2DM risk in Uyghur population of China [37] and Bangladeshi population [38] respectively. Li and Fan observed an association of *ABCA1* C69T polymorphism with T2DM in a Chinese Han population, and T allele served as a protecting factor for T2DM

Table 7 Statistical analysis of clinical parameters in association with genotype distribution of SNP G-250A of LIPC gene

Parameters	T2DM Cases			Control Subjects		
	GG	GA + AA	*p*-value	GG	GA + AA	*p*-value
BMI (Kg/m^2)	24.59 ± 3.94	26.15 ± 3.95	**0.011***	24.49 ± 4.40	24.52 ± 4.09	0.973
SBP (mm/Hg)	131.91 ± 17.26	135.64 ± 16.46	0.154	133.27 ± 12.77	134.09 ± 12.53	0.763
DBP (mm/Hg)	86.6 ± 13.83	85 ± 14.74	0.468	85.68 ± 9.95	86.06 ± 9.08	0.762
FBG (mg/dl)	184.33 ± 66.30	183.09 ± 62.09	0.900	103.25 ± 9.99	104.55 ± 11.34	0.582
HbA1c %	7.62 ± 1.20	7.40 ± 1.15	0.227	5.45 ± 0.42	5.52 ± 0.52	0.509
TG (mg/dl)	156.01 ± 37.18	170.19 ± 39.62	**0.017***	151.86 ± 39.32	154.87 ± 57.67	0.488
HDL-C (mg/dl)	51.66 ± 12.25	47.33 ± 11.53	**0.019***	55.66 ± 10.20	57.82 ± 10.93	0.351
LDL-C (mg/dl)	105.75 ± 40.22	108.59 ± 47.26	0.674	81.51 ± 21.16	80.43 ± 17.83	0.791
Total Chl (mg/dl)	188.62 ± 45.44	190.02 ± 46.54	0.843	167.54 ± 23.43	169.22 ± 19.59	0.708
HOMA- IR index	2.6 ± 2.66	3.7 ± 4.31	**0.048***	1.33 ± 1.04	1.23 ± 0.70	0.571
Insulin (IU/ml)	5.94 ± 4.08	7.63 ± 6.62	**0.045***	4.94 ± 3.63	5.00 ± 3.20	0.933
HOMA β	22.73 ± 19.96	25.47 ± 20.41	0.386	47.38 ± 33.33	45.54 ± 46.18	0.841

Data shown as mean ± standard deviation

*$p < 0.05$ statistically significant

BMI, body mass index; *FBP*, fasting blood glucose; *HbA1c%*, glycated hemoglobin; *TG*, triacylglycerides; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein; *HOMA-IR*, homeostasis model assessment for insulin resistance; *HOMA-β*, homeostasis model assessment for assessing beta cell function

Table 8 Logistic regression analysis of polymorphism of SNPs G656A, C69T of ABCA1 gene, and G-250A of LIPC gene by using T2DM as dependent variable and SNPs as independent variables

SNPs	*p*-value[a]	OR (95% CI)	*p*-value[b]	OR (95% CI)
G656A	**0.000**	**1.24** (0.72–2.12)	**0.000**	6.71 (3.49–12.8)
C69T	0.432	5.36 (3.06–9.38)	0.556	0.82 (0.43–1.56)
G-250A	**0.006**	2.14 (1.24–3.67)	**0.034**	1.95 (1.05–3.63)

OR, odd ratio; *CI*, confidence interval

*$p < 0.05$ statistically significant

[a]*p*-value, unadjusted

[b]*p*-value, adjusted for demographic variables (age and sex) and clinical variables (HDL-C and HOMA-IR)

[39]. But, Du et al. observed that *C69T* polymorphism confers increased risk of T2DM in the Chinese Han Population [40]. Significant association could not be observed between *C69T* polymorphism and T2DM in different genetic models, genotypes, and clinical characteristics in our study.

G-250A polymorphism is present in the promoter region of LIPC gene, and a substitution in the promoter region has been reported to be related to altering the insulin sensitivity [41]. Todorova et al. observed that polymorphism at SNP *G-250A* of *LIPC* gene is a major risk factor for T2DM ($p = 0.032$) in Finnish population [42]. Zacharova et al. predicted that the G-250A polymorphism of *LIPC* gene is involved in conversion of person from impaired glucose tolerance (IGT) to T2DM and suggested that low hepatic lipase (HL) activity plays an important role in the development of insulin resistance and T2DM [43]. Grarup et al. noted elevated fasting HDL-C levels as possible mechanisms for increased T2D risk [44], and similar were the observations of Zhao et al. and Ou et al. in Chinese population [45, 46]. The frequency of A allele was also significantly higher in T2DM patients ($p = 0.001$) in the present study. Therefore, AA genotypes might be a predisposing factor in development of T2DM. Serum TG levels ($p = 0.017$), insulin levels ($p = 0.045$), and BMI ($p = 0.011$) have been found to be significantly higher in T2DM patients with GA + AA genotype as compared to GG genotype, while HDL-C level ($p = 0.019$) was significantly lowered in T2DM patients with GA + AA genotypes.

Conclusion

It can be concluded from the present study that *ABCA1* variant G656A might be a predisposing factor in the development of T2DM. In addition, it was also observed that HDL-C level was statistically lowered in T2DM patients having *LIPC* variants (GA + AA) of G-250A, and A allele of G-250A may increase susceptibility to T2DM in the North Indian population. Due to low power of sample size, more studies with larger size are still required to confirm the findings.

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Author contribution All authors contributed to this work significantly, and all authors are in agreement with the content of the manuscript.

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Declarations

Ethics approval and consent to participate The research work was approved by the Ethical committee of Kurukshetra University, Kurukshetra, and all the subjects who participated in the study had signed the informed consent form.

Competing interest The authors declare no competing interest.

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ORIGINAL ARTICLE

Comparative analysis of the transcriptome of T2DM Bama mini-pigs with T2DM patients

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Abstract

Objective To compare the transcriptional differences between the T2DM-susceptible and T2DM-tolerant Bama mini-pigs and to determine the utility of Bama mini-pigs as an animal model for T2DM by comparing the transcriptomes of the animals with T2DM patients.

Methods The skeletal muscle transcriptomes of healthy Guangxi Bama mini-pigs (CON), T2DM pigs (T2D), and non-T2DM pigs (NT2D) were determined by RNA sequencing.

Results A total of 290 differentially expressed genes (DEGs) were detected in NT2D relative to the CON groups, 572 DEGs in T2D compared to CON, and 300 DEGs in T2D compared to NT2D. The RNA-seq data was verified by qPCR analysis of *PGC1α*, *NR4A3*, *CSRP3*, *MYH7*, *MYH2*, *MYH1*, *GLUT4*, *PPARγ*, *LEP*, *ATP5H*, *UCP2*, and *MTOR* transcripts. The DEGs in T2D Bama mini-pigs were mainly involved in signaling pathways associated with metabolism, cardiovascular diseases, infectious disease, cancer, inflammation and immune responses, cytoskeleton, and signal transduction. Comparative analysis of the transcriptomes of T2DM Bama mini-pigs and T2DM patients (GSE29221 dataset) revealed 17 differentially co-expressed genes that were assigned to 11 GO terms and 6 annotated pathways, including hypertrophic cardiomyopathy, dilated cardiomyopathy, thyroid cancer, cardiac muscle contraction, tight junction, and calcium signaling pathway, suggesting that the T2D Bama mini-pigs could be used on T2DM research in those pathways.

Conclusion This study represents the first RNAseq transcriptome analysis in T2D Bama mini-pigs and provided a new perspective for the study on the human T2DM pathogenesis by using the T2DM model of Bama mini-pigs.

Keywords T2DM · Bama mini-pigs · Humans · Transcriptome · Skeletal muscle

Introduction

Type 2 diabetes mellitus (T2DM) accounts for ~ 90% of all diabetes cases and is characterized by insulin resistance. Due to the complexity of clinical samples, appropriate animal models are needed to elucidate the pathogenesis, complications, and the underlying genetic/environmental factors of T2DM [1]. To date, several transgenic and food- or drug-induced primate, feline and swine models of T2DM have been established [2–6].

Compared to non-human primates and rodents, the miniature pig shares several physiological similarities with humans, along with comparable organ size. In addition, it is easy to breed and handle and therefore a suitable animal model for preclinical studies [7]. Xenotransplantation of porcine organs has gained considerable attention in recent years to obviate the chronic shortage of suitable donors. To this end, Yang et al. [8] integrated CRISPR-Cas into the pig cell genome to prevent transmission of the porcine endogenous retrovirus (PERV) into human cells. In another study, Yan et al. [9] established a knock-in pig model of Huntington's disease (HD) that endogenously expresses full-length

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mutant huntingtin (HTT), using CRISPR/Cas9 and somatic nuclear transfer technology, and demonstrated for the first time that overt and selective neurodegeneration seen in HD patients can be simulated in large mammals. Furthermore, a pig model of cardiac instability has been established to investigate the regenerative potential of hESC and hESC-CM transplantation [10].

Miniature pigs are also highly suitable for simulating diabetes due to similar gastrointestinal structure and function, pancreatic morphology, overall metabolic status [11], and response to high-fat and high-energy diets between humans and pigs [12]. The Guangxi Bama mini-pig [13] has the advantages of small size, short growth cycle, adaptability, stable gene expression, and strong resistance to diseases, and is therefore suitable for T2DM research [14–16]. In a previous study, we developed the T2DM-susceptible (T2DM pigs) and -tolerant (non-T2DM pigs) Bama mini-pig models induced by a high-fat, high-sugar diet, and observed decreased glucose tolerance and significant changes in serum glucose and insulin levels in the T2DM pigs compared with non-T2DM pigs [17]. However, its genetic background has not been completely elucidated, and the transcriptional differences between the T2DM-susceptible and T2DM-tolerant pigs are not well documented. Therefore, we analyzed the skeletal muscle transcriptome of Bama mini-pigs with high-fat, high-sugar (HFHS) diet-induced diabetes and compared it to that of T2DM patients.

Materials and methods

Sample preparation

Healthy (CON), T2DM-susceptible (T2D), and T2DM-tolerant pigs (NT2D) Bama mini-pigs (3 animals per group) were obtained from the Bama miniature pig breeding center of Guangxi University. The relevant data of the T2D and N2TD models have been published elsewhere [17]. The skeletal muscle tissues were dissected and snap-frozen in liquid nitrogen, and stored at – 80 °C till further processing. All protocols were in accordance with the Guide for the Institutional Animal Care and Welfare Committee of the College of Animal Science and Technology, Guangxi University (Guangxi, China, permit no. GXU2013002).

Transcriptome sequencing and bioinformatics analysis

Transcriptome sequencing and bioinformatics analysis were performed by BGI Company (Shenzhen, China). After adjusting the data by Benjamini and Hochberg's approach for controlling the false discovery rate (FDR), the differentially expressed genes (DEGs) were identified using threshold values of $|\log_2 FC| > 1$ and FDR < 0.001. The DEGs were then functionally annotated on the basis of GO and KEGG databases.

Comparative transcriptome analysis

The transcriptome data of T2D Bama mini-pigs was selected from the dataset between T2D and NT2D groups. And, the GSE29221 skeletal muscle transcriptome dataset of T2DM patients was retrieved from the GEO (Gene Expression Omnibus) database of NCBI (National Center for Biotechnology Information Support Center). After transforming the gene symbols using the DAVID online software, the DEGs were screened with threshold values of $|\log_2 FC| > 1$ and $p < 0.05$. The transcriptome data of the T2D Bama mini-pigs and T2DM patients was compared and annotated using the Venn and DAVID programs.

Validation of RNAseq by qPCR

Total RNA was extracted from CON, T2D, and NT2D tissues using TransZol Up (TransGen, China) and reverse transcribed to cDNA by Trans Script All-in-one First-Strand cDNA Synthesis Super Mix for qPCR kit (TransGen, China) according to the manufacturer's instructions. The cDNA was amplified using specific primers (Table S1, Supplementary) and SYBR Premix Ex Taq II (TaKaRa, Japan). The relative gene expression levels were analyzed by the $2^{-\Delta\Delta Ct}$ method with 18 s rRNA as the internal control. Three biological replicates were tested per group, and each sample was tested in triplicate.

Results

Sequencing analysis and qPCR validation

Over 45.2 million raw reads per sample were generated, including more than 44.48 million clean reads (over 98.3%) (Table 1), of which 95.5% had quality scores greater than 20 (Q20) and 89.8% had quality scores greater than 30 (Q30). The expression levels of *PGC1α*, *NR4A3*, *CSRP3*, *MYH7*, *MYH2*, *MYH1*, *GLUT4*, *PPARγ*, *LEP*, *ATP5H*, *UCP2*, and *MTOR* analyzed by qPCR were almost consistent with the RNA-seq analysis (Fig. 1).

Skeletal muscle transcriptome analysis of T2DM Bama mini-pigs

(1) Identification of the DEGs

Using |log2FC|> 1 and FDR < 0.001 as the thresholds (Fig. 2), 572 DEGs were identified in the T2D group relative to the CON group, including 523 upregulated

Table 1 Statistical analysis of sequencing data

	CON group		T2D group		NT2D group	
	Raw data	Clean data	Raw data	Clean data	Raw data	Clean data
Number of Reads	45,244,160	44,534,896	45,245,188	44,489,220	45,242,306	44,570,534
GC (%)	57.605	57.75	56.71	56.845	56.68	56.81
Q20 (%)	96.36	96.535	96.29	96.48	95.505	95.665
Q30 (%)	90.765	90.985	90.645	90.88	89.805	89.98
N (%)	0.03	-	0.03	-	0.04	-
Low qual (%)	0	-	0	-	0	-
Adapter (%)	1.54	-	1.64	-	1.44	-
Clean reads (%)	98.43		98.33		98.52	

and 49 downregulated genes. Likewise, 290 DEGs (213 upregulated and 77 downregulated) were identified between NT2D and CON, and 300 DEGs (247 upregulated and 53 downregulated) between the T2D and NT2D groups.

(2) Pathway enrichment analysis for DEGs in T2D and CON

The biological importance of the DEGs was further determined by KEGG analysis. A total of 216 KEGG pathways were associated with 481 DEGs between T2D and CON, of which 25 pathways were significantly enriched ($p < 0.05$) (Fig. 3a). Nine of these pathways, including cardiac muscle contraction, vascular smooth muscle contraction, dilated cardiomyopathy, hypertrophic cardiomyopathy (HCM), *Salmonella* infection, HTLV-I infection, tight junction, Fc gamma R-mediated phagocytosis, and B cell receptor signaling pathways, are closely related to immunity and inflammation. Ten pathways were related to metabolism, including fructose and mannose metabolism, glycolysis/gluconeogenesis, fatty acid biosynthesis, pentose phosphate pathway, metabolic pathways, glycerophospholipid metabolism, galactose metabolism, lysine degradation, cysteine and methionine metabolism, and protein processing in the endoplasmic reticulum. The remaining pathways were related to gene modification and cell growth, such as spliceosome, mismatch repair, base excision repair, mRNA surveillance pathway, cell cycle, and progesterone-mediated oocyte maturation.

(3) Pathway enrichment analysis for DEGs in T2D and NT2D

Functional annotation of the 300 DEGs between T2D and NT2D revealed that 257 DEGs were significantly enriched in 14 KEGG pathways ($p < 0.05$), including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, cardiac muscle contraction, vascular smooth muscle contraction, viral myocarditis, *Salmonella* infection, prion diseases, thyroid cancer, tight junction, adherent junction, B cell receptor signaling pathway, calcium signaling pathway, regulation of actin cytoskeleton, and osteoclast differentiation (Fig. 3b). These pathways are associated with cardiovascular diseases, infectious diseases, cancer, inflammation, immune responses, cytoskeleton, and signal transduction.

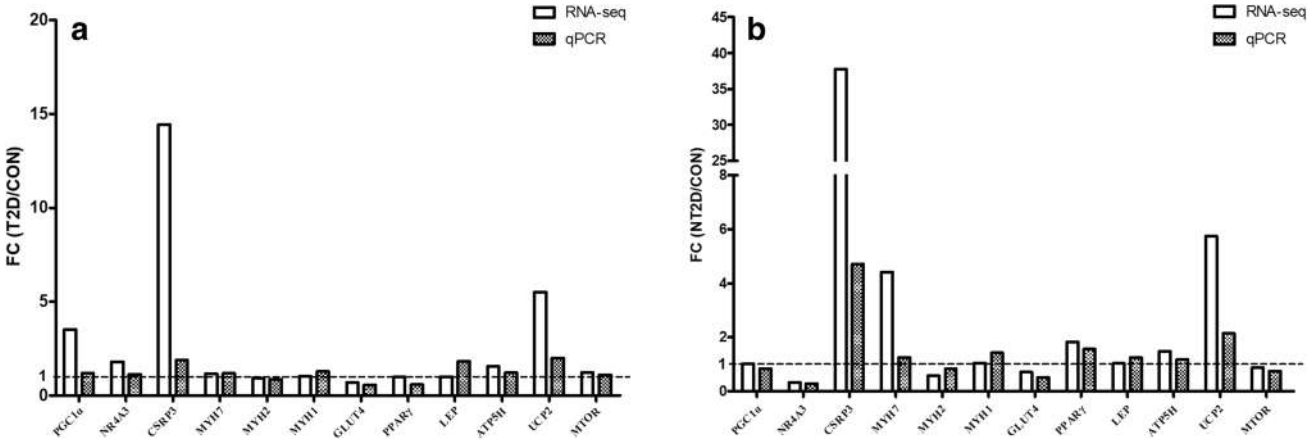


Fig. 1 The comparison of gene expression profiles by RNAseq and qPCR. The fold change in gene expression levels between **a** T2D and CON and **b** NT2D and CON. The dashed line indicates the baseline level of one

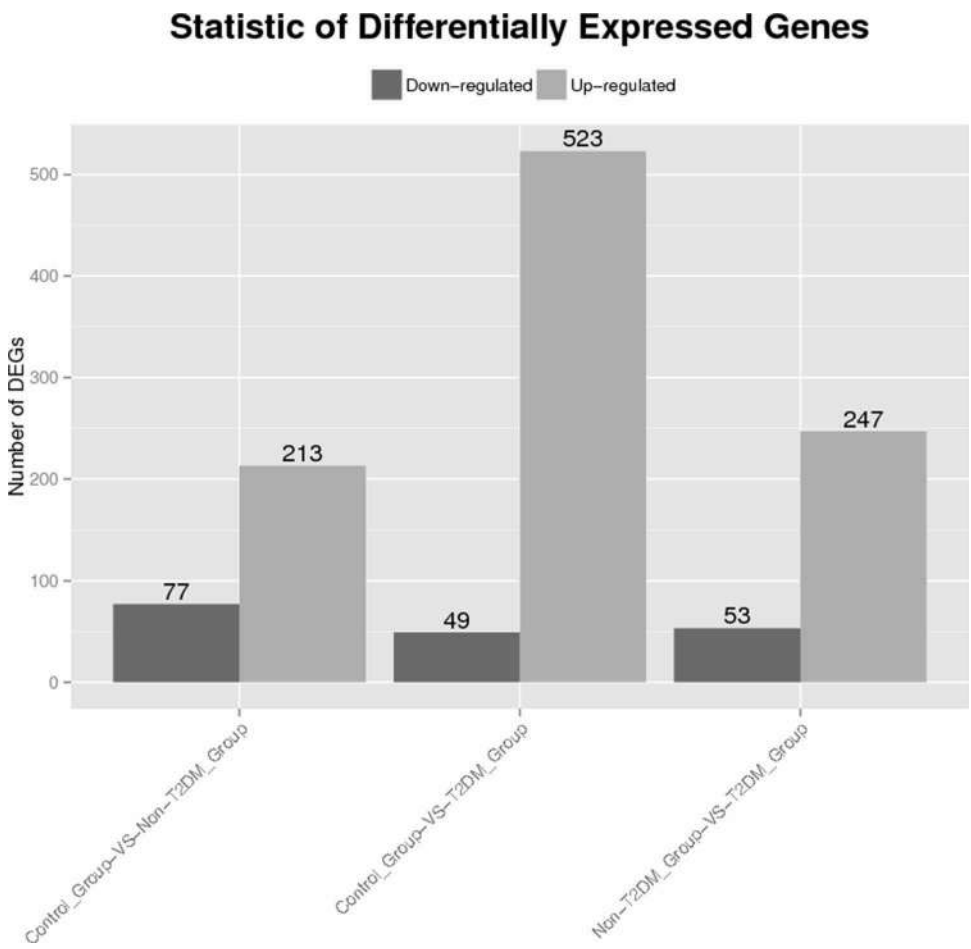


Fig. 2 Statistical analysis of differentially expressed genes (DEGs)

Comparative analysis of skeletal muscle transcriptome of T2D Bama mini-pigs and T2DM patients

(1) Overlapping DEGs between T2D Bama mini-pigs and T2DM patients

We retrieved the GSE29221 dataset including gene expression profiles of skeletal muscle biopsies from T2DM patients and non-diabetic controls [18]. According to the threshold values of |log2FC|> 1 and $p < 0.05$, 896 upregulated DEGs and 1872 downregulated DEGs were identified in the transcriptome data of T2DM patients. One hundred fifty-nine upregulated DEGs and 45 downregulated DEGs in transcriptome data of T2D Bama mini-pigs could be annotated the unique gene symbol which were consistent with T2DM patients. A comparative analysis of total DEGs revealed 17 co-DEGs between the T2DM patients and Bama mini-pigs, of which 13 were upregulated (1.2%) and 4 were downregulated (0.2%) (Fig. 4).

(2) Gene expression correlation analysis of the overlapping DEGs

The gene expressions of *ACTC1*, *AGL*, *AMOT*, *ARRDC4*, *ATP2B3*, *BVES*, *FAM46C*, *KIF1B*, *LRP4*, *NR4A3*, *PRKAB2*, *RBM20*, and *RXRG* were upregulated both in pigs and patients, and the gene expressions of *FAM134B*, *GBP2*, *RBP4*, and *SPHK2* were downregulated (Table 2). The Spearman correlation coefficient of the 17 co-DEGs between pigs and patients was 0.823 ($p < 0.01$), indicating that the expression of these genes was highly consistent between T2DM patients and Bama mini-pigs.

(3) GO analysis of the overlapping DEGs

The 17 co-DEGs were annotated to 7 biological processes (BP), 2 molecular functions (MF), and 2 cellular components (CC) as per GO analysis (Table 3). The majority of co-DEGs in the BP category was involved in embryonic morphogenesis and muscle tissue development process. The CC category included actomyosin and actin filament, and the MF terms were ATPase activity and steroid hormone receptor activity.

(4) Identical pathways between T2D Bama mini-pigs and T2DM patients

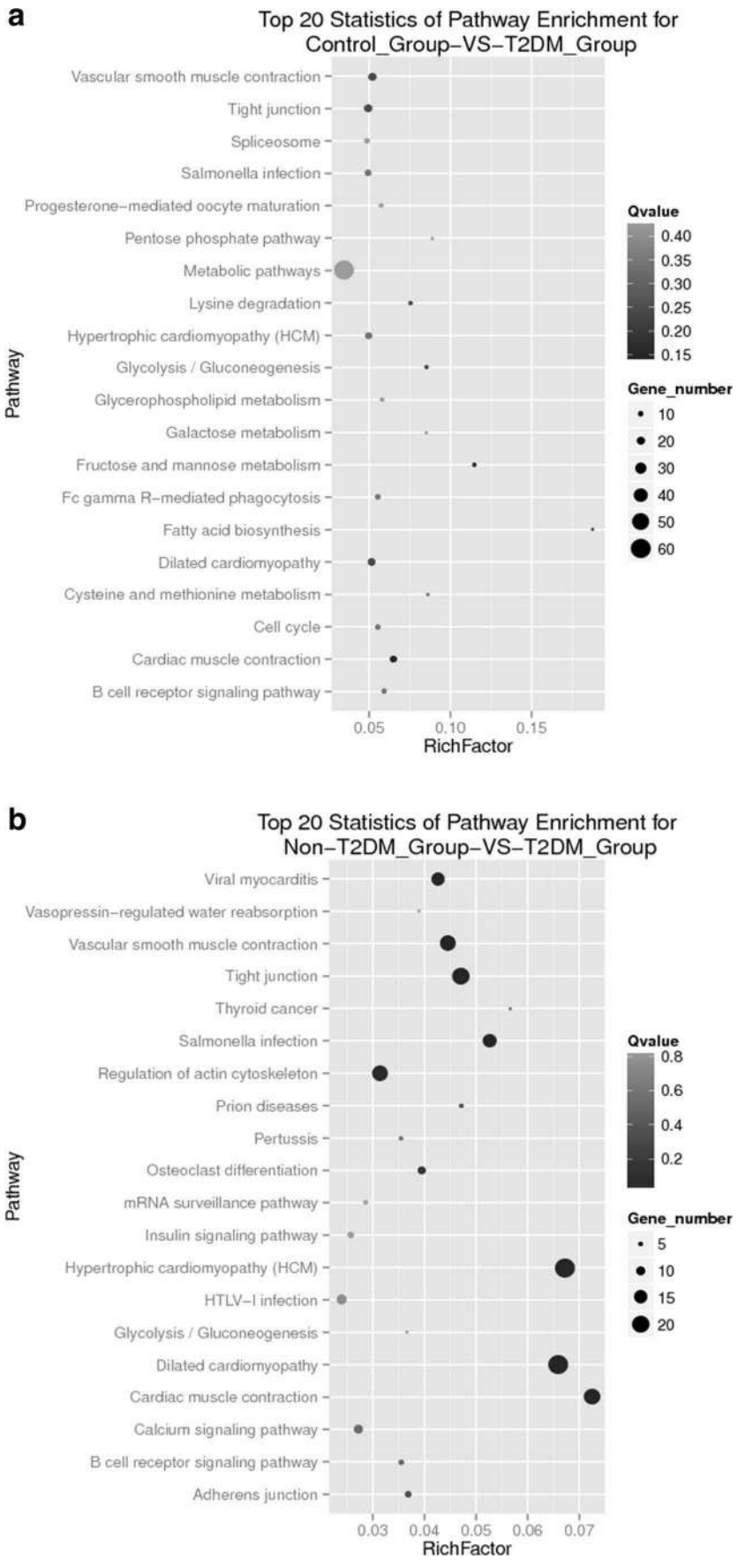


Fig. 3 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for DEGs. Enriched pathways between **a** CON and T2D and **b** NT2D and T2D

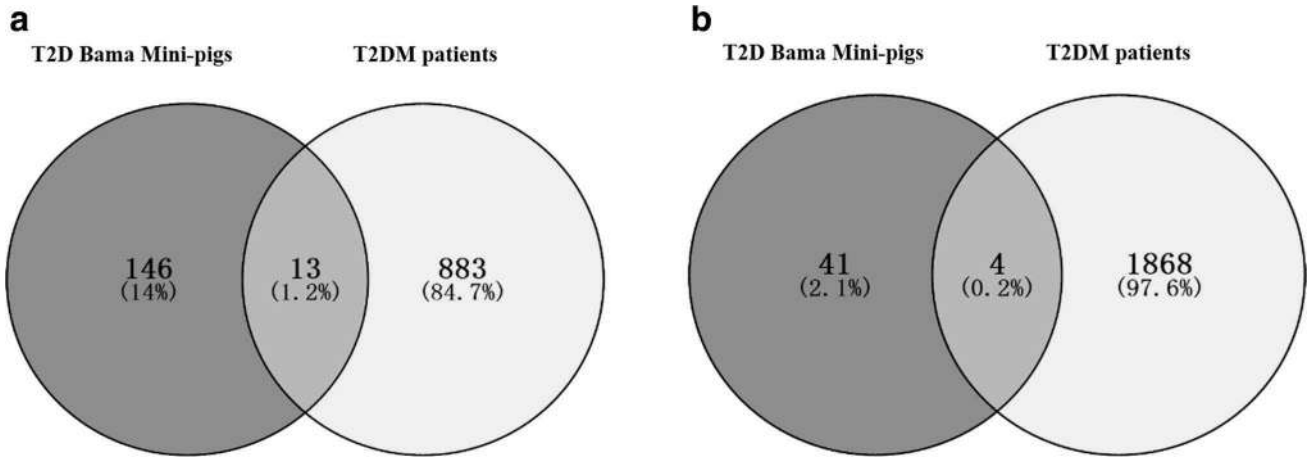


Fig. 4 Venn diagram of skeletal muscle DEGs between T2D Bama mini-pigs and T2DM patients. Overlapping **a** upregulated and **b** downregulated DEGs between T2D Bama mini-pigs and T2DM patients

KEGG enrichment analysis revealed that the DEGs of T2D Bama mini-pigs and T2DM patients were involved in 15 and 30 pathways respectively. There were six overlapping pathways, including hypertrophic cardiomyopathy, dilated cardiomyopathy, thyroid cancer, cardiac muscle contraction, tight junction, and calcium signaling pathway (Fig. 5).

Discussion

T2DM is a highly complex disease involving multiple tissues, disease states, and underlying factors [19]. High-throughput transcriptome analysis of human skeletal muscle, adipose, liver, pancreas, and blood has provided new insights into the possible causes and risks of T2DM

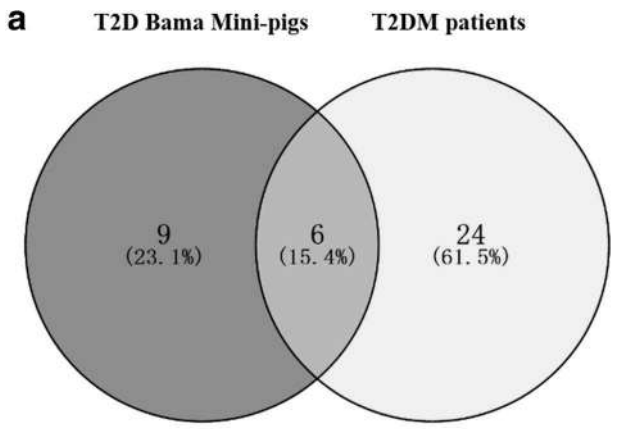
Table 2 Comparative analysis of differentially co-expressed genes (co-DEGs) between T2D Bama mini-pigs and T2DM patients

gene ID	Gene symbol	Bama mini-pigs		T2DM patients	
		LOG2	*p*-value	LOG2	*p*-value
ENSSSCG00000004803	*ACTC1*	1.68	1.45E-24	2.53	1.27E-02
ENSSSCG00000006872	*AGL*	1.45	3.45E-54	1.52	2.66E-02
ENSSSCG00000012591	*AMOT*	1.02	1.30E-17	1.29	3.72E-05
ENSSSCG00000002252	*ARRDC4*	1.31	2.01E-09	2.83	1.43E-02
ENSSSCG00000012769	*ATP2B3*	1.39	4.14E-13	1.25	2.04E-02
ENSSSCG00000004366	*BVES*	1.90	9.38E-10	1.89	1.36E-02
ENSSSCG00000016792	*FAM134B*	− 1.07	6.45E-10	− 2.71	1.82E-02
ENSSSCG00000006729	*FAM46C*	1.60	9.29E-10	1.19	5.53E-03
ENSSSCG00000006923	*GBP2*	− 2.52	6.16E-13	− 1.07	1.16E-03
ENSSSCG00000003401	*KIF1B*	1.76	1.25E-46	1.31	2.20E-03
ENSSSCG00000013248	*LRP4*	1.15	1.41E-05	1.13	1.97E-03
ENSSSCG00000005385	*NR4A3*	2.47	5.64E-35	3.82	1.13E-04
ENSSSCG00000006703	*PRKAB2*	1.11	1.91E-15	1.09	6.31E-03
ENSSSCG00000010626	*RBM20*	1.30	6.77E-09	1.61	6.08E-03
ENSSSCG00000010479	*RBP4*	− 2.72	2.62E-06	− 2.43	2.53E-02
ENSSSCG00000006328	*RXRG*	1.11	1.45E-40	1.11	1.42E-02
ENSSSCG00000030425	*SPHK2*	− 1.77	9.19E-10	− 1.43	1.19E-05

Table 3 GO analysis of co-DEGs between T2D Bama Mini-pigs and T2DM patients

GO term	*p*-value	Genes
Biological process		
Muscle organ development	7.42E-04	*RBP4*, *ACTC1*, *BVES*, *RXRG*
Striated muscle tissue development	4.78E-03	*RBP4*, *ACTC1*, *RXRG*
Muscle tissue development	5.26E-03	*RBP4*, *ACTC1*, *RXRG*
Embryonic morphogenesis	2.92E-02	*RBP4*, *AMOT*, *NR4A3*
Alcohol biosynthetic process	3.84E-02	*RBP4*, *SPHK2*
Cytoskeleton-dependent intracellular transport	4.52E-02	*ACTC1*, *KIF1B*
Cardiac muscle tissue development	4.94E-02	*RBP4*, *ACTC1*
Molecular function		
ATPase activity	3.67E-02	*ATP2B3*, *ACTC1*, *KIF1B*
Steroid hormone receptor activity	4.44E-02	*RXRG*, *NR4A3*
Cellular component		
Actomyosin	2.30E-02	*ACTC1*, *AMOT*
Actin filament	3.47E-02	*ACTC1*, *AMOT*

[20]. In this study, we found that the DEGs in the skeletal muscles of T2D mini-pigs relative to that of CON or NT2D animals were mainly involved in signaling pathways associated with metabolism, cardiovascular diseases, infections, cancer, inflammation immune responses, cytoskeleton, and signal transduction. Consistent with our results, Sun et al. [19] found the differentially expressed genes in the white adipose, skeletal muscle, and liver tissues of the T2DM Goto-Kakizaki rat model were mainly associated with inflammation and perturbed metabolism.



b

KO ID	CO-pathways
ko05410	Hypertrophic cardiomyopathy (HCM)
ko05414	Dilated cardiomyopathy
ko05216	Thyroid cancer
ko04260	Cardiac muscle contraction
ko04530	Tight junction
ko04020	Calcium signaling pathway

Fig. 5 The comparative analysis of pathways between T2DM Bama mini-pigs and T2DM patients. **a** Venn diagram showing overlapping pathways and **b** common pathways of T2D Bama mini-pigs and T2DM patients

Due to the limited availability of skeletal muscle biopsies from human subjects, few studies have performed transcriptome analysis of the skeletal muscle tissues from T2DM patients. One study reported that the vitamin D receptor, insulin-degrading enzyme, *INSR*, *Akt*, *IRS-1*, *IRS-2*, *GLUT4*, and glycolytic enzyme genes were downregulated in the muscle tissues of at least 50% of the T2DM subjects [21]. In addition, RNA-seq analysis of diabetic skeletal muscle showed decreased expression of genes related to insulin resistance, carbohydrate, energy and amino acid metabolism pathways, and an upregulation in apoptosis, immune response-related genes [22]. We have shown for the first time that the T2D Bama mini-pigs and T2DM patients have a high degree of similarity in terms of disease, cytoskeleton, and signal transduction-related pathways.

The pathological features of T2DM, such as hyperglycemia, hyperinsulinemia, high levels of circulating fatty acids and triacylglycerols, and increased inflammatory cytokines can impair myocardial function [23]. In addition, the diabetic state induces oxidative stress, intracellular calcium abnormalities, metabolic alterations, mitochondrial dysfunction, and inflammation, all of which can directly contribute to the development of cardiac abnormalities [24]. In fact, transcriptomes related to cardiovascular diseases (CVD) and T2DM are enriched in cardiomyopathy-related pathways, calcium signaling, axon guidance, cell adhesion, and extracellular matrix [25]. Consistent with this, the six overlapping pathways between T2D Bama mini-pigs and T2DM patients included

cardiomyopathy-related pathways, which suggested similar cardiovascular complications in human subjects and the diabetic model.

The 17 co-DEGs between the T2D mini pigs and diabetic patients included the orphan nuclear receptor *NR4A3*, also known as Nor-1 (neuron-derived orphan receptor), which mediates transcriptional responses to β-adrenergic signaling and is overexpressed in insulin-sensitive mice and humans [26, 27]. Since *NR4A3* is associated with an increase in insulin-stimulated glucose transport and *AKT* phosphorylation in muscle cells, it is a potential pharmacological target for diseases characterized by insulin resistance, such as T2DM and metabolic syndrome [28]. *NR4A3* is also implicated in β-cell function, although it was not covered by the SNP arrays in genome-wide association studies for T2DM [29]. NR4A3 knockout mice show an increased pancreatic β-cell mass and better glucose tolerance compared to wild-type animals, indicating that *NR4A3* inhibits β-cells [30, 31]. Taken together, *NR4A3* is a promising target for alleviating insulin resistance in diabetics and should be further investigated in the Bama mini-pig model.

Conclusion

The DEGs in T2D Bama mini-pigs were mainly involved in signaling pathways associated with metabolism, cardiovascular diseases, infectious disease, cancer, inflammation and immune responses, cytoskeleton, and signal transduction. And, the skeletal muscle transcriptomes of T2D Bama mini-pigs and T2DM patients share six pathways, including hypertrophic cardiomyopathy, dilated cardiomyopathy, thyroid cancer, cardiac muscle contraction, tight junction, and calcium signaling pathway, suggesting that the T2D Bama mini-pigs could be used on T2DM research in those pathways.

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Author contribution Ganqiu Lan conceived the study and revised the manuscript. Xueyu Yan performed the experiments and wrote the manuscript. Jinglei Si assisted the experiments. Fangjie Zhong analyzed the data. Yanjun Wu assisted the experiments. Qinyang Jiang provided instructions and revised the manuscript. Yafen Guo provided instructions and advice. Xiurong Yang provided instructions and advice. Jing Liang provided instructions and advice.

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

Declarations

Ethics statement All protocols were in accordance with the Guide for the Institutional Animal Care and Welfare Committee of the College of Animal Science and Technology, Guangxi University (Guangxi, China, permit no. GXU2013002).

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

SMOTE-SMO-based expert system for type II diabetes detection using PIMA dataset

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Abstract

Background Medical data, which is critical to human existence, is used to identify potential people prone to any specific complication or disease by the application of appropriate data mining (DM) techniques. DM is specifically applied to extract details for diagnosis, prediction, prevention, and treatment of various diseases. According to the International Diabetes Federation (IDF) 2019 atlas report, diabetes caused 4.2 million deaths over the globe, and hence, it is critical to diagnose diabetes at an early stage.

Material and method Even though many techniques are available to diagnose diabetes, the methods are not efficient to find hidden patterns with the desired accuracy for correct decision-making. Thus, this paper presents an integrated approach of synthetic minority oversampling technique (SMOTE) and sequential minimal optimization (SMO) algorithms for predicting diabetes. In this proposed two-phase classification model, the first step is pre-processing of data using the SMOTE algorithm, and the second step is SMO classifier. The output of the pre-processing is given to SMO to increase the performance of the classifier.

Result This classification model achieved an accuracy rate of 99.07% on the PIMA Indian diabetes dataset (PIDD) using our proposed approach. PIDD has been taken from UCI repository for this proposed work; however, the National Institute of Diabetes and digestive kidney disease owned the PIDD. The dataset contains 768 female patients, details each with 8 numeric and one decision class attribute.

Conclusion The output of the study confirms that the proposed integrated approach of DM could be used as an expert system for diagnosing diabetes in patients at an early stage. The extracted features from this study will be used for the development of a prognostic tool in the form of a mobile application for early diabetes detection.

Keywords Risk assessment · SMOTE algorithms · SMO algorithms · Data mining · Expert systems · PIMA dataset

Introduction

Diabetes mellitus is a medical condition resulting from a metabolic disorder in which the body is not in a condition to make use of carbohydrates and also affected by a sedentary lifestyle [1]. Diabetes has emerged as a major intimidating disease for both developed and developing countries. Diabetes is a disease in which the body either does not produce insulin or responds at all. Further, diabetes gives birth to other severe complications as it raises the risk of coronary diseases like heart disease, kidney failure, and retinal eye disease. Diabetes is categorized into three main parts: type I, type II, and gestational diabetes. When the pancreas is not able to construct insulin, then this condition causes type I diabetes, when the pancreas does not construct enough insulin or process it properly, then this condition causes type II diabetes. It can be said that type II diabetes accounts for 90% of cases all over the globe and is the most common type of diabetes in adults [2]. It is generally categorized as insulin resistance in this type of diabetes; the body does not adequately respond to the insulin produced because insulin does not work properly. In some of the type II diabetes cases, the pancreas is exhausted, which results in the lesser production of insulin and can further cause high blood sugar levels. In earlier times, type II diabetes was mostly diagnosed in older people. However, gradually this is observed to be also occurring in younger generations and children due to the

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improper diet, lack of physical activities, and rising levels of obesity [1]. It will not be incorrect to state that the keystone of type II diabetes management is proper diet, daily workout, increased physical activities, and maintaining steady body weight [3]. There are several distinct factors, which can lead to type II diabetes including the following:

- Overweight of a person
- Family history of diabetes
- Increasing age
- Stress
- Poor and unhealthy diet
- Obesity

As per the IDF Atlas 2019 report, the occurrence of type I and type II diabetes increased from 151 to 577 million in people aged between 20 and 79 years during the period from 2002 to 2019 [3]. It can be said that if not well managed, then about 578 million people could have diabetes by 2030, and as shown in Fig. 1, the number of patients would likely increase to 700 million by 2045. It has been known for years that people having diabetes are slightly at a higher risk for emerging other infections than a healthy person. Therefore, diabetic patients need to keep all necessary safeguards to avoid other complications [2]. Diabetes can be considered as a serious global threat because the spread of diabetes neither considers the socioeconomic status of a country nor its national boundary. World over, diabetes is considered as one among the top 10 reasons for premature deaths. However, many countries still lack a national diabetes prevention plan; hence, the partial population of the world does not obtain full coverage of healthcare services till now. So, it will not be wrong to say that most of the countries are falling short of the WHO (World health organization) 2025 target of halting diabetes globally [4]. It is essential to take urgent action to stop the spread of type II diabetes and to improve the prevention of this disease.

In 2000, the IDF diabetes prevalence estimation of 151 million is close to the WHO estimation of 150 million at the time. The global estimation has shown the alarmingly increase in the number of diabetes cases from 2000 to 2019 which is almost a tripling from 151 to 463 million people [3]. Figure 1 shows the global prevalence of diabetes from 2000 to 2019 between the ages of 20 and 79 and, the IDF also projected the global diabetes prevalence in upcoming years as per the current situation and preventive measures taken by the developed and developing countries, which is represented in Fig. 2.

Medical data is critical to human existence. With the advancement in medical technology, even the minor details of the patient are stored in the healthcare databases for diagnoses and analysis of complications confronted by the patient later. However, that vast data can lead to inconsistency in decision-making. Healthcare organizations accumulate a massive amount of data, which leads to information overload, and decision-making on such type of data can mislead the diagnosis and, consequently, the treatment of the patient [5]. DM is Knowledge Discovery in Database (KDD) process, which is the procedure of computational extraction of knowledge in the form of models and patterns from large databases and altering it in a reasonable format for further use. It helps to identify the hidden patterns of successful medical therapies for distinct illnesses. Classification algorithms are considered as a widely used DM methodology that is implemented to classify and predict the prearranged data for a definite class. Numerous researchers are working in disease prediction area using different machine learning (ML) algorithms like neural network (NN), decision tree (DT), deep learning (DL), and naive Bayes (NB) [6]. Researchers also proved that ML algorithms tend to produce more accurate results in diagnosing diverse diseases.

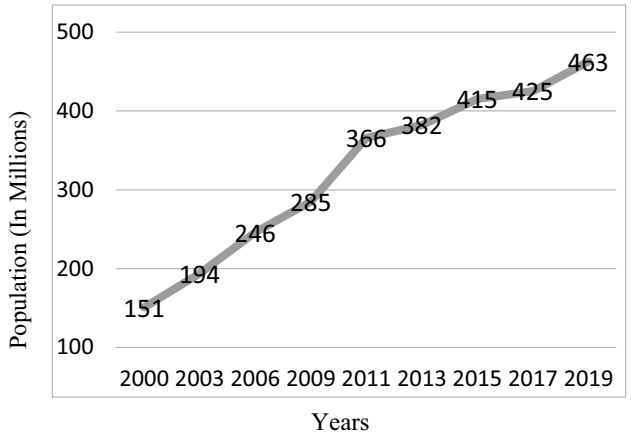

Fig. 1 Estimations of global prevalence of diabetes in 20–79 age groups (2000–2019)

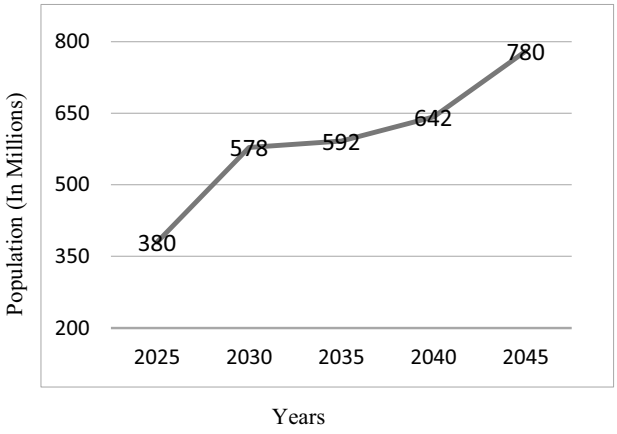

Fig. 2 Projection of global prevalence of diabetes (2025–2045)

Therefore, this proposed work suggests an approach of SMOTE and SMO algorithm for the diagnosis of diabetes. In DM, the majority of the data is collected from distinct sources, and that data is often distorted, which leads to the imbalance problem. Class imbalance is highly prevalent in healthcare datasets because most of the instances belong to one labeled class called the majority class, while the others belong to another labeled class called minority class [7]. Class imbalance further leads to misinterpretation in the decision and accuracy of the classification algorithm. That is where the SMOTE algorithm came into existence. It resolves the problem of class imbalance and improves the performance of the classifier. In this proposed work, the processed output of the SMOTE algorithm is provided to SMO classifier as input, which further classifies the data into diabetic and non-diabetic patients.

The second section elaborates on the related work done on the detection of diabetes. The objective of the proposed work is specified in the third section. The fourth section presents the conceptual outline, including a dataset description and methodology. The fifth section presents the experimental outline used to examine the performance of the model. Finally, the paper concludes in the sixth section.

Previous studies

DM plays a vital role in medical diagnosis and healthcare domain for discovering the hidden patterns. Utility DM techniques have superseded the existing analytical and statistical techniques in terms of accuracy. In recent times, researchers also highlighted the potential of DM techniques for the development of predictive models, which could help in decision-making based on the patient's historical records. With time, ML has become an integral part of healthcare for predicting disease timely. In that process, Alghamdi et al. [8] proposed an ensemble model-based predictive model by implementing multiple linear regression and information gain ranking methods. To deal with the oversampling of the data, the authors applied SMOTE algorithm, and the model has been applied on FIT project study. This study consists of 32,555 patients with 13 attributes for predicting diabetes onset.

Igor Kononenko [9] proved the significance of ML techniques in medical diagnosis and disease prediction. The author made use of ML techniques NB, DT, and NN for disease diagnosis. The author also suggested that ML algorithms, along with other algorithms, could enhance the capability and the accuracy of medical diagnosing systems. DM techniques thus not only produce more accurate results in the healthcare domain but also perform as a predictive tool for healthcare officials.

Dursun et al. [10] verified the importance of DM with their proposed model, which used three DM algorithms named linear regression statistical method, artificial neural network (ANN), and DT for diagnosing breast cancer. Among those three algorithms, DT produced the best results with an accuracy rate of 93.6% followed by a NN with an accuracy rate of 91.2%. The logistic regression provided results with an accuracy rate of 89.3%. Meanwhile, Kemal et al. [11] proposed a novel cascaded learning model by implementing a least-square support vector machine (SVM) and generalized discriminant analysis algorithm. In their proposed work, the first stage was using the discriminant analysis algorithm to find the discriminate features, among diabetic and non-diabetic patients. In the second stage, the author applied the least-square SVM classifier to classify the diabetes data and achieved an accuracy rate of 78.21% using a tenfold cross-validation method. The achieved results are most prominent in comparison with the available methods.

Barakat et al. [12] compared the diverse DM and ML algorithms for diabetes diagnosis, and it identified that SVM provided the most promising results for conducting diagnostic decisions. The results achieved on a real-life dataset showed that intelligent SVM could be used to attain promising results for diabetes detection. Experimental results showed that classifiers achieved an accuracy rate of 94%, a specificity of 93%, and a sensitivity of 94%. In the field of artificial intelligence, genetic algorithms perform best with the heuristic and search problems. These algorithms tend to produce more accurate results for real-life problems that mimic the process of natural selection.

Therefore, Aslam et al. [13] suggested a fuzzy-based genetic approach for diabetes detection. In their proposed three-phase approach, the first stage was a feature selection method, which was implemented through preserving an ordered list of features, which were sustained in decreasing rank orders. In the second phase of the model implementation, novel features were generated by picking new features from each subclass of the original dataset features. In the final stage, testing was performed by using a k-nearest neighbor and SVM classifier. The performance of genetic algorithm is being tested on the PIMA, and preliminary results showed that suggested methods exhibited superior performance in comparison to other available methods.

Ahmed [14] suggested a novel approach to classify the type II diabetes data through the datasets collected from Jaber Abn Abu Aziz clinic center. J48 was implemented for diabetes prediction on the Waikato Environment for Knowledge Analysis (WEKA) tool, and performance was evaluated based on classification accuracy. WEKA is an authentically tested open-source tool which consists of diverse ML algorithm for real-world data mining problems. It provides an easy interface for model implementation. The accuracy rate achieved on the proposed model

was 70.8%. Sisodia and Sisodia [15] proposed a model for diagnosing diabetes at an early stage; they compared the three ML algorithms and evaluated those on different performance measures of precision, accuracy, recall, and f-measure. The three classifiers, viz. DT, NB, and SVM, were evaluated on the PIDD. The outcome was that NB outperformed others with an accuracy of 76.30%.

Hemanth et al. [16] proposed a hybrid approach for the detection of diabetic retinopathy from retinal fundus images. The proposed hybrid method combined DL and image processing techniques for obtaining better prediction outcomes. The proposed solution for detecting diabetic retinopathy at an early stage combined the image processing through contrast-limited histogram equalization technique. Convolutional neural network (CNN) was used further for diagnosis. The proposed approach achieved an accuracy rate of 97%, which was validated on 400 retinal fundus images. The performances of other parameters measured were sensitivity at 94%, precision at 94%, and specificity at 98%. The outcome was then compared with the available studies, and its results presented that the projected approach was adequately competent and reliable for detecting diabetic retinopathy.

Perveen et al. [17] recommended hidden Markov model (HMM) as an attractive tool for predictive analysis in the healthcare domain and therefore proposed Newton's divided method-based approximation technique with the implementation of HMM. The methods regulated the risk of diabetes occurrences in an individual using sparse and asymmetrical electronic medical record data. The suggested approach was proficient in detecting hidden categorizations of clinical trials recorded form the patient's data for effective diagnosis and also for improved performance. The achieved outcomes showed that the proposed approach surpassed the traditional HMM in terms of effective diagnosis with a better accuracy rate.

However, no one implemented the integrated approach of a pre-processing technique like SMOTE and the advanced DM classifier that could diagnose diabetes effectively. In all the previously implemented diabetes detection approaches, most of those were time incompetent, since all work with the weighted approach. The need for a better approach has therefore become overdue, for providing an accurate, reliable, and time-efficient outcome for diabetes detection. In our proposed work, we shall attempt to detect diabetic patients using an integrated approach of pre-processing and classification.

Objective of the study

The objectives of this proposed study are as follows:

- To propose a novel integrated algorithm for diabetes detection at an early stage
- To improve the accuracy rate of existing hybrid algorithms in the healthcare domain
- To provide a prognostic tool to the healthcare officials for effective and efficient decision-making

Methodology

Dataset description

Various experiments have been done using the PIDD. The dataset was originally from the National Institute of Diabetes and Digestive and Kidney (NIDDK). PID was taken from the UCI ML repository for this work [18], and the reason for selecting this dataset is that most of the people in modern times are living with an identical type of lifestyle that includes a high reliance on the processed food coupled with declining physical activities. PIMA is a group of Native Americans who lived in an area now known as Central Arizona. Due to their genetic predisposition, they could survive on low carbohydrates for many years. However, during recent past, PIMA group suddenly shifted from their traditional diet towards processed food, followed with a decrease in their physical activities too [19]. Consequently, they were detected with high levels of type II diabetes, and for this reason, since 1965, their health data have been used in many diabetes studies.

PIDD includes a certain number of medical predictors and one variable target. The predictor variables are the number of pregnancies, BMI, blood pressure, skin thickness, insulin level, age, glucose, and diabetes pedigree function shown in Table 1. All the participants in the PIDD study are females up to the age of 21. The dataset has 768 instances divided into 268 non-diabetic instances and 500 diabetic instances. The target variable identifies whether a person is non-diabetic (represented by 0) or diabetic (represented by 1). The description of different parameters of each attribute in the dataset, including max value, min value, standard deviation, missing value, mean, and the median which are given in Table 2.

Data pre-processing

Pre-processing is the method applied to the dataset before commencing its processing. Typically, pre-processing modifies the raw data, which can enhance the classification ability of processing. It also includes detection and removal of outliers where outliers are the inconsistent type of data. It also performs attributes' extraction, normalization, integration, aggregation, and discretization [20]. The dataset consists of

Table 1 Description of PIDD attributes

Sr. no.	Predicators	Description of predicators	Unit
1.	Preg	Number of times a female participant is pregnant	–
2.	Plasma glucose	Glucose concentration in 2 h in an oral glucose tolerance test	Mg/dl
3.	Diastolic blood pressure	Diastolic blood pressure (upper blood pressure)	mmHg
4.	Triceps skinfold thickness	Skin thickness of participant in mm It is concluded by the collagen content	Mm
5.	Insulin	Participant's 2-h serum insulin	Mm U/Ml
6.	Body mass index	Weight of a participant in Kg/(height in m)^2)	Kg/m2
7.	Diabetes pedigree function	Appealing attributes used for diabetes diagnosis	–
8.	Age	Age of participants	–
9.	Outcome	Diabetes onset with diabetic and non-diabetic patients	–

Table 2 Detailed description of PIDD attributes

Sr. no	Predicators	Missing values	Mean	Std. dev	Range	Data type
1	Pregnancy	0	3.845	3.370	0–17	Integer
2	Glucose	0	120.89	31.973	0–199	Integer
3	Diastolic blood pressure	0	316.56	1096.927	0–122	Integer
4	Skinfold thickness	0	51.697	88.690	0–99	Integer
5	Insulin	0	819.49	3873.732	0–846	Integer
6	Body mass index	0	60.769	92.015	0–67.1	Real
7	Diabetes pedigree function	0	0.472	0.472	0.078–2.42	Real
8	Age	0	33.241	11.760	21–81	Integer
9	Outcome	(Diabetic instances, 268) (Non-diabetic instances, 500) (Total instances, 768)				Polynomial

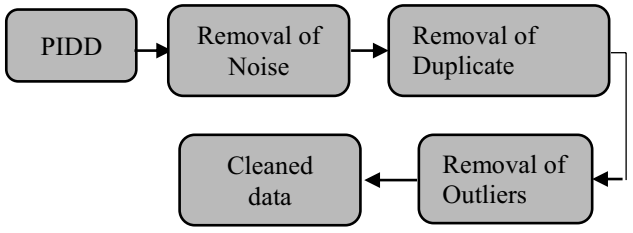

Fig. 3 Steps for traditional pre-processing of data

Table 3 Detailed description of PIDD after pre-processing

Sr. no	Predicators	Mean	Std. dev	Data type
1	Pregnancy	0.845	0.362	Integer
2	Glucose	120.89	30.624	Integer
3	Diastolic blood pressure	70.775	12.327	Integer
4	Skin thickness	29.241	10.553	Integer
5	Serum insulin	153.63	111.361	Integer
6	BMI	32.762	6.497	Real
7	Diabetes pedigree function	0.527	0.338	Real
8	Age	30.646	10.050	Integer

8 attributes with 768 total number of instances, considered to be analyzed using our proposed approach.

However, it is being analyzed that the dataset contains inconsistent values, and those values are processed applying diverse available pre-processing methods to recover the quality of the data. Therefore, data cleaning operation was performed on the dataset to remove the outliers and integrate the various attributes. In this paper, we used some traditional approaches to remove the outliers and integrate the attributes, as shown in Fig. 3. After the removal of noise and inconsistent data, SMOTE algorithm was applied on PIDD to eliminate the class imbalance problem [21].

After passing through the traditional pre-possessing steps, we had the processed data, whose mean and standard deviation (SD) were lesser and different from that of the unprocessed data, as shown in Table 3. Lesser mean and SD implied that data was clustered rather than spread out. In our approach, the first step of pre-processing was the removal

of noise as represented in Fig. 3; this was done by eliminating the irrelevant data which did not secrete its attribute [21]. The second step was the removal of duplicate values to eliminate identical data for reducing the overhead. The last step was to remove the outliers, which was done by using diverse available DM techniques like DBSCAN, Z-score.

Class imbalance problem

At present, in DM classification, class imbalance problem is the most crucial problem being discussed. The data that we used in our classification problem was imbalanced as the instances of one class outnumbered the instances of the other class by a large ratio [22]. The DM algorithms used for prediction in the medical domain perform well when provided with evenly distributed data for training. However, most of the datasets that we practiced were not evenly distributed, which led to a class imbalance in datasets.

In most of the cases, the dataset contained more negative class instances than the positive ones. However, this class being more was a good sign as it represented the disease class. Further, feeding the imbalanced data would make a classifier, biased to the majority class because it would not have enough data to train the minority class [23]. This bias would further bias the classifier to produce a higher performance for majority class and lesser performance for the minority one. Data imbalance problem is exceptionally predominant and can be resolved by using resampling methods. However, these kinds of methods solve the problem of class imbalance by either reducing the instances of majority class or increasing the instance of minority one through duplicate values. However, that leads to data loss and reduces the possibility of higher accuracy. In the dataset used for our proposed work, the negative instances outnumbered the positive one, which was solved using SMOTE algorithm [24]. Among the diverse oversampling methods available, SMOTE has proved to be the most promising and therefore used by many scholars in medical research.

SMOTE

SMOTE is an oversampling technique suggested to avoid the class imbalance problem in the dataset. It increases the performance of the classifier and proceeds with joining the minority class points to line segments along with the fictitious points placed on these lines. In SMOTE, new instances are created by replicating the data points of the minor class synthetically, as was done in the traditional oversampling approach [25]. It is different from the traditional approach in that it operates in feature space instead of operating in the data space by considering the minority class instance to its closest vector [26]. The new synthetic parameters can be created using two different approaches: first is using oversampling rate, and another is the number of nearest neighbor (k). In other words, SMOTE generates the artificial data points for minority class to shift the learning bias of classifier from majority class to minority class [24]; according to Chawla et al. [24], new synthetic points can be produced for continuous features using the following steps.

Synthetic minority oversampling technique (SMOTE)

Step 1: Calculate the distance between the minority class feature sample under consideration and its k-nearest neighbor

Step 2: Then multiply the obtained difference with any random number ranges among 0 to 1

Step 3: Finally add the results obtained from step 2 to the considered feature vector; it will cause the selection of a new synthetic point on the line segments between two distinct features. The new feature will be generated in this form

$$\gamma_{NEW} = \gamma_0 + (\gamma_{0i} - \gamma_0) \times \partial$$

Here,

γ_{NEW} represents the newly generated synthetic sample.

γ_0 represents each instance's feature vector of the minority class.

γ_{0i} signifies the ith nearest neighbor of γ_0.

∂ signifies the random number generated among 0 and 1.

Sequential minimal optimization (SMO)

The updated version of the SVM classifier is known as SMO. SMO is an algorithm that was introduced in late 1998 by John Plat to solve the problem of quadratic programming (QP) raised during the SVM training. The main difference between both of them is that unlike earlier SVM classifier [27], SMO practices a logical QP step in place of numerical QP as an inner loop for solving the problem of QP. It resolves the problem of QP without using additional matrix storage and numerical QP calculation. SMO divides the problem of QP into a series of sub-problems and selects the smallest one to ensure the convergence. This statistical classifier can be considered to be computationally faster with a lower average error rate than the other available classifiers [28]. The steps for solving an optimization QP problem using SMO classifier are given below.

Sequential minimal optimization (SMO)

Step 1: Select the langrage multiplier that interrupts the property of Karush–Kuhn–Tucker (KKT) for an optimization problem

Step 2: Select the second multiplier $\propto 2$ and improve the pair $(\propto 1, \propto 2)$

Sequential minimal optimization (SMO)
Step 3: Repeat steps 1 and 2 until finding the best convergence

Proposed approach for diabetes detection

The proposed approach combined SMOTE and SMO algorithm, which initially, pre-processes the imbalanced data, followed by improving its classification performance by SMO classifier. The processing approach is represented in Fig. 4.

Experimental results and analysis

Our proposed approach model has been validated on the test data of PIDD, the performance of PIDD measured using diverse evaluation metrics like classification accuracy, recall, precision, and F-measure. The accuracy rate achieved by applying the integrated approach of SMOTE and SMO algorithms was 99.07%. It was then compared with the NB, J48, random forest, and PART algorithm using the WEKA toolkit [29]. Our proposed model can enable the medical practitioner for improving the decisions based on extracted features. Numerous researchers performed diverse algorithms using distinct features on the PIDD. Table 4 shows some of the previous work done till date with their proposed methods and accuracy rates.

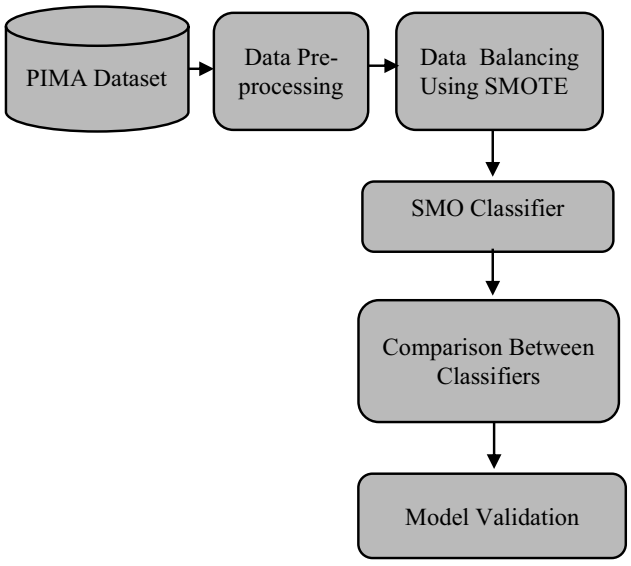

Fig. 4 Flow chart of the proposed approach for diagnosing diabetes

Table 4 Relative study of previous work done on diabetes detection using PIDD

Reference No	Applied methods	Accuracy rate (in %)
Ref No. [30]	Hybrid approach of firefly and cuckoo search	81%
Ref No. [31]	Feedforward NN	82%
Ref No. [32]	NB algorithm	79.56%
Ref No. [33]	LDA, MWSVM	89.74%
Ref No. [34]	Genetic algorithm with NN	87.46%
Ref No. [35]	K-means with DT	90.03%
Ref No. [36]	K-means with PCA algorithm	72%

Experimental analysis of diabetes prediction using proposed approach

In this experiment, the combined approach of SMOTE-SMO was implemented using PIDD on WEKA toolkit for diagnosing diabetes. Four distinct performance measures named accuracy, precision, recall, execution time (in milliseconds), and F-measure were calculated for all classification algorithms using PIDD. Table 5 shows that our proposed integrated approach outperformed in every performance measure and provided the best result for the detection of diabetes with an accuracy rate of 99.07%. Figure 5 shows the executive time comparison, and Fig. 6 presents the comparison between the distinct performance matrices for diabetes detection.

As presented in Figs. 5 and 6, our proposed integrated approach provided the highest accuracy and lowest execution time among all algorithms on the PIDD. The achieved accuracy rate of 99.07% shows that the approach can be used as a prognostic tool for early diabetes detection. Those features, which do not contribute to the study, need to be pruned. In this study, we have used the best-selected attributes for achieving an accurate diagnosis of diabetes onset. The execution time makes a difference in any disease prediction tool. Compared with previous diabetes detection approaches, our proposed approach provided results with a better accuracy rate, in lower execution time.

Conclusion and future scope

The proposed study presented a medical decision support system based on SMOTE-SMO to predict diabetes. In particular, the PIDD identified several inconsistent and irrelevant values, which could be improved using data preprocessing techniques. Then, the model has been trained using 70% data, and 30% has been used for test purpose. Then the model has been designed using an integrated approach of SMOTE and SMO algorithms which attained

Table 5 Combined performance measures of algorithms

Performance measures	Methods				
	Proposed approach	J48	Random forest	Part	Naive Bayes
Accuracy (In %)	99.07	96.62	90.34	76.33	77.43
Precision (In %)	96.23	95.06	89.09	60.72	60.23
Recall (In %)	98.24	96.35	84.43	65.81	65.09
F-measure (In %)	97.71	93.21	86.78	62.76	62.88
Specificity (In %)	99.14	97.86	91.43	84.29	85.28
Sensitivity (In %)	95.52	94.03	88.06	59.70	60.65
Execution time (in milliseconds)	0.1	0.2	3.66	0.25	0.5

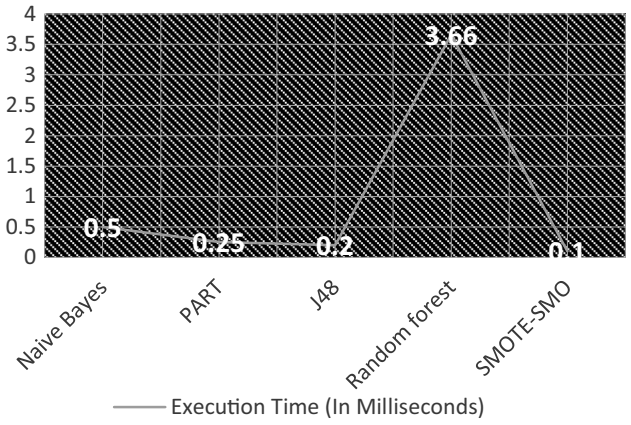

Fig. 5 Execution time of different classification algorithm

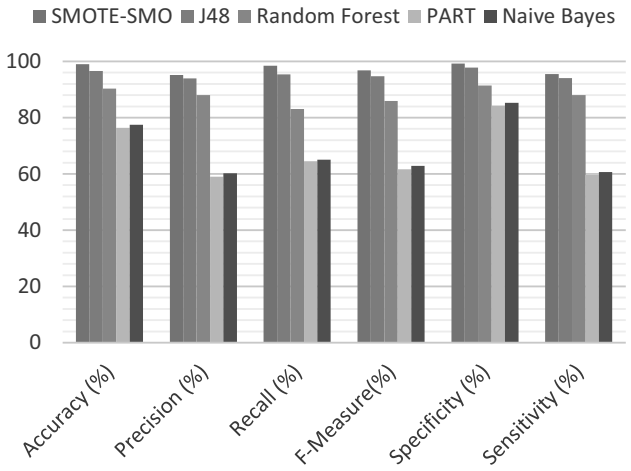

Fig. 6 Comparison of performances various classification methods

99.07% accuracy with an execution time of 0.1 ms. Diverse assessment metrics named accuracy, sensitivity measure, and specificity have been used to compute the performance of the proposed system. The outcome presented that our proposed approach attained a highly efficient prediction outcome. Besides this, the improvement in the quality of data is achieved using data pre-processing and data balancing techniques, which in turn improved the prediction accuracy for the diabetes onset. In future, we aim to develop a robust system in the form of an application by using our proposed approach with more complex data. This system would enable healthcare officials in the early prediction of diabetes of their patients.

Declarations

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Utilization of Indian diabetes risk score (IDRS) in steroid-induced diabetes

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Abstract

Introduction Steroid-induced diabetes (SID) can be defined as the occurrence of hyperglycemic state in non-diabetic individuals following steroid therapy. The traditional risk factors for type 2 DM, such as age, abdominal obesity, family history, and physical activity, have been incorporated in the Indian diabetes risk score (IDRS). We conducted this study to determine the use of IDRS in the prediction of SID.

Materials and methods A prospective observational, cohort study on non-diabetic subjects, aged between 18 and 70 years, on oral or parenteral steroid therapy for different diseases was conducted. Anthropometric records were collected. Baseline biochemical parameters in the blood (FPG, PPG, and HbA1c) were measured before initiating steroid therapy. The biochemical parameters, except HbA1c, were measured again on day 3 after steroid therapy initiation. Based on FPG and PPG of day 3, participants were categorized as normal, pre-diabetic, and diabetic. Based on IDRS, patients with SID were categorized as low-risk, moderate-risk, and high-risk IDRS groups for further analysis.

Results Out of 317 subjects, SID was found in 132(42%) subjects. We observed significantly higher age (p value = 0.04) and BMI (p value = 0.03) in diabetes group compared to normal and pre-diabetes groups. There was no significant difference (p = 0.6) in mean waist circumference across steroid-induced diabetic groups. A sedentary lifestyle (p = 0.359) and family history (p value = 0.388) have no association with SID. The incidence of SID was significantly high among high-risk IDRS (58%) when compared to that of low-risk (34.8%) and medium-risk (36%) IDRS (p < 0.001). IDRS more than 60 showed odds ratio of 1.69 (95% C.I, 1.24–2.16, p < 0.001) for steroid-induced diabetes. IDRS of more than 30 had 71.4% sensitivity and 44.6% specificity for SID (p value = 0.001).

Conclusions The cumulative scores of IDRS were higher in patients with SID. This score may be used as an initial screening test to select patients for blood sugar monitoring in those treated with steroids on OPD basis.

Keywords Steroid-induced diabetes · Indian diabetes risk score · IDRS · SID · Glucocorticoids

Introduction

Steroid-induced diabetes (SID) can be defined as the occurrence of the hyperglycemic state in non-diabetic individuals following steroid therapy. Ingle, in the 1940s, termed "steroid diabetes" to describe hyperglycemia observed in rats upon steroid treatment [1]. Steroids such as prednisolone, methylprednisolone, dexamethasone, etc. are commonly used in acute and chronic disease due to their anti-inflammatory properties. Their therapeutic uses frequently pose side effects, which include osteoporosis, puffy face, and metabolism-related adverse effects resulting in glucose intolerance and SID. Higher doses (30 mg/day or more of prednisolone) of steroids significantly affect glycemic status

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[2]. The incidence of SID varies across studies, ranging from 1.5 to 47% [3].

Steroids raise glucose levels in the blood by elevating glucose production in the liver and inhibiting glucose uptake into muscles due to their effect of increasing insulin resistance (IR). The risk factors of SID in various studies are dose of steroid, advanced age, and obesity. The Indian diabetes risk score (IDRS) was derived by Mohan et al. from the Chennai Urban Rural Epidemiology Study to predict the risk of type 2 DM in Indian population [4]. IDRS includes traditional risk factors for type 2 DM, such as age, abdominal obesity, family history, and physical activity. Studies have shown that IDRS is a cost-effective strategy in screening diabetes mellitus in Indian population. In the background of high incidence of SID, we conducted this study to identify the risk factors and role of IDRS in predicting SID.

Materials and methods

This was a secondary analysis of data from another study, the role of osteocalcin in steroid-induced diabetes. A prospective observational, cohort study on non-diabetic subjects, aged between 18 and 70 years, on oral or parenteral steroid therapy for different diseases was conducted in Kasturba Hospital Manipal, Karnataka, from December 2015 to December 2017. A total of 317 patients were recruited from outpatients or inpatients. Subjects who were on other drugs known to cause hyperglycemia, those who were already on steroid treatment before enrolment, those who were acutely ill, or those having major organ dysfunction were excluded from the study as shown in Fig. 1. Osteoporosis subjects and pregnant women were also excluded.

Anthropometric records were collected at the time of enrolment. Baseline biochemical parameters in the blood (FPG, PPG, and HbA1c) were measured before initiating steroid therapy. The biochemical parameters, except HbA1c, were measured again on day 3 after steroid therapy initiation. Based on FPG and PPG of day 3, participants were categorized as normal, pre-diabetic, and diabetic. IDRS score was calculated using the parameters shown in Table 1. Based on the IDRS, participants from normal, pre-diabetes, and diabetes groups were further categorized into low-, moderate- and high-risk groups [4].

Sample size

Assuming 50% of the subjects in the study population develop diabetes after steroid administration and expected dropout of 15% during follow-up period, the study would require a sample size of 314 for estimating the expected proportion with 12% precision relative to the expected proportion and 95% confidence.

Research ethical clearance

Ethical clearance for conducting the proposed study is obtained from the "Institutional ethics committee" (IEC) of "Kasturba Medical College and Hospital," Manipal, Karnataka (IEC: 207/2015).

Operational definitions

Steroid-induced diabetes Incident cases of diabetes were diagnosed on day 3 after initiating steroid therapy.

Diabetes/pre-diabetes "Diabetes was defined as FPG ≥ 126 mg/dL or PPG ≥ 200 mg/dl or HbA1c ≥ 6.5% or taking oral anti-diabetic drugs (after diagnosis of diabetes). Pre-diabetes was defined as FPG 100–125 mg/dL or PPG 140–199 mg/dL or HbA1c 5.7–6.4%" [5].

Obesity Based on BMI, subjects were categorized into "underweight (< 18.5), normal (≥ 18.5 to < 23), overweight (≥ 23 to < 27.5), and obese (≥ 27.5)" according to the proposed cut-off for the diagnosis in Asians [6].

Abdominal obesity "Abdominal obesity was defined as waist circumference (WC) ≥ 90 cm for males and ≥ 80 cm for females," according to the proposed cut-off for the diagnosis in Asians [6].

Data collection

A total of 317 non-diabetes subjects as per inclusion and exclusion criteria were recruited. Information on diabetes history in their family, history of steroid use, and comorbidities were collected by using a questionnaire designed as per a standard, structured pro forma during enrolment. Anthropometric data such as height, weight, and WC were noted at the time of enrolment as explained below:

Height To measure the height, the subjects were made to stand on the floor against a vertical metric rule. Care was taken to see if the subjects were barefoot, arms hanging freely by the sides, line of vision straight to the body, the head straight, and heels contact with measuring board. Horizontal headboard was pulled down to touch the head at the uppermost point. Reading on the topmost point of the head was taken nearest to 0.1 cm when the participant inhaled deeply at erect position.

Weight ISI certified machine was used to measure with variation nearest of 100 g. Participants were wearing light

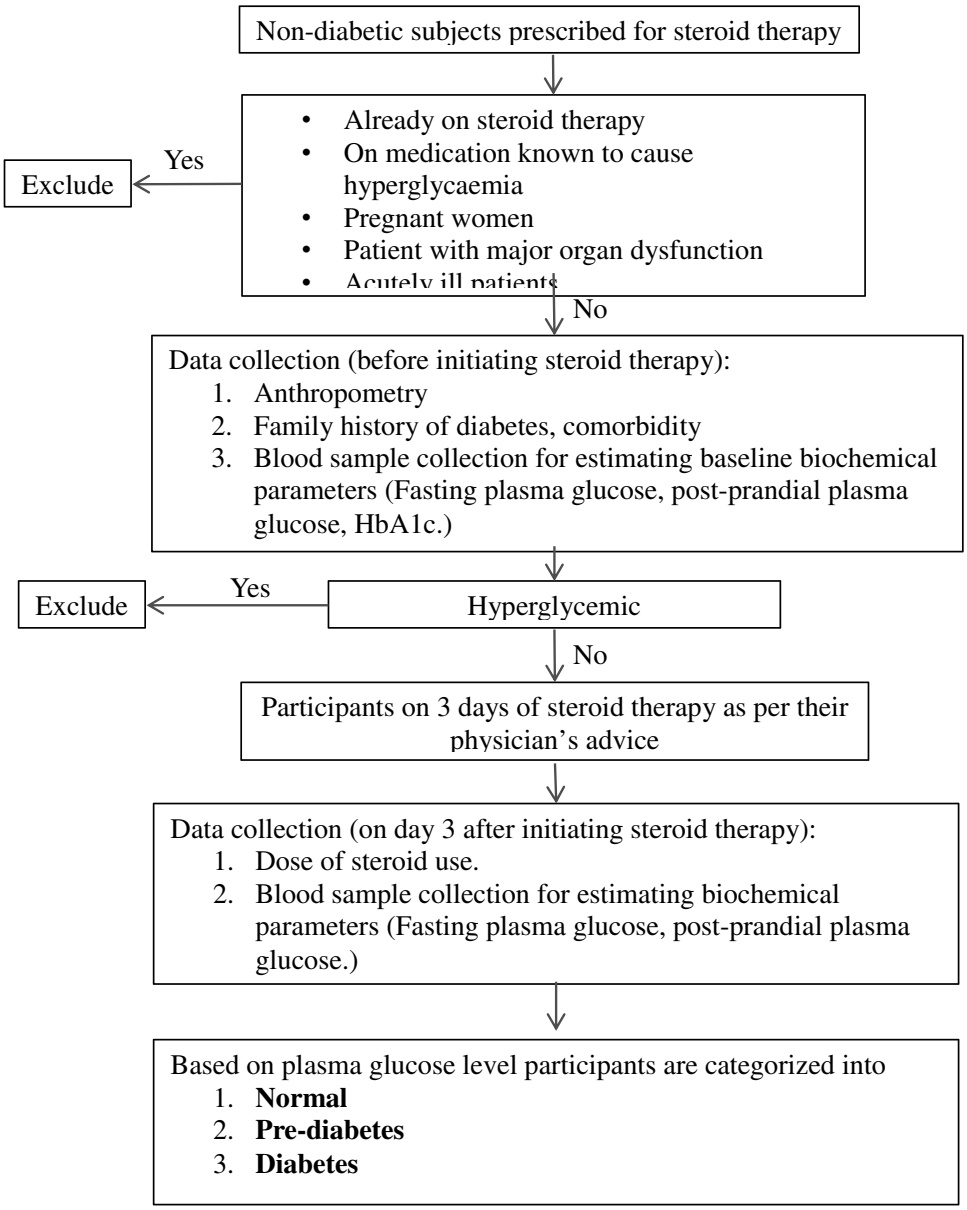

Fig. 1 Schematic representation of the protocol of this cohort study

clothes, without footwear, and standing straight with evenly distributed body between the feet on the middle of the weighing machine.

Body mass index (BMI) BMI was estimated using the formula (Quetelet index) [7] mentioned below:

$$\text{BMI} = \text{weight (kg)}/\text{Height (m}^2\text{)}.$$

Waist circumference It was measured midway between the lowest rib and the iliac crest at the end of normal expiration (as per WHO STEPS protocol) [8].

Detailed physical evaluation General and systemic examination was carried out in a structured manner.

Physical activity measurement Physical activity level was subjectively assessed by the WHO guidelines for measuring physical activity, i.e., the Global Physical Activity Questionnaire (GPAQ). Based on the GPAQ outcomes, participants were classified into three groups: active, moderately active, and sedentary. GPAQ data will be expressed as metabolic equivalents (METs). MET is the ratio of a person's working metabolic rate relative to the resting metabolic rate. One MET is defined as the energy cost of sitting quietly and is equivalent to a caloric consumption of 1 kcal/kg/h. During GPAQ data analysis, 4 METs get assigned to the time spent in moderate activities and 8 METs to the time spent in vigorous activities [9].

Table 1 Components of IDRS and risk stratification

Categorized risk factors	Score
Age	
< 35 years	0
35–49 years	20
≥ 50 years	30
Abdominal obesity	
Waist circumference female < 80 cm, male < 90 cm (Reference)	0
Female 80–89 cm, male 90–99 cm	10
Female ≥ 90 cm, male > 100 cm	20
Physical activity	
Vigorous exercise or strenuous at work	0
Moderate exercise at work/home	10
Mild exercise at work/home	20
No exercise and sedentary at work/home	30
Family history	
Two non-diabetic parents	0
Either parent diabetic	10
Both parents diabetic	20
Maximum score	100

Score ≥ 60, high-risk; 30–50, medium-risk, < 30, low-risk

Blood sample collection

Blood was collected by venipuncture after overnight fasting and 2 h after standard breakfast. Plain vacutainers, fluoride vacutainers, and EDTA vacutainers were used for collecting serum, plasma, and whole blood samples, respectively. Samples were allowed to clot in plain vacutainers for 30 min. Venous blood was then centrifuged at 3500 rpm for 10 min to separate plasma and serum from RBCs. FPG and PPG were measured in respective plasma samples.

Analytical methods

Automated auto-analyzer Hitachi P800 was used to measure FPG and PPG. The coefficient of variation for intra- and inter-batch was < 2% and < 5%, respectively. HbA1c was measured by HPLC method.

Statistical analyses

The incidence of steroid-induced diabetes and pre-diabetes was calculated and presented in percentage. The continuous variables such as age, BMI, and waist circumference, which were normally distributed, were expressed as mean ± SD. Data such as dose of steroids used, which were not normally distributed, were expressed as the median and interquartile range (IQR). Categorical variables such as abdominal obesity physical activity etc. were expressed in percentage. A comparison between glycemic status groups was tested by one-way ANOVA. Chi-square test was used to compare the categorical variables across the groups. Utilization of IDRS across three study groups was evaluated by using Chi-square test. $p < 0.05$ was considered to be statistically significant. ROC curve was used to derive cut-off value of IDRS for predicting SID. Ordinal regression analysis was used to derive IDRS score having odds of steroid-induced diabetes. Statistical analysis was performed using SPSS software version 20.0.

Results

SID was considered based on FPG and PPG levels measured on day 3 of steroid use. Out of 317 subjects, SID was found in 132 (42%) subjects and pre-diabetes in 105 (33%) subjects. The remaining 80 (25%) subjects did not show any abnormal glycemic changes. Predisposing factors like age, obesity, abdominal obesity, diabetes history in the family, and physical activity were compared across different groups of steroid-induced diabetes as follows. The subjects' mean age in pre-diabetes and diabetes groups was significantly high when matched to a normal group (p = 0.04). The data indicates that age may be a significant risk factor for diabetes among subjects on steroid therapy (Table 1). A comparison of BMI across steroid-induced diabetic groups was made in the current study. The data revealed significantly higher BMI in the diabetes group when compared to that of pre-diabetes and diabetes groups (Table 1). There was no significant difference (p = 0.6) in mean waist circumference across steroid-induced diabetic groups, tested by two-way ANOVA (Table 2). However, data on the incidence of diabetes showed a significant increase in steroid-induced diabetes among subjects with abdominal obesity when compared to those with normal waist circumference (51% vs. 34%).

A sedentary lifestyle is a known risk factor for developing diabetes. As per WHO guidelines for measuring physical activity, the study subjects were divided into four groups: active, moderate, mild, and sedentary. The incidence of steroid-induced diabetes was compared across physical activity groups. Though there is a clinically meaningful increase in the incidence of diabetes among sedentary subjects when compared to that of active subjects (52% vs. 34%), the Chi-square test failed to show the significant association of SID with the level of physical activity (p = 0.359). The detailed data are presented in Table 3.

The presence of a diabetes history in the family is one of the diabetes risk factors. In the current study, this was found in 27 (8.5%) subjects only. There was no significant relationship of history of diabetes in the family with the incidence of SID (p value = 0.388).

Table 2 Comparison of predisposing factors (age, BMI, and abdominal obesity) across steroid-induced diabetic groups

SID groups	Age (years)#	BMI (kg/m^2)#	Waist circumference (cm)#		Abdominal Obesity (%)
			Male	Female	
Normal	38.02 ± 13.63	21.8 ± 5.09	83.03 ± 11.58	78.9 ± 8.6	24 (17.8)
Pre-diabetes	42.41 ± 13.83	22.4 ± 4.67	83.37 ± 8.5	82.28 ± 10.64	42 (31.1)
Diabetes	42.68 ± 13.86	23.3 ± 4.45	84.90 ± 8.7	81.88 ± 10.92	69 (51.1)
p value	**0.04***	**0.03***	0.6		**0.005***

#, mean ± SD

*, statistically significant

The values in bold are statistically significant results.

Table 3 Comparison of the incidence of steroid-induced diabetes across physical activity groups

Physical activity	Normal	Pre-diabetes	Diabetes	Total	*p* value
Active (3000 MET/week)	18 (32.1)	19 (33.9)	19 (33.9)	56	0.359
Moderate (1500 MET/week)	29 (21.8)	48 (36.1)	56 (42.1)	133	
Mild (600 MET/week)	25 (30.5)	24 (29.3)	33 (40.2)	82	
Sedentary (Not meeting above criteria)	8 (17.4)	14 (30.4)	24 (52.2)	46	

Values are counted (%). *MET*, metabolic equivalents

Table 4 IDRS profile of the study population

IDRS category	No. of subjects (%)	Dose (mg/kg)
Low-risk (< 30)	115 (36.3)	0.9 (0.7, 1.11)
Medium-risk (30–50)	116 (36.6)	0.97 (0.83, 1.6)
High-risk (> 60)	86 (27.1)	0.88 (0.6, 1.3)

Table 5 Incidence of steroid-induced diabetes across IDRS risk categories

SID groups	Low-risk	Medium-risk	High-risk	*p* value
Normal	40 (34.8)	26 (22.4)	14 (16.3)	**0.001***
Pre-diabetes	35 (30.4)	48 (41.4)	22 (25.6)	
Diabetes	40 (34.8)	42 (36.2)	50 (58.1)	

Values are counted (%)

*Statistically significant

The values in bold are statistically significant results.

IDRS scores were prepared using four parameters as described previously. Based on the IDRS, the study participants were categorized into "low-risk (< 30), medium-risk (30–50), and high-risk (> 60) groups." As shown in Table 4, the study population consisted of 115 low-risk, 116 medium-risk, and 86 high-risk individuals. The distribution of prednisolone equivalent dose (mg/kg) was the same across IDRS category.

The incidence of SID after 3 days of steroid therapy was compared across IDRS categories. The incidence of SID was significantly high among high-risk category (58%) when compared to that of low-risk (34.8%) and medium-risk (36%) categories. Chi-square test showed a significant association of IDRS with the incidence of SID with *p* value < 0.001(Table 5).

Ordinal regression was done to calculate odds ratio. The odds of developing steroid-induced diabetes were 1.69 times higher with IDRS score of 60 and higher. This was statistically significant, Wald X^2 (1) = 64.7 and *p* value < 0.001 (95% C.I, 1.24–2.16). Receiver operating curve (ROC) analysis was performed to derive cut-off value of IDRS for identifying subjects with hyperglycemia and SID. IDRS of more than 30 had 71.4% sensitivity and 44.6% specificity for SID (*p* value = 0.001) as shown in Fig. 2.

Discussion

IDRS, a simple screening tool for the prediction of undiagnosed diabetes, was developed by Dr. Mohan and colleagues at the Madras Diabetes Research Foundation (MDRF), Chennai. IDRS was derived from the Chennai Rural Epidemiology Population Study (CURES) and was internally validated using the data from the Chennai Urban Population Study (CUPS). IDRS has 2 modifiable components, namely, physical activity and waist circumference, and 2 non-modifiable risk factors, age and family history of diabetes. IDRS uses a scoring system of 0 to 100. Individuals were classified as high-risk (score ≥ 60), moderate-risk score (30–50), and low-risk (score < 30) [4]. Several studies have confirmed that IDRS is a cost-effective screening tool for

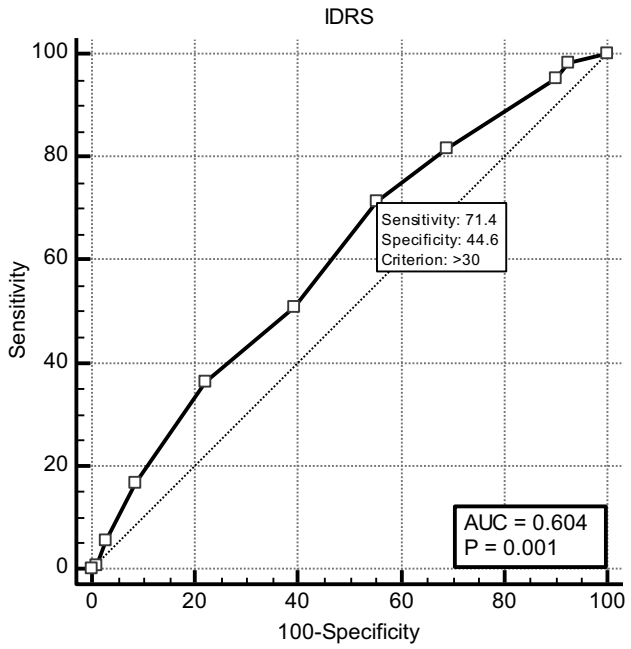

Fig. 2 ROC curve for predicting cut-off value of IDRS in SID

type 2 diabetes. Since its inception, the role of IDRS has been expanding. Its present applications include the detection of incident diabetes, non-alcoholic fatty liver disease [10], metabolic syndrome and coronary artery disease [11], and for classifying the type of diabetes [12].

In our study, we analyzed all patients for the presence of risk factors included in IDRS. An IDRS > 30 had 71.4% sensitivity and 44.6% specificity in predicting SID in our study. IDRS of more than 60 had 1.69 odds of developing SID. In a study by Adhikari et al., IDRS of more than 60 had sensitivity and specificity of 62.2% and 73,7%, respectively, for detecting type 2 diabetes in community [13]. In a study by Nagarathna R et al. on assessment of risk factors in diabetes using IDRS, a value of > 50 had sensitivity of 78.05% and specificity of 62.68%, respectively [14]. However, similar studies of utility of IDRS in predicting SID are lacking.

We proved IDRS score was useful in predicting the risk of developing SID by comparing the incidence of diabetes with the IDRS categories across three groups and found steroid-induced diabetes was significantly high among high-risk category (58%) when compared to that of low-risk (34.8%) and medium-risk (36%) categories. There was a significant association of IDRS with the incidence of steroid-induced diabetes (p value < 0.001). These results are unlike from another two Indian studies by Vikram et al. and Kartik et al. where they observed no correlation between IDRS and SID [15, 16] and they stated IDRS score was not useful in predicting the risk of developing SID, but it was not confirmatory since the sample size was not large in their studies. These results have potentially significant clinical implications because, for several diseases, steroids are still the first-line drug of choice.

The incidence of SID in our study was 42%. In comparison, the incidence of SID has been variable ranging from 1.2 to 47% in several studies [3]. Risk factors for SID include the dose of steroid, type of steroid, and steroid treatment duration, age, abdominal obesity, and history of diabetes in the family. While we know the older age is the risk factor for diabetes, but in the case of SID, the data is not clear. The mean age of our study population is 41.40 ± 13.89. Further, the maximum number of subjects (72.8%) is aged less than 50 years in our study. Previous studies have stated that aging is a risk factor for the development of SID [3, 17, 18]. The reason behind this may be a decrease in glucose tolerance with aging. Beta cell function also decreases with aging leading to decreased basal insulin level. In addition to these, factors such as decreased physical activity, obesity, and several medications increase IR with age [19]. In our study, subjects who developed pre-diabetes (42.4 ± 13.8) or diabetes (42.6 ± 13.8) after 3 days of steroid therapy are older when compared to those who retained normal glycemic status (38 ± 13.6). The Japanese study by Takayuki et al. (65.2 vs. 50.4) [17] and South Korean study by Kim et al. [18] (65 vs. 53) also found similar observations.

Obesity and abdominal obesity are often associated with impaired glucose tolerance and increased risk of type 2 diabetes. The current study also found a significantly higher mean BMI value in SID (23.3 ± 4.45) and pre-diabetes (22.4 ± 4.67) compared to those with normal blood glucose (21.8 ± 5.09). The incidence of steroid-induced diabetes was also high among subjects with high waist circumference when compared to those with normal waist circumference (51% vs. 34%) as revealed by Chi-square analysis with p = 0.005. This data indicates that both obesity and abdominal obesity increase the risk of diabetes in steroid users. However, some studies have shown no correlation with steroid-induced diabetes [15, 20, 21].

The family history of diabetes is one of the well-known risk factors for type 2 diabetes. Overall, only 27 subjects in the current study had a family history of diabetes, and only 10 of the 127 diabetics and 13 of the 99 pre-diabetics had a family history of diabetes. The results failed to show a significant association of SID with a family history of diabetes. This indicates that steroid treatment is not unmasking hidden diabetes; instead, the newly developed diabetes may be mainly due to steroid-induced adverse mechanism which is independent of a family history of diabetes. Our results are similar to the study by Simmons et al. which showed no significance of family history of diabetes in the development of SID [3].

The study has some limitations. The sample size may not be adequate, as this is a secondary analysis of data collected from another study. The study has 71.4% sensitivity,

but the specificity is 44.6%. Heterogeneous dose of steroids across the study groups could have impacted the results. Further studies with appropriate sample size and robust study designs focused on SID and IDRS as primary objective may be needed to confirm these conclusions.

Conclusions

As the therapeutic benefits of steroids continue to expand across medical specialties, the incidence of steroid-induced diabetes will continue to rise. In our study, steroid-induced diabetes was seen in 42% and pre-diabetes in 33% of our subjects. Increased age and obesity are maybe the predisposing factors for steroid-induced diabetes. The cumulative scores of IDRS were found to be higher in patients with SID, despite the traditional risk factors for diabetes, such as family history and sedentary life not found to be significantly associated. In the light of emerging evidence regarding steroid use and diabetes among COVID patients and increased incidence of mucormycosis, IDRS can also be used to screen individuals for SID. This score could be used as a preliminary screening test for patients treated with steroids on OPD basis, which should be confirmed by further specific tests.

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Declarations

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

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

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Two nested syndromes: fibromyalgia and neuropathic pain in prediabetes—a pilot study

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Abstract

Background This study aimed to determine the prevalence of neuropathic pain (NeP) and fibromyalgia syndrome (FMS) in prediabetics and to compare them with normoglycemic controls, to disclose the impact of NeP and FMS in disease burden.
Methods People, 18–65 years old, who were admitted to a tertiary hospital's internal medicine outpatient clinic for routine health check-ups and then those who were newly diagnosed with prediabetes were recruited as a prediabetic group and those who were normoglycemic were recruited as a control group. Participants' demographics and clinical data were recorded. The ShortForm-36 and Hospital Anxiety-Depression Scale were answered by the participants. The 2016 ACR Fibromyalgia Diagnostic Criteria and the painDETECT questionnaire were used for evaluation of FMS and NeP, respectively. One hundred nine prediabetics and 53 controls were enrolled.
Results Eighty-four (77.1%) of 109 prediabetics and 37 of 53 controls (69.8%) were female. The mean age of the prediabetics was 48.85 (SD = 9.8) and the controls was 47.37 (SD = 11.11). Age, gender, BMI, smoking status, occupational, marital, and educational status were similar between the groups. FMS was more common in prediabetics 32 (29.6%) of 109 than in normoglycemics 7 (13.2 %) of 53 ($p = 0.022$). Eight of the prediabetics (7.4%) and 2 of the normoglycemics (3.8%) had possible or likely NeP ($p = 0.273$). The frequency of possible or likely NeP was higher in prediabetics with FMS than without FMS ($p = 0.001$). The prevalence of FMS was higher in prediabetics with NeP (87.5%) than without NeP (25.5%) ($p = 0.001$). Prediabetics with FMS or NeP had lower QoL than without FMS or NeP ($p < 0.001$, $p = 0.014$, respectively).
Conclusion While evaluating prediabetics, it is important to assess both FMS and NeP.

Keywords Fibromyalgia syndrome · Quality of life · Neuropathic pain · Prediabetes

Introduction

Fibromyalgia syndrome (FMS) has controversies in terms of definition, pathogenesis, and diagnosis in its evolving historical process. However, it is obvious that patients with FMS complain of chronic pain and non-pain symptoms such as fatigue and sleep disturbance [1]. In recent years, it was shown that neuropathic changes in skin small fibers have taken a part in the pathogenesis of pain in FMS [2]. While it was once necessary to exclude FMS in diagnosing neuropathic pain (NeP), new evidence raises the question of whether fibromyalgia is a neuropathic pain [3]. Both FMS and NeP are considered chronic pain syndromes with similar pathogenetic mechanisms. Another common aspect is that both FMS and NeP negatively affect quality of life (QoL) of patients and increase the burden of the disease, which they accompany [4, 5].

Diabetes mellitus (DM) leads to chronic vascular and non-vascular complications. One of the non-vascular complications is chronic pain syndromes, in which FMS and NeP are more frequent in the diabetic population, 17–23.3% [6–10] and 8–26% [11–14], respectively. Due to NeP and FMS being nested syndromes, it was shown in a study that peripheral neuropathy was detected in 61.9% of diabetics with FMS compared to only 2.5% in diabetics without FMS [8]. Prediabetes (PD) is an intermediate stage between DM

This paper was not presented anywhere

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and normoglycemia [15] and may be a window of opportunity to struggle DM and its complications. Although many studies evaluated NeP and FMS in DM, there was only one study evaluating NeP in PD [16] and there was no study evaluating FMS in PD. Simultaneous assessment of the two interrelated diseases, FMS and NeP, is essential to obtain reliable results. Therefore, this study aimed to determine the prevalence of NeP and FMS in prediabetics and to compare them with normoglycemic controls, to disclose the impact of NeP and FMS in disease burden.

Materials and methods

A cross-sectional study was conducted from December 2018 to April 2019. Fasting plasma glucose (FPG) and HbA1c levels were measured for all 18–65-year-old participants, who were applied to a tertiary hospital's internal medicine outpatient clinic for routine health check-ups and who agreed to participate in the study. The glucose values of 0 and 2nd hour of the oral glucose tolerance test (OGTT), which is a 2-h test that checks participants' blood sugar levels before and 2 h after participants drink a 75 g anhydrous glucose solution, were conducted for all participants without diagnosed diabetes. Participants, whose plasma glucose levels were in the prediabetic range according to the American Diabetes Association Criteria [15], were recruited to the study as the prediabetic group. Participants, whose plasma glucose levels were in the normal range, were recruited to the study as the normoglycemic control group. PD was defined as OGTT-0 value of 100–125 mg/dl (impaired fasting glucose; IFG) and/or OGTT-2nd hour value of 140 to 199 mg/dl (impaired glucose tolerance; IGT) [15]. HbA1c value of 5.7 to 6.4% was also considered PD [15]. Participants' demographics and clinical data were recorded. General outcome measures, such as the Short Form-36 (SF-36) and the Hospital Anxiety and Depression Scale (HADS), were answered by participants.

Exclusion criteria were as follows: having a previous diagnosis of psychiatric disease, chronic neurologic disease, inflammatory rheumatic disease, hypothyroidism, hyperthyroidism, vitamin B12 deficiency, and pregnancy.

A total of 162 participants (109 prediabetic and 53 normoglycemic control group participants) were enrolled in the study.

Health indicators

Height and weight were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. BMI was categorized as normal (BMI < 30 kg/m^2) and obese (BMI: 30 kg/m^2 and above) [17].

Measurement of laboratory parameters

A fasting venous blood sample was collected after an overnight fast of at least 12 h for biochemical investigations, and samples were processed in the hospital laboratory on the same day. Fasting plasma insulin (FPI) and glucose were estimated using a Roche Cobas 8000 immunoassay analyzer (Roche Diagnostics, USA). Plasma glucose values at 0 and the 2nd hour were conducted by an oral glucose tolerance test (OGTT), and glycated hemoglobin (HbA1c) levels were measured for all participants. The level of HbA1c was estimated using an Adams A1c HA-8180 V automatic analyzer (Arkray Diagnostics, USA). All assays were performed with specific kits and calibrators supplied by the manufacturers.

Insulin resistance (IR)

Twelve-hour fasting blood samples were obtained for FPI and FPG determinations in order to calculate the homeostasis model assessment of insulin resistance (HOMA-IR). It was determined by the formula [18]:

HOMA-IR = FPI (mU/L) × FPG (mmol/l)/22.5. If the result is ≥ 2.5, it indicates the presence of insulin resistance. The higher the score, the greater the insulin resistance is measured.

Hospital Anxiety and Depression Scale

The HADS is a self-reported questionnaire to evaluate and measure the risk of depression and/or anxiety [19]. HADS is a reliable and validated psychometric scale, which includes 14 questions; half of them assess anxiety and the other half assess depression with four possible answers (score 0–3). According to the Turkish validation study of the HADS, scores ≥ 11 were accepted as having anxiety, and ≥ 8 were accepted as having depression in the current study [19].

Short Form-36

The SF-36 is a valid and reliable questionnaire to assess both physical and mental components of QoL [20, 21]. The SF-36 contains 36 items associated with 8 dimensions: physical functioning for limitations in performing all physical activities, role-physical for problems with work or other daily activities, bodily pain, general health, vitality, social functioning, role-emotional, and mental health [20]. The SF-36 is also a valid and reliable questionnaire in Turkish people [22].

Assessment of neuropathic pain

The painDETECT questionnaire (PDq) was used to evaluate the presence of NeP by the same experienced physician (KE), who was blinded to the clinical findings of the

participants. The PDq, a Turkish validated and reliable NeP screening tool [23, 24], consists of 3 parts and 12 items. The first section contains 3 items and evaluates the intensity of pain at the moment and the average and maximum pain intensity during the past 4 weeks on a 0- to 10-point numerical rating scale. This first section is not included in the scoring. In the second section, one of the 4 pain course patterns related with nociceptive or neuropathic components are determined. The other item is related to radiating pain. Sensations (burning, tingling or prickling, allodynia, pain attacks, temperature evoked pain, numbness, and pressure-evoked pain) are scored with a 0- to 5-point numerical rating scale in the third section. The total score of the PDq (from 1 to 38) is calculated by summing the scores of the items. A total score of 0–12 indicates "unlikely," 13–18 indicates "possible," and ≥ 19 indicates "likely" for NeP.

Assessment of fibromyalgia syndrome

The participants were evaluated and examined for the classification of FMS, by the same experienced physician (KE), who was blinded to the clinical findings of the participants. Participants were classified as having FMS according to the 2016 American College of Rheumatology Fibromyalgia Classification Criteria [25]. To assess the current health status of participants with the FMS, a Turkish validated and reliable version of Fibromyalgia Impact Questionnaire (FIQ) was used [26].

Statistical analysis

A power analysis program, G*Power version 3.0.10 software (Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany), was used to calculate the post hoc power analysis. It was done considering the presence of FMS. The effect size was 0.512. The power was calculated as 0.84 for $\alpha = 0.05$ with a sample size of 53 in the control group and of 109 in the study group.

Statistical analyses were performed using the SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Number of cases and percentages were used for categorical variables. Categorical data was analyzed by chi-square or Fisher's exact test, where appropriate. Shapiro–Wilk test and histograms were used to determine whether continuous variables were normally distributed. Normally distributed variables were presented as means and standard deviations (SD), non-normally distributed variables were presented as medians and interquartile ranges (IQR: per 25–75). Two independent groups of parametric variables were compared using the Student *t*-test. For non-parametric variables, the Mann–Whitney *U* test was administered. The relationship between non-parametric variables was analyzed by Spearman correlation tests and the relationship between parametric variables was analyzed by a Pearson correlation test. A p value of < 0.05 was considered to indicate statistically significant differences.

Results

Eighty-four (77.1%) of 109 prediabetics and 37 (69.8%) of 53 normoglycemic control group subjects were female. The mean age of the prediabetic group was 48.85 (SD = 9.8) years and the control group participants was 47.37 (SD = 11.11) years. The median BMI value of the prediabetics was 32.46 kg/m^2 and the control group participants was 30.67 kg/m^2. Age, gender, BMI, smoking status, professions, marital status, and educational status were similar between the prediabetic and control groups. Two people in both prediabetic (1.8%) and control (3.8%) groups were one unit weekly alcohol drinker. Sociodemographic data is summarized in Table 1. HOMA-IR values were also similar between groups. In prediabetic group, 53 patients (48.6%) had IFG, 15 patients (13.8%) had IGT, 32 patients (29.4%) had both IFG and IGT, and 9 patients (8.3%) had isolated elevated HbA1c.

Presence of comorbidity in participants was as follows: 5 patients (4.7%) in PD group and 1 participant (1.9%) in control group had hyperlipidemia, 2 patients (1.9%) in PD group and no one in control group had coronary artery disease, 2 patients (1.9%) in PD group and no one in control group had chronic obstructive lung disease, and 3 patients (2.8%) in PD group and no one in control group had gastroesophageal reflux. These data were not appropriate for statistical analyses. A total of seven patients (6.5%) in PD group and 6 participants (11.5%) in control group had asthma ($p = 0.281$); 22 patients (20.6%) in PD group and 7 participants (13.5%) in control group had hypertension ($p = 0.277$).

FMS was more common in prediabetics (32 (29.6%) of 109 prediabetics) than in normoglycemics (7 of 53 normoglycemics (13.2)) ($p = 0.022$). FIQ total scores were similar between prediabetics with FMS and normoglycemics with FMS ($p = 0.266$). Eight of prediabetics (7.4%) and 2 of normoglycemics (3.8%) had NeP according to the PDq, but this difference was not statistically significant ($p = 0.273$). PDq total scores were also similar between prediabetics and normoglycemics ($p = 0.095$). Comparison of clinical variables of prediabetic and normoglycemic groups is summarized in Table 2.

When comparing prediabetic patients with or without FMS, age, BMI, OGTT for the 0 and 2nd hour values, HbA1c, and HOMA-IR values were similar between the groups. Female gender was more common in prediabetics with FMS (96.9%) than without FMS (68.4%), ($p = 0.001$). Patients with FMS had lower SF-36 scores than without FMS ($p < 0.001$). Frequency of risk of anxiety and/or

Table 1 Sociodemographic data of prediabetic patients and control group participants

	Prediabetics (n = 109)	Controls (n = 53)	*p* value
Gender (F/M), n (%)	84 (77.1)/25 (22.9)	37 (69.8)/16 (30.2)	0.319
Age (year), mean (SD)	48.85 (9.80)	47.37 (11.11)	0.337
Occupation, n (%)			0.212
Housewife	77 (72.0)	26 (55.3)	
Officer	10 (9.3)	6 (12.8)	
Worker	9 (8.4)	8 (17.0)	
Retired	11 (10.3)	7 (14.9)	
Marital status			0.821
Married	99 (92.5)	44 (93.6)	
Divorced	1 (0.7)	0 (0)	
Single	7 (6.5)	3 (5.4)	
Education			0.392
Illiteracy	4 (3.7)	0 (0)	
Primary school	82 (76.7)	32 (68.1)	
High school	11 (10.3)	6 (12.8)	
University	10 (9.3)	9 (19.1)	
Menopausal status, n (%)			0.865
Premenopause	44 (52.4)	20 (54.1)	
Postmenopause	40 (47.6)	17 (45.9)	
Smoking, n (%)			0.053
Never	82 (75.2)	38 (71.7)	
Quit	17 (15.6)	3 (5.7)	
Smoker	10 (9.2)	12 (22.6)	
BMI (kg/m^2), median (per 25–75)	32.46 (28.45–38.56)	30.67 (27.34–36.79)	0.150
Obesity (+), n (%)	74 (67.9)/35 (32.1)	31 (60.8)/20 (39.2)	0.378

BMI, body mass index; *F/M*, female/male

depression was higher in prediabetics with FMS than without FMS ($p < 0.001$). VAS-fatigue levels were also higher in prediabetics with FMS than without FMS ($p < 0.001$). Frequency of possibly or likely NeP was higher in prediabetics with FMS than without FMS ($p = 0.001$) (Table 3).

To compare prediabetics with or without NeP, patients having possible or likely NeP were grouped as NeP (+) and patients having unlikely NeP were grouped as NeP (−). Age, gender, BMI, OGTT for the 0 and 2nd hour values, HbA1c, and HOMA-IR values were similar between groups. Patients with NeP had lower SF-36 scores than those without NeP. Frequency of risk of anxiety and/or depression was similar between prediabetics with or without NeP. VAS-fatigue levels were also quite similar between prediabetics with or without NeP. Prevalence of FMS was higher in prediabetics with NeP (87.5%) than in those without NeP (25.5) ($p = 0.001$) (Table 3).

Discussion

In this study, we had three main findings. First, while FMS occurred more in prediabetics than in normoglycemic subjects, NeP occurred similarly between both groups. Second, NeP prevalence was higher in prediabetics with FMS than in those without FMS, and vice versa, where FMS frequency was higher in prediabetics with NeP than in those without NeP. Third, while glycemic levels were similar in prediabetics with or without FMS and in prediabetics with or without NeP, impaired QoL was observed in prediabetics with FMS or NeP.

Whether FMS is a rheumatic disease, a psychosomatic disorder, a central sensitization syndrome, a neuropathic pain syndrome, or a psycho-cultural movement is still being debated [27]. However, the truth is that FMS leads to a serious decrease in QoL, reduces work productivity, and increases the burden of the disease accompanied by it [4]. Based on this, FMS prevalence was investigated in DM, as with many chronic diseases. Wolak et al. found the prevalence of FMS was 23.3% in women with type 2 DM and Tishler et al. found it was 15.5% [6, 7]. Both found that the prevalence of FMS was higher in diabetics than in healthy subjects. Yanmaz et al. found the prevalence of FMS was 18% in patients with type 2 DM [10]. In this study, the first study evaluating FMS in PD shows that the prevalence of FMS was 29.6% in prediabetics and was 13.2% in normoglycemic subjects and the difference was statistically significant. The prevalence of FMS was higher in our study than in abovementioned three studies that evaluated FMS in type 2 DM [6, 7, 10]. In all three studies, FMS was

Table 2 Comparison of clinical variables of prediabetic patients to control group participants

	PD (n = 109)	Control (n = 53)	*p* value
OGTT-0 (mg/dl), mean (SD)	104.86 (8.24)	91.36 (5.53)	**< 0.001**
OGTT-2 (mg/dl), mean (SD)	131.08 (32.50)	107.17 (16.90)	**< 0.001**
HbA1c, median (per 25–75)*	5.9 (5.7–6.2)	5.5 (5.3–5.6)	**< 0.001**
HOMA-IR, median (per 25–75)**	2.36 (1.59–3.83)	2.12 (1.33–3.26)	0.218
IR (+), n (%)	45 (46.9)	18 (42.9)	0.663
SF-36 dimensions, median (per 25–75)			
SF-36/ PCS	54.06 (31.72–83.91)	87.50 (57.50 94.06)	**< 0.001**
SF-36/ MCS	52.56 (35.38–76.56)	78.65 (67.44 87.00)	**< 0.001**
SF-36/ TS	51.59 (35.08–79.64)	80.66 (60.99 90.27)	**< 0.001**
HADS			
Anxiety (+), n (%)	27 (25.2)	1 (2.2)	**< 0.001**
Depression (+), n (%)	39 (36.4)	6 (13)	**0.004**
VAS-fatigue, median (per 25–75)	6 (4–7)	3 (2–6)	**< 0.001**
FMS (+), n (%)	32 (29.6)	7 (13.2)	**0.022**
FIQ-t, mean (SD)	54.75 (16.86)	45.41 (12.87)	0.266
PDq-t, median (per 25–75)	1 (1–3)	1 (0–3)	0.095
PDq-NeP			0.273
Unlikely, n (%)	101 (92.7)	51 (96.2)	
Possible, n (%)	5 (4.6)	0 (0)	
Likely, n (%)	3 (2.8)	2 (3.8)	

FIQ-t, fibromyalgia impact questionnaire-total score; *FMS*, fibromyalgia syndrome; *HADS*, Hospital Anxiety and Depression Scale; *HbA1c*, glycated hemoglobin; *HOMA-IR*, homeostasis model assessment of insulin resistance; *IR*, insulin resistance; *NeP*, neuropathic pain; *OGTT*, oral glucose tolerance test; *PDq-t*, painDETECT questionnaire total score; *SF-36*, Short Form-36; *SF-36/MCS*, Mental Component Score; *SF-36/PCS*, Physical Component Score; *SF-36/TS*, Total Score

*Coefficient of variations of HbA1c are 5.57 for PD group and 3.31 for control group

**Coefficient of variations of HOMA-IR are 82.51 for PD group and 64.01 for control group

classified according to the ACR 1990 Criteria and this may be the reason for this difference. This study determined FMS according to the ACR 2016 Diagnostic Criteria, which is a more sensitive diagnostic criteria compared to the ACR 1990 Criteria [28]. It should be noted that we also evaluated control group participants with the ACR 2016 Criteria. Consequently, increased FMS prevalence was found in the prediabetics when compared to the normoglycemic subjects, in this study. Other possible reason of the higher prevalence of FMS in the groups may be the mean age of participants which were 48.85 years in PD group vs 47.37 years in control group. Topbaş et al. noted that the prevalence of FMS was found to increase with age which was the highest in the 50–59 age group (10.1%) [29]. In the study, FMS prevalence was found 3.7% in 20–64 years women in Turkey [29]. Another possible reason of the higher prevalence of FMS in the groups may be the mean BMI of the participants which were 32.46 kg/m^2 in PD group vs 30.67 kg/m^2 in control group. Several mechanisms have been proposed to explain "the hidden link between FMS and higher BMI values," but at this time, it is not possible to ascertain whether obesity is cause or consequence of FMS [30]. Branco et al. found the prevalence of FMS was 2.2–6.6% in general population of five European countries [31]. They concluded that the prevalence of FMS was age- and gender-related and varied among countries [31]. In our study, the PD group was similar with the normoglycemic control group in terms of age, gender, occupation, marital status, educational status, BMI, and insulin resistance. This provides a favorable condition for comparing groups. Thus, it could be thought that increased FMS prevalence in PD is associated with own nature of PD.

Patients suffering from FMS have impaired QoL compared with the general population. These patients also suffer from anxiety, depression, and fatigue, possibly related to a central sensitization disorder [27]. In this study, although having similar OGTT and HbA1c values, prediabetic patients with FMS had worse QoL and more common anxiety, depression, and fatigue levels compared to prediabetic patients without FMS. We think that underlying and/or accompanying pathogenetic mechanisms of prediabetes such as dysregulated inflammatory cytokines and sleep disturbance may be the reason of similar metabolic variables between prediabetic patients with FMS and without FMS [27, 32, 33]. Another issue that needs to be clarified is that there are increased anxiety and/or depression risks and impaired QoL cause or consequence of the presence of FMS in prediabetes. These questions are also related to complex

Table 3 Comparison of clinical variables of prediabetic patients in terms of presence of FMS and NeP

	FMS (+);(n = 32)	FMS (−);(n = 76)	*p* value	NeP (+); (n = 8)	NeP (−); (n = 101)	*p* value
Gender (F/M), n (%)	31(96.9) / 1(3.1)	52(68.4)/24(31.6)	**0.001**	8 (100) / 0(0)	76 (75.2)/25(24.8)	0.194
Age (year), mean (SD)	50.28 (9.44)	48.25 (10.01)	0.320	49.63 (10.11)	48.79 (9.82)	0.828
Obesity (+), n (%)	24 (75)	49 (64.5)	0.286	5 (62.5)	69 (68.3)	0.710
BMI (kg/m^2), mean (SD)	34.56 (6.36)	33.40 (8.02)	0.427	36.98 (7.13)	33.47 (7.53)	0.217
OGTT-0 (mg/dl), mean (SD)	104.22 (8.29)	105.35 (8.09)	0.529	101.50 (8.57)	105.11 (8.16)	0.283
OGTT-2 (mg/dl), mean (SD)	130.34 (30.32)	132.04 (33.31)	0.836	122.88 (18.23)	131.50 (33.27)	0.259
HbA1c, median (per 25–75)	5.9 (5.75–6.2)	5.9 (5.7–6.2)	0.659	5.9 (5.75–6.3)	6.0 (5.7–6.2)	0.667
HOMA-IR, median (per 25–75)	2.94 (1.65–4.22)	2.26 (1.59–3.71)	0.384	3.51 (2.15–6.05)	2.28 (1.60–3.81)	0.295
IR (+), n (%)	14 (30)	56 (42.9)	0.258	4 (80)	41 (45.1)	0.183
SF-36, median (per 25–75)	27.5 (16.88–37.81)	67.81 (44.69–88.66)	**< 0.001**	26.25 (16.88–48.75)	58.13 (32.81–85.00)	**0.011**
SF-36/PCS	34.13 (23.63–44.48)	66.06 (40.31–82.78)	**< 0.001**	35.25 (20.75–57.19)	53.63 (35.81–88.78)	**0.031**
SF-36/MCS	31.44 (20.47–39.56)	66.59 (43.28–84.89)	**< 0.001**	33.44 (19.13–51.31)	52.81 (36.65–81.49)	**0.014**
SF-36/TS						
HADS	16 (51.6)	11 (14.7)	**< 0.001**	2 (28.6)	25 (25)	1.000
Anxiety (+), n (%)	20 (64.5)	19 (25.3)	**< 0.001**	4 (57.1)	35 (35)	0.255
Depression (+), n (%)						
VAS-fatigue, median (per 25–75)	7 (6–9)	5 (3 7)	**< 0.001**	8 (4–8.5)	6 (4–7)	0.052
FMS (+), n (%)	-	-	-	7 (87.5)	25 (25)	**0.001**
FIQ-t, mean (SD)	-	-	-	61.81 (16.38)	53.22 (16.92)	0.331
PDq-t, median (per 25–75)	3 (1–5)	1 (0.25–1)	**< 0.001**	-	-	-
PDq-NeP	25 (78.1)	75 (98.7)	**0.001**	-	-	**-**
Unlikely, n (%)	4 (12.5)	1 (1.3)				
Possible, n (%)	3 (9.4)	0 (0)				
Likely, n (%)						

BMI, body mass index; *FIQ-t*, fibromyalgia impact questionnaire-total score; *FMS*, fibromyalgia syndrome; *F/M*, female/male; *HADS*, Hospital Anxiety and Depression Scale; *HbA1c*, glycated hemoglobin; *HOMA-IR*, homeostasis model assessment of insulin resistance; *IR*, insulin resistance; *NeP*, neuropathic pain; *OGTT*, oral glucose tolerance test; *PDq-t*, painDETECT questionnaire total score; *SF-36*, Short Form-36; *SF-36/MCS*, Mental Component Score; *SF-36/PCS*, Physical Component Score; *SF-36/TS*, Total Score

nature of FMS. These outcomes are the first data regarding the impact of FMS to disease burden in PD and this sheds light for future studies on this topic.

NeP, caused by a lesion or disease of the somatosensory nervous system, contains a cluster of symptoms and signs such as burning, tingling, and numbness [34]. Sometimes the etiology of NeP is not determined in routine clinical procedures. Although affected by factors such as disease duration, disease severity, and diagnostic method, the prevalence of NeP was found to be 8–30% in various studies in type 2 DM [11–14, 35]. Ziegler et al. found that the prevalence of NeP was 8.7% in patients with IGT, 4.2% in patients with IFG, and 1.2% in normoglycemic subjects [16]. In accordance with this study, the prevalence of possible or likely NeP was 7.4% in the prediabetics. However, the prevalence of NeP in prediabetics was similar with the normoglycemic subjects in our study. The reason for this similarity may be that the prevalence of NeP in normoglycemic subjects was a little higher in our study than in Ziegler et al.'s study [16]. In Ziegler et al.'s study, there was only a statistically significant difference between the prevalence of NeP in IGT and in the normoglycemic group [16]. Thus, another possible reason of similar NeP prevalence in this study is that the number of patients is not enough to compare the prevalence of NeP in subgroups of PD such as IGT and IFG with normoglycemic groups. That this study group included newly diagnosed patients with PD may be another reason for the similarity.

It is well known that NeP is related to lower levels of QoL [36–38]. Lower QoL levels were also reported in diabetic patients with NeP than in those without NeP [39, 40]. However, there is no study evaluating the burden of disease including QoL in prediabetic patients with or without NeP. Although the presence of anxiety and/or depression was similar in prediabetics with or without NeP, we found lower QoL levels in prediabetics with NeP than in prediabetics without NeP in our study.

With a new NeP definition by the International Association for the Study of Pain, FMS was not considered a NeP because it was thought that there was no somatosensorial nervous system disease in FMS [41]. However, new evidence shows a role of small fiber neuropathy in pathogenesis of FMS [2]. Additionally, central sensitization takes a role in the pathogenesis of both NeP and FMS [3]. It was shown in a study based on the question "Is fibromyalgia a

NeP?" that FMS may have a NeP component [42]. In this study, prediabetics with FMS had more common NeP than prediabetics without FMS and vice versa that the prediabetics with NeP had more common FMS than prediabetics without NeP. Thus, it has been demonstrated in prediabetics that FMS and NeP are nested syndromes.

The present study has a few limitations. This is a monocentric study performed in a small number of patients, who were admitted to a tertiary center. Small fiber tests and comprehensive neurologic examination were not performed. Sleep disturbance and physical activity level were not evaluated separately. The strengths of this study include that we diagnosed FMS and NeP with a valid and reliable method and we investigated laboratory and clinical markers. In addition, our results should be complemented by future large studies, to obtain stronger results.

In conclusion, many prediabetic patients suffer from FMS that reduces their QoL. While NeP prevalence was similar between prediabetics and normoglycemics, prediabetics who had NeP had a lower QoL. While evaluating prediabetic patients, it is important to assess both FMS and NeP because they are nested syndromes.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analyses were performed by all authors. The first draft of the manuscript was written by KE and all authors commented on previous versions of the manuscript.

Data availability Available.

Code availability N/A.

Declarations

Ethics approval Erciyes University Local Ethical Committee approval was received (Date: 20.02.2019; No: 2019/141).

Consent to participate This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice. The patient's written informed consent to publish the clinical information and materials was obtained.

Consent for publication All authors have seen and approved the revised manuscript for publication.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

WISP1 is increased in the maternal serum, adipose tissue, and placenta of women with gestational diabetes mellitus

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Abstract

Objective This study aimed to investigate the concentration of Wingless-type (Wnt)-inducible signaling pathway protein-1 (WISP1) in the serum, and its expression in the abdominal subcutaneous adipose tissue (SAT), and placenta of women with gestational diabetes mellitus (GDM).

Methods A total of 69 patients with GDM and 71 pregnant women with normal glucose tolerance (NGT, control) were recruited. Carbohydrate metabolism, alanine aminotransferase (ALT), lipid profiles, thyroid function, interleukin-6 (IL-6), and WISP1 levels were assessed. Fasting sera were collected between 25 and 30 weeks of gestation. Tissues of placenta and abdominal SAT samples were obtained from 24 women who had undergone cesarean section and were divided into a GDM group and a control group. Reverse transcription polymerase chain reaction (RT-PCR) and western blot were used to detect the WISP1 expression.

Results The serum WISP1 concentrations were higher in the GDM group than in the control group ($p < 0.01$) and positively associated with body mass index (BMI), fasting glucose, fasting insulin, HOMA-insulin resistance (HOMA-IR), and IL-6 levels. BMI, fasting glucose, and HOMA-IR independently and positively predicted WISP1 levels. Further, WISP1 mRNA and protein expression were higher in tissues from the placenta and abdominal SAT from the GDM group ($p < 0.01$).

Conclusions WISP1 may be an important adipokine in modulating carbohydrate metabolism in women with GDM.

Keywords Gestational diabetes · Insulin resistance · Placenta · Subcutaneous adipose tissue · WISP1

Introduction

Gestational diabetes mellitus (GDM) is a common condition that occurs in up to 16% of pregnant women in high-risk populations [1]. Hyperglycemia during pregnancy can result in adverse pregnancy outcomes in both fetus and the mother. Macrosomia, premature rupture of membranes, and preeclampsia are more common in patients with GDM [2]. A good mental health status as well as regular exercises and a good diet will help the diabetic mother to achieve good glycemic control [3–5]. In addition, women with previously diagnosed GDM are approximately 30% more likely to develop type 2 diabetes within 10 years [6]. It is worth noting that the incidence of GDM has increased rapidly over the past decade as the global rate of obesity has increased [7].

The pathophysiology of GDM is not fully understood currently. Accumulating evidence indicates that the maternal adipose tissue plays an important role in pathogenesis of GDM [8]. Maternal obesity is considered to be one of the important variable risk factors. Recently, the role of inflammation as a regulator of metabolic disorder in obesity has received increasing attention [9]. Adipose tissue not only stores energy, but also modulates the endocrine function by secreting molecules known as adipokines or adipocytokines. Dysfunction of adipose tissue can cause low grade chronic inflammation and insulin resistance [10]. The risk of GDM is higher in overweight and obese women, and this may be partly related to adipokines released from the adipose tissue [11].

The placenta is not only an additional source of systemic cytokines during pregnancy, but also a target of cytokine action [12]. Studies have confirmed that chronic inflammation in the placenta tissue during pregnancy with

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obesity and GDM play an important role in determining the fetal environment [13, 14]. Moreover, maternal obesity and GDM have been related to changes in maternal–fetal transport of nutrients [15].

Wingless-type (Wnt)-inducible signaling pathway protein-1(WISP1) was recently identified as a novel adipokine. The expression of WISP1 correlates with index of insulin resistance and inflammation. In addition, treatment of macrophages with WISP1 may lead to pro-inflammatory responses [16]. Barchetta et al. [17] indicated that WISP1 expression increased in obese subjects and was linked with higher levels of C-reactive protein and interleukin (IL)-8 in adipocytes. Wang et al. [18] found that the level of serum WISP1 was correlated to increased adiponectin, leptin, and IL-18 expression, as well as with markers of insulin resistance. They confirmed that higher WISP1 expression was linked with increased adipocyte inflammation. In another study, Jung et al. [19] found that treatment with WISP1 significantly induced inflammation in hepatocytes and in the skeletal muscle cells of mice. These studies suggest that WISP1 may play an active part in the pathogenesis of chronic inflammation-related diseases such as obesity.

WISP1 mRNA expression has also been detected in the placenta and pancreas [20]. However, there is a lack of data regarding expression of WISP1 in adipose tissue and the placenta during pregnancy, particularly under conditions such as GDM. In this study, we measured the levels of WISP1 in serum and changes of WISP1 expression in the abdominal subcutaneous adipose tissue (SAT) and placenta of patients with GDM and pregnant women with NGT.

Material and methods

Study design and subjects

The subjects were divided into a GDM group and a normal glucose tolerance (NGT, control) group. Inclusion criteria for pregnant women include the following: (1) women who had singleton pregnancies; (2) women who had no current regular medications; and (3) women without history of glucose intolerance. Exclusion criteria are patients with adverse medical conditions (pregnancy-induced hypertension, preeclampsia, premature membrane rupture). GDM was diagnosed according to the criteria of the International Association of Diabetic Pregnancy Study Group (IADPSG). Oral glucose tolerance test (OGTT) was conducted using 75 g glucose loading at 24–28 weeks, and diagnosis of GDM was made by either a fasting venous glucose level of ≥ 5.1 mmol/L, or 1 h ≥ 10.0 mmol/L, or 2 h ≥ 8.5 mmol/L [21].

Study 1 population: effect of GDM on circulating maternal WISP1 levels

A total of 140 serum samples were collected from Chinese pregnant women with regular follow-up between 25 and 30 weeks of gestation visiting the outpatient department of Shengjing Hospital of China Medical University. Body mass index (BMI) was assessed at the day of inclusion. Whole blood samples were immediately centrifuged at 1,000 rpm for 5 min, and then serum samples were collected and stored at − 80 ℃ for further analysis.

Fasting plasma glucose (FPG) was detected by an automated glucose oxidase method using Olympus AU5800 (Tokyo, Japan). Fasting serum insulin (FIN) levels were determined using chemiluminescence assays (Huamei Biotech, Wuhan, China). Hemoglobin A1c (HbA1c) level was examined by high performance liquid chromatography (HPLC) using D-10 Hemoglobin Testing System (Bio-Rad, Shanghai, China). Blood triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and alanine aminotransferase (ALT) levels were determined by routine methods with an Olympus AU 5400 Automatic Analyzer (Tokyo, Japan). Thyroid function was detected by chemiluminescence immunoassay with commercially available diagnostic kits (Mingde Biotech, Wuhan, China). Insulin resistance was expressed by the HOMA-insulin resistance (HOMA-IR) index (HOMA-IR = FPG (mmol/L) × FIN (μU/mL) / 22.5). The concentrations of serum WISP1 and IL-6 were evaluated with the enzyme-linked immunosorbent assay (ELISA) method (Cloud-Clone Corp, Wuhan, China) according to the manufacturer's instructions.

Study 2 population: effect of GDM on adipose tissue and placental WISP1 gene expression

All participants in this study signed written informed consent. Pregnant women were selected for cesarean section in Shengjing Hospital of China Medical University, including 12 cases in GDM group and 12 cases in NGT group, respectively. All pregnant women in Study 2 have clinical indications to perform cesarean section. The maternal side of the placenta tissue and abdominal SAT (~ 1 cm^3) were collected from the pregnant woman shortly after delivery.

RNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was isolated from the placental tissue and abdominal SAT with Trizol reagent (Ambion, TX, USA). Then, 1 μg of total RNA was converted to cDNA using a cDNA synthesis kit (Transgen, Beijing, China). The cDNA was diluted tenfold and 2 μL was taken for RT-PCR using an Exicycler 96 (Bioneer, South Korea) and 100 nM of primers. The samples

were incubated in the Exicycler 96 for 40 PCR cycles according to the manufacturer's instructions. The primer sequences were shown in Table 1.

Western blot

Total protein of placental tissue and abdominal SAT was extracted with radioimmunoprecipitation assay lysis buffer. Protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Beyotime, Shanghai, China). Protein samples were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes. After 1 h of blocking with 5% non-fat milk at 2 5 ℃, the membranes were incubated with anti-WISP1 polyclonal antibody (1:1000; Boster, Wuhan, China) overnight at 4 ℃. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000; Wanleibio, Shenyang, China) for 2 h at 25 ℃. The immune complexes were observed with enhanced chemiluminescence reagent kit. Band strength was quantified using Gel-Pro-Analyzer software, and data were expressed as a ratio of the WISP1 and GAPDH integral optic density (IOD) values.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) and Student's t tests were used to analyze two sample comparisons. Univariate and multivariate regression analyses were utilized to identify independent variables of WISP1. The level of significance was considered at $p < 0.05$. All statistical analyses were performed using SPSS 13.0 program (SPSS, Chicago, IL, USA).

Results

Clinical and demographic characteristics of the subjects in Study 1

A total of 140 subjects were enrolled, including 69 patients in the GDM group and 71 in the control group. The clinical and biochemical characteristics of the two groups are shown in Table 2. Compared to the control group, GDM women had higher fasting glucose levels (5.45 ± 1.37 vs. 4.82 ± 1.41, $p = 0.008$), fasting insulin levels (13.89 ± 8.07 vs. 10.23 ± 7.64, $p = 0.007$), HbA1c (5.32 ± 0.84% vs. 4.97 ± 0.73%, $p = 0.009$), HOMA-IR (3.63 ± 1.34 vs. 2.42 ± 0.97, $p < 0.001$), and IL-6 (2.25 ± 0.51 vs. 1.92 ± 0.45, $p < 0.001$) values. However, there was no difference with respect to age, BMI, ALT, FT3, FT4, sTSH, TG, LDL-C, or HDL-C levels between the two groups.

Table 2 Demographic and clinical data of participants—*Study 1 population*

Category	GDM ($n = 69$)	Control ($n = 71$)	*P*
Age (years)	31.45 ± 3.23	31.23 ± 3.64	0.706
Gestational age (weeks)	26.0 ± 4.0	27.0 ± 3.7	0.127
BMI (kg/m^2)	26.53 ± 3.34	26.12 ± 3.23	0.462
ALT (U/L)	16.4 ± 9.83	19.2 ± 10.72	0.110
Fasting glucose (mmol/L)	5.45 ± 1.37	4.82 ± 1.41	0.008**
Fasting insulin (mU/mL)	13.89 ± 8.07	10.23 ± 7.64	0.007**
HOMA-IR	3.63 ± 1.34	2.42 ± 0.97	< 0.001**
HbA1c (%)	5.32 ± 0.84	4.97 ± 0.73	0.009**
FT3 (pmol/mL)	4.31 ± 0.42	4.42 ± 0.54	0.182
FT4 (pmol/mL)	12.18 ± 3.90	13.12 ± 3.28	0.125
sTSH (mIU/L)	1.66 ± 0.87	1.67 ± 0.85	0.945
TG (mmol/L)	1.85 ± 0.56	1.98 ± 0.71	0.231
LDL-C (mmol/L)	2.57 ± 0.59	2.73 ± 0.66	0.133
HDL-C (mmol/L)	1.52 ± 0.56	1.65 ± 0.69	0.223
IL-6(pg/mL)	2.25 ± 0.51	1.92 ± 0.45	< 0.001**

BMI body mass index; *ALT* alanine aminotransferase; *HOMA-IR* HOMA-insulin resistance; *HbA1c* glycosylated hemoglobin; *FT3* free triiodothyronine; *FT4* free thyroxine; *sTSH* sensitive thyroid stimulating hormone; *TG* triglyceride; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; *IL-6* interleukin-6. Data represent the mean ± SD. $^{**}p < 0.01$

Serum WISP1 concentration was higher in the GDM group

As shown in Fig. 1, the serum WISP1 concentrations were higher in the GDM group compared with that in the control group (2.29 ± 0.35 vs. 1.40 ± 0.30, respectively, $p < 0.01$).

Correlation between serum WISP1 levels and clinical and demographic characteristics

By using bivariate correlation analyses, we found that WISP1 was positively correlated with BMI ($r = 0.332$, $p < 0.001$), fasting glucose ($r = 0.306$, $p < 0.001$), fasting

Table 1 Primer sequences for real-time PCR

Primer	Forward primer (5'-3')	Reverse primer (5'-3')
WISP1	AGGTATGGCAGAGGTGCAAG	TTGCCTTTGGTATTTGTGTCC
GAPDH	TATTAACGGATTCGGTCG	CACATTAAGGGTGGTGCA

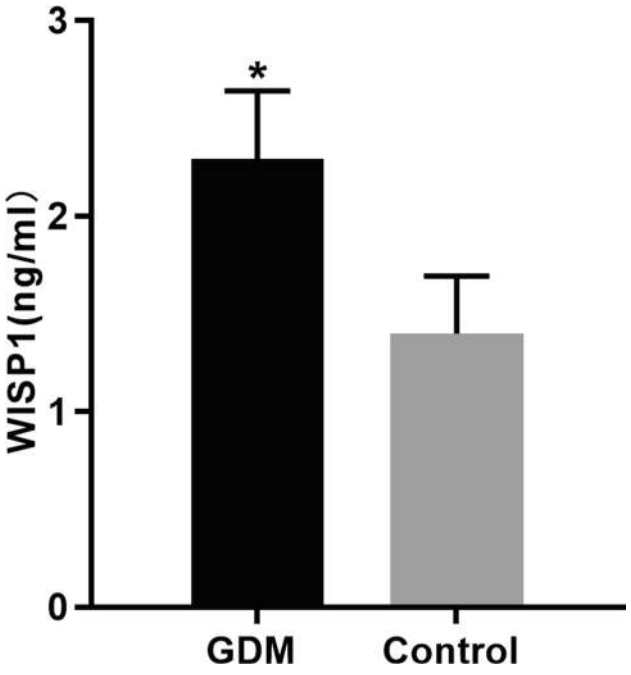

Fig. 1 Maternal serum WISP1 concentrations in the GDM ($n=69$) and control ($n=71$) groups between weeks 25 and 30 of gestation. *$p<0.01$, compared with the control group

insulin ($r=0.246$, $p=0.003$), HOMA-IR ($r=0.296$, $p<0.001$), and IL-6 ($r=0.182$, $p<0.031$). In contrast, circulating WISP1 levels were not significantly correlated with age, HbA1c, FT3, FT4, sTSH, ALT, TG, HDL-C, or LDL-C levels (Table 3). Multivariate regression analysis demonstrated that BMI ($\beta=0.142$, $p<0.001$), fasting glucose ($\beta=0.216$, $p=0.003$), and HOMA-IR ($\beta=0.125$, $p=0.005$) independently and positively predicted serum WISP1 levels (Table 3).

Table 3 Univariate and multivariate regression analyses of WISP1

Category	Univariate		Multivariate regression analyses	
	r	*P*	*β*	*P*
Age (years)	0.015	0.860		
BMI (kg/m^2)	0.332	< 0.001	0.142	< 0.001
ALT (U/L)	0.134	0.114		
Fasting glucose (mmol/L)	0.306	< 0.001	0.216	0.003
Fasting insulin (mU/mL)	0.246	0.003		
HOMA-IR	0.296	< 0.001	0.125	0.005
HbA1c (%)	0.112	0.186		
FT3 (pmol/mL)	− 0.111	0.192		
FT4 (pmol/mL)	− 0.079	0.351		
sTSH (mIU/L)	− 0.084	0.323		
ALT (U/L)	0.082	0.331		
TG (mmol/L)	0.040	0.641		
LDL-C (mmol/L)	0.052	0.542		
HDL-C(mmol/L)	− 0.131	0.123		
IL-6(pg/mL)	0.182	0.031		

Demographics of the subjects at cesarean section in Study 2

The demographics of the Study 2 population are shown in Table 4: there were no statistical differences between the control and GDM groups ($p>0.05$).

WISP1 expression was upregulated in the placenta and SAT of GDM group

As shown in Fig. 2, compared to the control group, the WISP1 mRNA expression was significantly upregulated in the maternal placenta and abdominal SAT of the GDM group ($p<0.01$). Similarly, the WISP1 protein expression was significantly upregulated in the maternal placenta (0.532 ± 0.162 vs. 0.323 ± 0.128, $p<0.01$) and abdominal SAT (0.398 ± 0.032 vs. 0.201 ± 0.053, $p<0.01$) of the GDM group than that in the control group (Fig. 3).

Discussion

In this study, we analyzed WISP1 levels in a population of women with GDM and a group of age and gestational week-matched NGT pregnant controls. Our results revealed that circulating WISP1 level was higher in GDM patients and correlated with fasting glucose and insulin levels, indicating that WISP1 is positively correlated with glucose intolerance.

WISP1 is thought to contribute to inflammatory events in metabolic diseases and insulin resistance through different pathways [22]. Barchetta et al. [17] found that WISP1 level was increased in obese individuals and was directly related to adiposity. A recent study on GDM in China showed that WISP1 level was positively correlated with fasting blood glucose, ALT, and systolic blood pressure and was negatively correlated with HDL-C [23]. Another study on GDM in the USA showed that WISP1 was positively correlated with BMI, fasting blood glucose and fasting insulin, HOMA-IR, and TG [24]. Our findings are consistent with

Table 4 Demographic and clinical data of participants—*Study 2 population*

Category	Control ($n=12$)	GDM ($n=12$)	*P*
Age (years)	31.78 ± 2.65	31.23 ± 2.43	0.601
BMI (kg/m^2)	26.25 ± 3.14	26.54 ± 3.24	0.826
Gestational age (weeks)	37.18 ± 2.37	38.24 ± 2.62	0.310
Birth weight (kg)	3.65 ± 0.23	3.82 ± 0.26	0.103

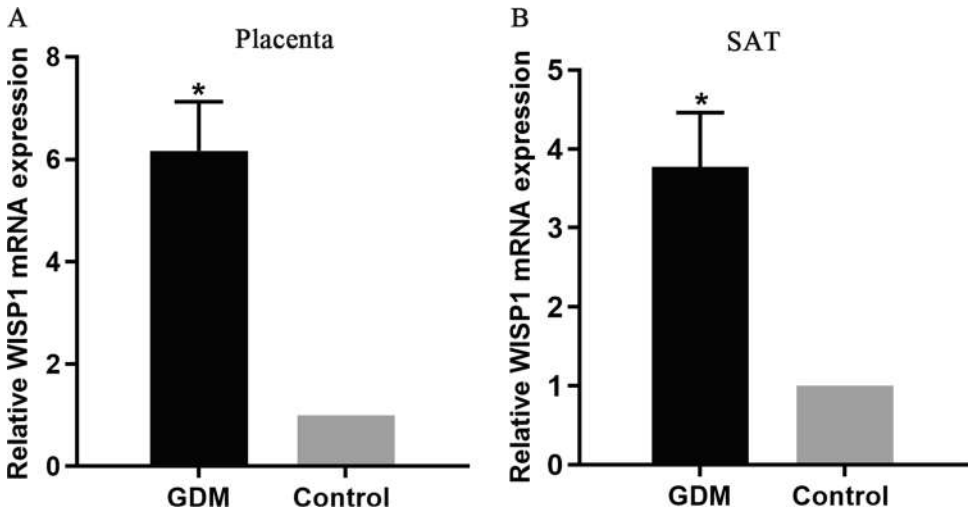

Fig. 2 Relative WISP1 mRNA levels in the placentae and SAT of the GDM and control groups. SAT: subcutaneous adipose tissue. **A** and **B** Quantification of the differences in the WISP1 mRNA levels in the placenta and SAT between the GDM and control groups ($n = 12$ for each of the placenta and SAT sample per group). GAPDH served as an internal control. *$p < 0.01$, compared with the control group

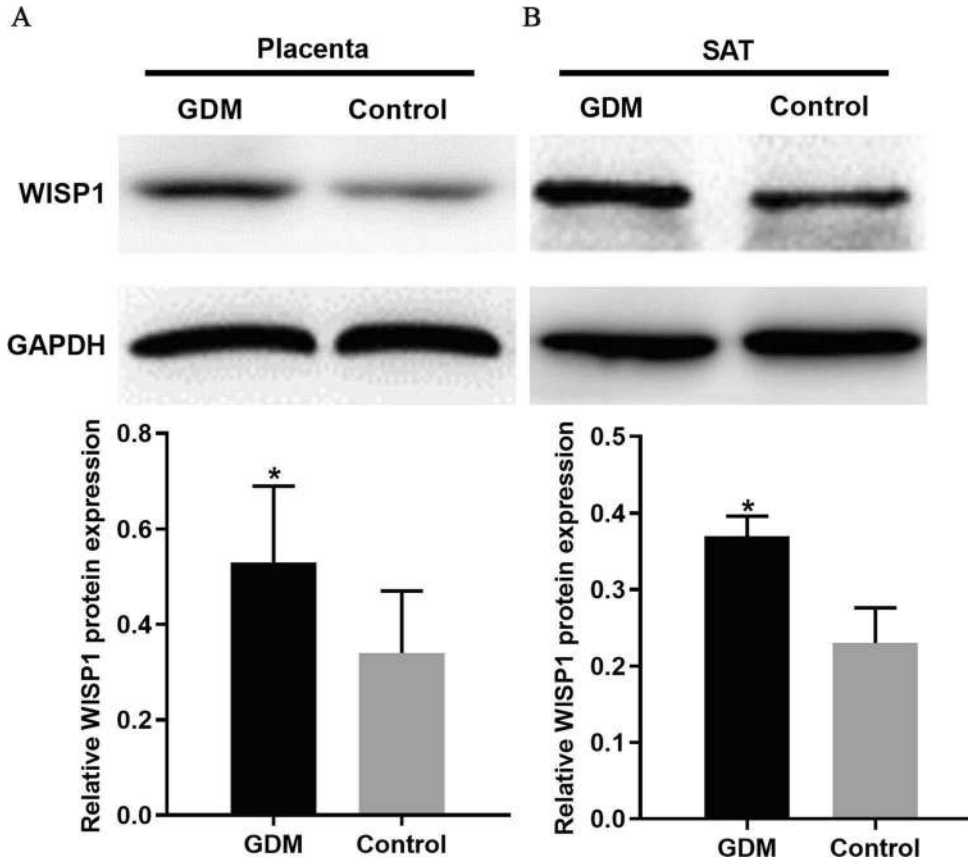

Fig. 3 Western blot analysis of the WISP1 protein levels in the placentae and SAT of the GDM and control groups**.** SAT: subcutaneous adipose tissue. **A** and **B** Quantification of the differences in the WISP1 protein levels in the placenta and SAT between the GDM and control groups ($n = 12$ for each of the placenta and SAT sample per group). GAPDH served as an internal control. *$p < 0.01$, compared with the control group

these studies with respect to the association of WISP1 and fasting glucose and fasting insulin levels. The discrepancies between our study and previous researches may be related to differences in the sample size, study population, and GDM severity.

WISP1 is a recently discovered adipokine that is secreted by adipocytes and stimulates cytokine responses in macrophages. The release of WISP1 was significantly increased during adipocyte differentiation; thus, fat cells are probably a main source of WISP1 released into the blood circulation [16]. Our study supports this hypothesis as we found that SAT from women with GDM displayed significantly higher WISP1 expression and release. The mechanisms underlying increased circulating WISP1 levels in women with GDM may be related to unknown factors, while the relationship between WISP1 and deterioration of glucose metabolism

and insulin sensitivity remains to be elucidated. It is plausible that the increased WISP1 levels with adipose tissue inflammation may induce adipose tissue remodeling and aberrant fibrogenesis, which, in turn, may be responsible for the loss of adipose tissue function, insulin resistance, and downstream consequences [25, 26].

Our data are also consistent with the notion that WISP1 may interfere with insulin signaling in insulin target tissues. For instance, WISP1 can inhibit insulin-mediated protein kinase B (Akt) phosphorylation, which regulates multiple important aspects of glucose metabolism [27, 28]. Contrary to this, during pancreatic regeneration, WISP1 is one of several overexpressed genes, suggesting that WISP1 may exert reparative effects during GDM [29]. Moreover, during oxidative stress WISP1 can upregulate phosphoinositide 3-kinase (PI3-K) and Akt to protect against DNA damage [30]. These studies suggest that WISP1 may play dual roles in modulating glucose homeostasis, and further studies are needed to elucidate its underlying pathophysiological mechanisms.

Our study found that WISP1 expression also significantly increased in the placentas of the GDM group compared to that in the control group. As an endocrine organ, the placenta can secrete many cytokines through adipocytes, which are well known to be essential for maintaining a normal pregnancy [31]. The placenta plays an important role in the mediation of chronic inflammation response in women with GDM. For instance, in the adaptive response to obesity during pregnancy, the function and structure of the placenta may be changed, and some adipocytokines are also co-secreted by the placenta [32]. Numerous researches have indicated an increase in inflammatory mediators in the placentas of women with obesity-related GDM [33, 34]. Some changes found in the placenta when maternal obesity may be adaptations to limit the fetal exposure to inflammation and oxidative stress [35]; however, inflammatory mediators may also work in utero, causing the fetal adipose tissue, skeletal muscle, and liver to develop insulin resistance in later life [36]. Recently, in a pregnant rat model with a predisposition to obesity, maternal obesity was shown to decrease placental efficiency and cause significant placental lipid accumulation by aberrantly activating placental Wnt signaling, indicating that the placentas of obese rats were less effective at supporting fetal development compared to those of lean rats. Wnt signaling can also contribute to obesity-associated metabolic disorder by increasing placental inflammation [37]. As a downstream target of Wnt signaling, WISP1 has been shown to impact multiple other signal transduction pathways to affect cellular injury and cellular proliferation [38].

To our knowledge, this is the first study to compare the expression of WISP1 in the abdominal SAT and placental tissue in individuals with GDM and controls. There are two major limitations of this study. Firstly, since this is a descriptive study, further studies are needed to fully elucidate the pathophysiological processes underlying our observations and their possible clinical implications. Secondly, the sample size in this study was limited; therefore, similar researches with a larger cohort are needed to confirm our observations.

Conclusion

In conclusion, this study presents novel data showing increased plasma WISP1 levels and increased WISP1 expression in the abdominal SAT and placenta of women with GDM. Our current findings may support the hypothesis that WISP1 plays a role in the pathogenesis of GDM. Although the physiological and pathological significance of these findings remains unclear, they may explain a mechanism by which insulin resistance occurs in pregnant women with GDM.

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

Declarations

Ethics approval This study was approved by the Human Ethics Committee of the Shengjing Hospital of China Medical University. It was designed in accordance with the principle of the Helsinki Declaration.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Predictive low-glucose suspend system and glycemic variability

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Abstract

Introduction Predictive low-glucose suspend (PLGS) system helps prevent hypoglycemia.

Aim To evaluate the effect of PLGS therapy on GV and percentage of time in range (TIR), time below range (TBR), and time above range (TAR) in pediatric type 1 diabetic patients.

Method HbA_{1c}, coefficient of variation (CV), standard deviation (SD), and percentage of TIR, TBR, and TAR were evaluated in type 1 diabetic (T1D) pediatric patients followed up between Jan 2016 and Mar 2020 using PLGS system.

Results Mean ages of diagnosis and duration of diabetes were 6.7 ± 4.1 and 8.2 ± 4.3 years, respectively. Nineteen of the patients were male (46.3%) and 22 were female (53.7%). Twenty-two (53.7%) of the patients were using low-glucose suspend system and 19 (46.3%) were on multiple daily injection therapy (MDI). On PLGS therapy, the 3rd, 6th, 9th, and 12th months' HbA_{1c} of patients were not different from previous years' mean HbA_{1c} in all participants. In the 3rd, 6th, 9th, and 12th months of PLGS therapy, % of TIR were 65.34 ± 14.75%, 65.80 ± 14.67%, 66.58 ± 11.21%, and 70.04 ± 10.16%, respectively ($p = 0.01$). Although statistically insignificant, CV decreased from 36.33 to 34.30% and SD decreased from 60.14 to 58.60 in the 1-year follow-up period ($p = 0.062$ and $p = 0.246$).

Conclusion With PLGS therapy, TIR was > 70% and the time spent in hypoglycemia was very low.

Keywords Insulin pump therapy · Predictive low-glucose suspend · Time in range · Glycemic variability · Coefficient of variation

What is known
- Time in range is provided at the recommended levels with predictive low-glucose suspend systems.
- Severe hypoglycemic event is prevented with the use of predictive low-glucose suspend systems.

What is new
- The use of predictive low-glucose suspend systems reduces glycemic variability parameters, coefficient of variation (CV), and standard deviation (SD) values.
- Patients send their downloads weekly and recommendations are made according to these downloads so they can be the better control.

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Introduction

Children and adolescents are seen to have more glycemic variability (GV), hypoglycemia, and glycemic excursions than adults due to their unpredictable activity, eating habits, and hormonal changes [1]. Continuous subcutaneous insulin infusion (CSII) is the preferred method of insulin administration for young children (aged <7 years) with type 1 diabetes (T1D) due to its effects of decreasing HbA_{1c} and hypoglycemia and improving the quality of life [2, 3]. Continuous glucose monitoring (CGM) provides a continuous measurement of the interstitial glucose over time and offers the opportunity to detect glucose variations and hypoglycemic events and it has been shown that it helps reduce HbA_{1c} levels and provides essential glucose metric information in T1D [4]. Time in and out of the target range and measures of glucose variability derived from CGM data can provide more comprehensive indicators of glycemic control than A_{1C} alone. For the interpretation of glucose data, time in range (TIR), coefficient of variation (CV), and standard deviation (SD), metrics use is recommended [5].

Sensor-augmented pump therapy with predictive low-glucose suspend (PLGS) system interrupts insulin delivery if the sensor glucose is predicted to reach 20 mg/dL (1.1 mmol/L) above the preset low-glucose limit within 30 min and automatically resumes basal insulin delivery after recovery from hypoglycemia in clinical practice [6]. We aimed to show the effectiveness of PLGS (Minimed®640G Medtronic, Northridge, CA) system in the everyday treatment of children with type 1 diabetes in real-world conditions.

Method

A descriptive study was conducted between January 2016 and March 2020. T1 diabetic patients aged between 1 and 18 years, on treatment either with low-glucose suspend (LGS) using Paradigm®Veo™ (Medtronic, Northridge, CA) or with multiple daily insulin (MDI) treatment, were switched to PLGS therapy with Minimed®640G and CGM system Enlite™ Sensor (Medtronic, Northridge, CA). Participants were included in the analysis if they completed at least 1 year of follow-up, if CGM data from the 14 days prior to the 1-year follow-up visit were complete, and if the sensor was used for more than 90% of the time with three or more self-monitoring blood glucose per day.

The demographic information and baseline characteristics of the population were extracted from systematically collected medical records. All auxological data calculations were made by an automatic calculator [7]. For participants on treatment with LGS, the information of the interstitial monitoring data of the 3 months previous to the start of PLGS was downloaded using CareLink Personal software version 3.0 (Medtronic, Minneapolis, MN). For participants on MDI, data from self-monitoring blood glucose records were recorded. In all patients, the MiniMed 640G® insulin pump, associated to enhanced Enlite® sensor and Guardian 2 Link transmitter (Medtronic, Northridge, CA), was started after completing a training program directed by a pediatric diabetologist, dietician, and diabetes nurse. The participants, their families, and caregivers learned about the insulin pump device, the CGM, and the carbohydrate counting through personal sessions. The low limit was configured to 60 mg/dL (3.3 mmol/L), and PLGS function was activated in all patients [8]. Adjustments were made at the 3rd, 7th, 14th, and 28th days and then the patients were requested to assist monthly.

At each outpatient visit, venous HbA_{1c} levels were measured according to DCCT/NGSP (Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program) (%) by turbidimetric inhibition immunoassay (Roche Cobas c513 analyzer using the Tina quant® HbA1c Gen. 3 assay, Germany: reference range, 4.8–5.9%). One-year HbA_{1c} level means were calculated pre- and post-PLGS.

Metrics of hypoglycemia, hyperglycemia, and GV were calculated. Metrics calculated included mean glucose and time below range (TBR is defined as percentage of CGM readings below target level < 70 mg/dL [3.9 mmol/L]); hypoglycemic events (defined as glucose levels below 70 mg/dL lasting at least 15 min); hyperglycemic events (defined as glucose levels above 180 mg/dL [10 mmol/L] lasting at least 15 min); time above range (TAR is defined as percentage of CGM readings above target level > 180 mg/dL); TIR (% time spent in the target range between 70 and 180 mg/dL); and SD and coefficient of variation (CV: (SD/mean glucose value) × 100). Nocturnal hypoglycemia episodes were not investigated.

Those children who gave assent and whose parents gave signed consent were enrolled in the study. The study was approved by the Ege University Medical Ethics Committee. Approval number is 20-T/83. Written informed consent was obtained from all participants and their parents.

Statistical analysis

Analyses were carried out using SPSS for Windows 25.0; descriptive statistics are reported using mean ± SD for normally distributed variables, and median for skewed data. Groups are compared by independent samples *t* test for normally distributed variables and the Mann-Whitney U test for skewed data. Trends across time were analyzed using linear polynomial contrasts (ANOVA). p value < 0.05 was considered statistically significant, and no adjustment was made for multiplicity of statistical tests.

Results

The study included 55 participants with T1D using PLGS for at least 1 year; 14 of them were excluded from the study due to onset of pump therapy at diagnosis or not using CGM regularly with PLGS (Fig. 1). In the remaining 41 participants, 19 were male (46%), and 22 were female (54%). Mean ages of diagnosis and duration of diabetes were 6.7 ± 4.1 and 8.1 ± 4.3 years, respectively. Mean age of the patients during the study was 14.9 ± 5.7 years. Twenty-two (54%) of the participants were previously on LGS and 19 (46%) were on MDI (Table 1).

No significant difference was found in body mass index (BMI) SDS of the participants pre- and post-PLGS therapy ($p = 0.21$) (Table 1).

In pre-PLGS and post-PLGS, the 3rd, 6th, 9th, and 12th months' mean insulin doses (U/kg) were similar in all participants. There was no significant difference between basal and bolus insulin ratios before and after PLGS therapy in participants previously using LGS therapy (Table 2).

At the beginning of PLGS therapy, HbA_{1c} was 7.6 ± 1.4% (58 ± 14 mmol/mol) for the participants (Table 1). HbA_{1c} values of all participants using PLGS therapy were similar in the 3rd, 6th, 9th, and 12th months (7.4 ± 0.8% [57 ± 8 mmol/mol], 7.2 ± 0.9% [55 ± 9 mmol/mol], 7.3 ± 0.7% [56 ± 7 mmol/mol], and 7.5 ± 0.9% [58 ± 9 mmol/mol], $p = 0.190$). Previous year's mean HbA_{1c} 7.3 ± 0.6% (56 ± 10 mmol/mol) and the 3rd, 6th, 9th, and 12th months' HbA_{1c} of patients using LGS therapy were not different on PLGS therapy ($p = 0.309$). Before PLGS, mean HbA_{1c} values were < 7.5% (58 mmol/mol) in 49 % of all participants. A total of 61% of the participants achieved mean HbA_{1c} values < 7.5%, 1 year after switching to PLGS.

In the 3rd, 6th, 9th, and 12th months of PLGS therapy, TIR rates were 65 ± 15%, 66 ± 15%, 67 ± 11%, and 70 ± 10%, respectively ($p = 0.011$) (Fig. 2). There is gender-wise difference in HbA_{1c} levels and TIR using PLGS therapy (p values > 0.05). In the 1-year follow-up period, none of the participants had severe hypoglycemic event or diabetic ketoacidosis.

Although there was no statistically significant decrease in the average CV values, it decreased below desired 36%, 9 months after switching to PGLS (p=0.063) (Fig. 3).

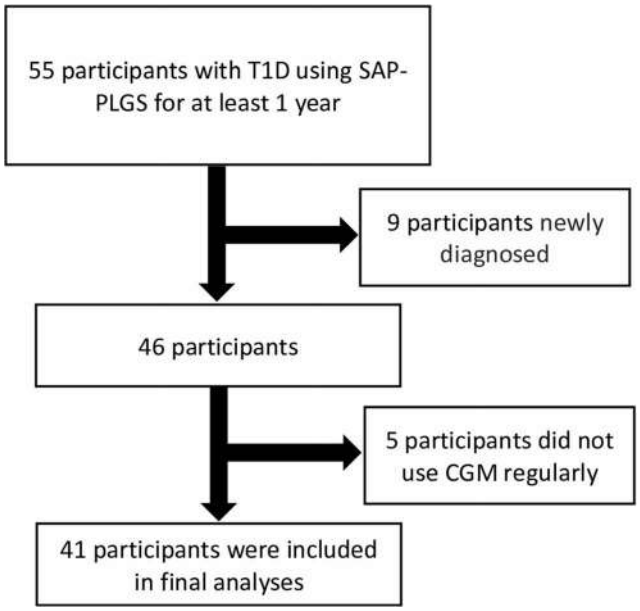

Fig. 1 Flow diagram showing the selection of the study population. T1D, type 1 diabetes; SAP-PLGS, sensor-augmented pump/predictive low-glucose suspend; CGM, continuous glucose monitoring

Similarly, there was no statistically significant decrease in SD value achieved on the 12th month of PGLS ($p = 0.246$) (Fig. 4).

Discussion

Attaining target glycemic control is challenging for youth with T1D throughout childhood and especially during adolescence due to a transient period of insulin resistance, unpredictable eating, activity patterns, and psychosocial factors [9]. CGM has introduced new terms and core metrics to the literature to assess GV [5]. The first of these terms is TIR and was defined as the percentage of CGM readings between 70 and 180 mg/dL. Vigersky et al. showed a clear association between TIR and HbA_{1c} by analyzing paired TIR and HbA_{1c} data from 18 clinical trials that involved both type 1 and type 2 diabetes [10]. Beck et al. studied the relationship between these two measures in datasets from four clinical trials involving 545 patients with type 1 diabetes, and formed a significant linear

Table 1 Baseline characteristics of patients

	Total (*n*: 41)	MDI to SAP-PLGS (*n*: 19)	SAP-LGS to SAP-PLGS (*n*: 22)
Gender (F/M)	22/19	10/9	12/10
Mean age of diagnosis (yrs)	6.7 ± 4.1	6.8 ± 3.3	6.7 ± 4.7
Mean duration (yrs)	8.1 ± 4.3	6.1 ± 3.9	9.9 ± 4.0
Previous years' mean HbA_{1c}, %	7.7 ± 1.2	8.2 ± 1.6	7.3 ± 0.6

MDI, multiple daily injection therapy; *SAP-PLGS*, sensor-augmented pump–predictive low-glucose suspend; *SAP-LGS*, sensor-augmented pump–low-glucose suspend; *F*, female; *M*, male

Table 2 Outcomes after 1-year follow-up with SAPT-PLGM in patients with previous treatment with SAP-LGS (group 1)

	Baseline SAP-LGS	3rd month	6th month	9th month	12th month	*p* value
BMI, mean	−0.28 ± 0.83	−0.28 ± 0.73	−0.28 ± 0.72	−0.12 ± 0.72	−0.08 ± 0.66	0.106
Insulin doses (U/kg)	1.04 ± 0.22	0.81 ± 0.16	0.82 ± 0.17	0.83 ± 0.21	0.82 ± 0.19	0.410
Insulin ratio, % (basal/bolus)	36.55/63.45	38.35/61.65	38.52/61.48	38.05/61.95	38.04/61.96	0.554
HbA_{1c} (mmol/mol), mean	56 ± 10 (7.3 ± 1.0%)	56 ± 9 (7.3 ± 0.9%)	55 ± 10 (7.2 ± 1.0%)	56 ± 8 (7.3 ± 0.8%)	58 ± 10 (7.5 ± 1.0%)	0.309
SD of glucose (mg/dL), mean	-	58.63 ± 16.12	60.13 ± 15.81	58.18 ± 13.83	58.09 ± 14.81	0.775
CV, mean	-	35.60 ± 4.67	35.10 ± 6.58	33.56 ± 5.67	33.78 ± 5.94	0.301
Time in range 3.9–10 mmol/L (%), mean	-	64.50 ± 14.70	65.27 ± 16.02	66.04 ± 11.78	69.13 ± 10.53	0.143
Percentage of time < 3.9 mmol/L, (%), mean	-	2.09 ± 1.50	2.86 ± 2.43	2.68 ± 2.19	2.86 ± 1.80	0.523
Percentage of time>10 mmol/L (%), mean (	-	33.40 ± 14.87	32.31 ± 18.25	31.27 ± 12.56	28.00 ± 11.00	0.132
Hospitalization for diabetes in the last year, *n* (%)	2 (9%)	0	0	0	0	-

SAP-LGS, sensor-augmented pump–low-glucose suspend; *BMI*, body mass index; *SD*, standard deviation; *CV*, coefficient of variation

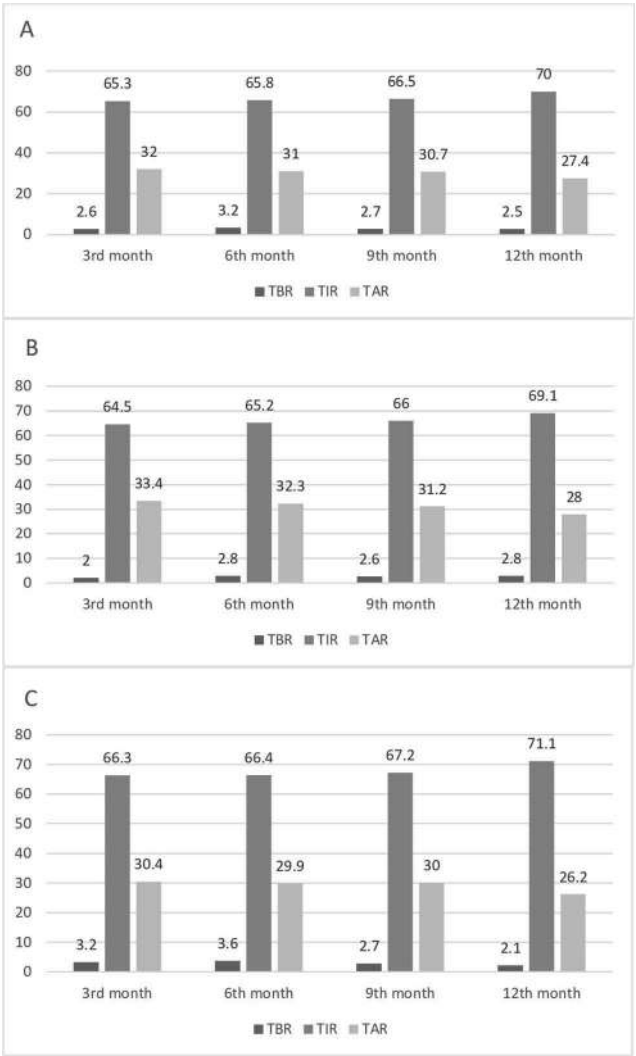

Fig. 2 Percentage of time above range (TAR), time in range (TIR), and time below range (TBR) 1-year follow-up with PLGM. **A** All participants; **B** previously on LGS (group 1); **C** previously on mDi (group 2)

relationship between TIR and HbA_{1c} [11]. According to a consensus report, a TIR > 70% is the recommended target for T1D [5, 12]. But targets should be personalized [5]. At the end of the 1-year follow-up, mean TIR was 70 ± 10% for all the patients and 58.5% of all patients were within the expected range. The change in TIR was not evaluated in the pre- and post-PLGS because the baseline data was not available for all patients. Few studies have specifically addressed the advantages of PLGS in patients who already experienced the use of LGS. In pediatric patients with a 59% reduction in time spent, 3.9 nmol/L (< 70 mg/dL) was achieved without increasing hyperglycemia [13]. In 851 adult users who had switched from LGS to PLGS with a 40% reduction in the time spent, < 3.9 nmol/L (70 mg/dL) was found [14].

Hypoglycemia, and fear of hypoglycemia, due to its acute feature of seizures/death and long-term effect of neurological damage is another concern limiting appropriate glycemic control in many children and adolescents with T1D [15]. One of the benefits of real-time CGM is the ability of these devices to alarm and suspend for hypoglycemia. Low-glucose suspend feature is crucial for decreasing hypoglycemic events. Richard et al. showed nocturnal hypoglycemic events to occur 31.8% less frequently in the threshold-suspend group than in the control group, without significant changes in glycated hemoglobin levels [16]. A more effective approach of new Minimed®640G and CGM system Enlite™ Sensor is to suspend insulin delivery earlier on the basis of a prediction algorithm before hypoglycemia happens: PLGS. Although CGM and PLGS are known to prevent hypoglycemia, target values for TBR are not certain. In the last consensus, TBR < 70 mg/dL (level 1 hypoglycemia) of < 4% and a TBR < 54 mg/dL (level 2 hypoglycemia) of < 1% are recommended as targets [5]. Also target can be described TBR < 70 mg/dL to less than 1 h per day and TBR < 54 mg/dL to less than 15 min per day. Quispe et al. found an increase in TIR from 61.7 ± 8.5 to 65.2 ± 9% in the 3rd month and a decrease in TBR from 10.4 ± 5.2

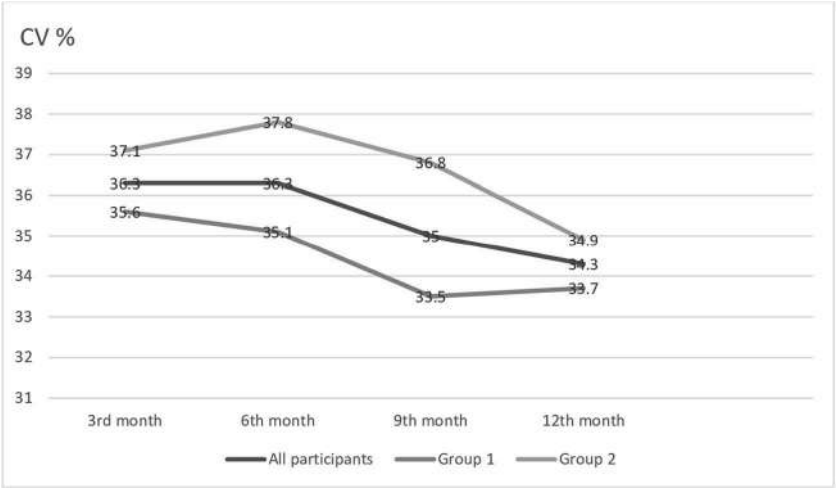

Fig. 3 Change of coefficient of variation (CV) in the entire population

to 7.6 ± 3.3% [17]. In a study, Choudhary et al. analyzed this method in children and adolescents and reported that it is safe for reducing hypoglycemic episodes in this population with no associated deterioration of metabolic control in terms of HbA_{1c} [18]. In our study, mean TBRs < 3.9 mmol/L were 2.61 ± 0.41%, 3.22 ± 0.64%, 2.70 ± 1.99%, and 2.53 ± 1.56% at the 3rd, 6th, 9th, and 12th months, respectively, within recommended cutoffs. Our patients send their downloads weekly and recommendations are made according to these downloads which can explain the better control in our patients.

The risk of diabetic ketoacidosis is one of the major drawbacks of preventing insulin infusions for long periods of time. In this regard, in a study, researchers focused on the possibility of ketosis after nighttime suspension of the PLGS, even when blood glucose levels were normal and no significant changes observed in fasting-hydroxybutyrate levels [19]. No patient in our study reported ketosis/ketoacidosis episodes while using the system.

GV is a metric that provides an integrated picture of postprandial hyperglycemia and hypoglycemic episodes. GV has been hypothesized to be an independent risk factor for vascular disease independent of HbA_{1c} [20–22]. The most used within-day and between-day variability indices are SD and CV [23]. Increased GV is consistently associated with mortality and is a consistent predictor of hypoglycemia, both in prospective studies and randomized clinical trials [24, 25]. For CV**,** 36% threshold has been adopted as the primary metric to separate stable from unstable diabetes [26]. Lu et al. concluded in their study that GV assessed by CV significantly affects the correlation between TIR and $eHbA_{1c}$ and GV should be taken into consideration when setting an individualized goal of TIR [27].

In our patients, CV was 36% at the 3rd month, 36% at the 6th month, 35% at the 9th month, and 34% at the 12th month within recommended limits, demonstrating PLGS's perfect effect on GV in T1D children. GV decreases were not statistically significant. However, these changes were clinically significant.

As the known relationship between CGM-derived glycemic variables and the corresponding HbA_{1c} levels, Hirsh et al. studied associations between HbA_{1c} levels and various

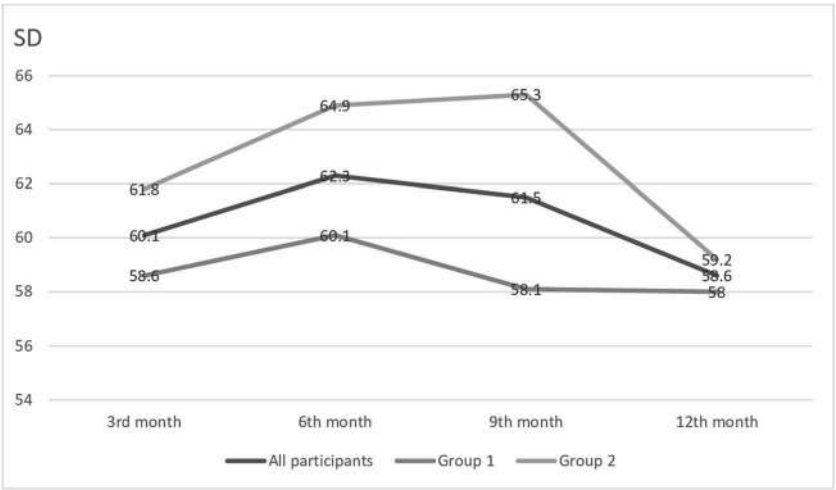

Fig. 4 Change of standard deviation (SD) in the entire population

continuous glucose monitoring-derived metrics. There was a strong inverse correlation between HbA_{1c} and TIR, such that every 10% change in TIR was associated with a 0.7% (7 mmol/mol) change in HbA_{1c}. There were strong positive correlations between HbA_{1c} and both mean glucose and the percentage of glucose values >250 mg/dl (13.9 mmol/L), and a weak inverse correlation between HbA_{1c} and the percentage of glucose values >70 mg/dl (28).

In our study, GV and TIR data before PLGS were not available and also the participants were retrospectively reviewed. Nocturnal hypoglycemia episodes were not investigated.

Conclusion

No data have yet been published regarding clinical experience with the PLGS systems in the Turkish pediatric population. Since T1DM is one of the most significant chronic diseases in terms of social and healthcare, the results indicate that the PLGS mechanism can have good glycemic control in a developing country. PLGS systems aim to reduce exposure to hypoglycemia and hyperglycemia in children and adolescents with T1D without compromising glycemic control or quality of life. For cases using PLGS therapy in the literature, the rate of normoglycemia >70% is defined as good glycemic control. In our clinical study, at the end of 1-year follow-up, the average normoglycemia rate obtained by calculating sensor data was found to be 70 ± 10%. The fact that HbA_{1c} values do not correlate with this rate can be explained by the high levels of level 2 hyperglycemia. However, longer term monitoring is needed to predict glycemic control and late complications. Future trials with larger sample size and long-term observation are necessary to confirm the generalizability of the study findings.

Abbreviations CGM, Continuous glucose monitoring; CSII, Continuous subcutaneous insulin infusion; CV, Coefficient of variation; DCCT/NGSP, Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program; GV, Glycemic variability; LGS, Low-glucose suspend; MDI, Multiple daily insulin; PLGS, Predictive low-glucose suspend; SD, Standard deviation; TAR, Time above range; TBR, Time below range; TIR, Time in range; T1D, Type 1 diabetes

Author contribution Ferda Evin: Methodology, conceptualization, writing
Eren Er: Software, investigation
Aysun Ata: Software, investigation
Günay Demir: Investigation
Hafize Çetin: Investigation
Yasemin Atik Altınok: Investigation
Samim Özen: Methodology, supervision
Şükran Darcan: Project administration
Damla Gökşen: Writing—reviewing, editing

Declarations

Ethics approval The study was approved by the Ege University Medical Ethics Committee. Approval number is 20-T/83.

Consent to participate Written informed consent was obtained from all the participants and their parents.

Consent for publication Written informed consent was obtained from all the participants and their parents.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Effect of constructing doctor-pharmacist joint pharmacy clinic for outpatients on the comprehensive management of type 2 diabetes mellitus: a pilot RCT

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Abstract

Background Comprehensive management of type 2 diabetes mellitus (T2DM) patients has a low control rate, and there is a lack of prospective studies involving clinical pharmacists in outpatient management of patients with T2DM. This study was designed to investigate the effectiveness of pharmacists participating in the comprehensive management of T2DM patients in the form of building doctor-pharmacist joint pharmacy clinic in the National Metabolic Management Center (MMC).

Methods A total of 204 patients with type 2 diabetes mellitus were enrolled and randomly divided into control group and intervention group. During the 3-month study period, both groups received standardized MMC diagnosis and treatment, and clinical pharmacists provided pharmaceutical care for the intervention group. Observation indicators include drug compliance, fasting blood glucose (FPG), glycosylated hemoglobin (HbA1c), blood pressure, blood lipid, and other comprehensive management indicators.

Results The baseline characteristics of the two groups were similar, and there was no significant difference ($p > 0.05$). After 3 months of follow-up, drug compliance, HbA1c, FPG, low-density cholesterol (LDL-c), and diastolic blood pressure in the intervention group were more significantly improved compared with those in the control group ($p < 0.05$). There was no significant difference in the incidence of adverse events between the two groups ($p = 0.825$).

Conclusion Clinical pharmacist can improve the comprehensive management of diabetes mellitus by building doctor-pharmacist joint pharmacy clinic for outpatients.

Keywords Clinical pharmacist · Metabolic management center · Pharmacy clinic · Effectiveness

Background

The total incidence of type 2 diabetes mellitus (T2DM)in China is 11.2%, of which the incidence of 65–74 years old is as high as 14.1% [1, 2]. The comprehensive management of metabolic indexes such as plasm glucose, blood pressure, and serum lipid has an important impact on the occurrence and development of macrovascular and microvascular complications [3, 4]. Among the different improvement strategies of comprehensive management of diabetes mellitus, improving the compliance of patients with the comprehensive management plan plays a key role in improving metabolic indicators [5, 6]. The National Metabolic Disease Management Center (MMC) is a nationwide standardized management system for metabolic diseases, led by Academician Ning Guang of the Chinese Academy of Sciences. The Department of Endocrinology of our hospital is an early member of MMC. Based on this center, the Department of Pharmacy has built a doctor-pharmacist joint pharmacy clinic to provide pharmaceutical care for patients with metabolic diseases, including diabetes [7]. At present, the management of diabetic patients is not optimistic. It has been reported that more than 50% of diabetic patients lack compliance with the comprehensive management plan, resulting in that the comprehensive compliance rate of blood glucose, blood pressure, and blood lipid is only 5.6%. The official data of MMC shows that the comprehensive compliance rate

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of metabolism of patients receiving the integrated diagnosis and treatment of the center is 17.3% [7]. Further improvement of comprehensive management is the common goal of doctors, pharmacists, nurses, and patients. There is a lack of prospective studies involving clinical pharmacists in outpatient management of patients with T2DM.

In this study, the influences of pharmaceutical care and medication education provided by the joint pharmacy clinic for outpatients on the comprehensive management effect of patients were evaluated, and the effectiveness of clinical pharmacists in building the joint pharmacy clinic for outpatients based on MMC was analyzed.

Methods

Study design

This was a single-center, prospective, open-label, randomized pilot study conducted over a period of 3 months evaluating the effectiveness of pharmacists participating in the management of outpatients with T2DM in the form of building doctor-pharmacist joint pharmacy clinic in the MMC.

The working mode of joint pharmacy clinic for outpatients

In 2013, our hospital began to explore the work mode of clinical pharmacist in the endocrinology department. Through years of clinical practice and standardized training, we have accumulated rich work experience in the ward of endocrinology department, and achieved good results in chronic disease management. In order to provide better pharmaceutical care to the outpatients, clinical pharmacists try to set up a joint pharmacy clinic in MMC and form a working partner with the chief physician of the Department of Endocrinology (Fig. 1).

Subjects

From April 2018 to March 2019, 220 patients with T2DM who met the inclusion and exclusion criteria were included in the study. According to the method of random number table, they were divided into control group (110 patients) and intervention group (110 patients). During the follow-up period, the control group lost 3 people, withdrew 7 people; the intervention group lost 2 people, withdrew 4 people. Finally, 204 patients completed the follow-up, 90

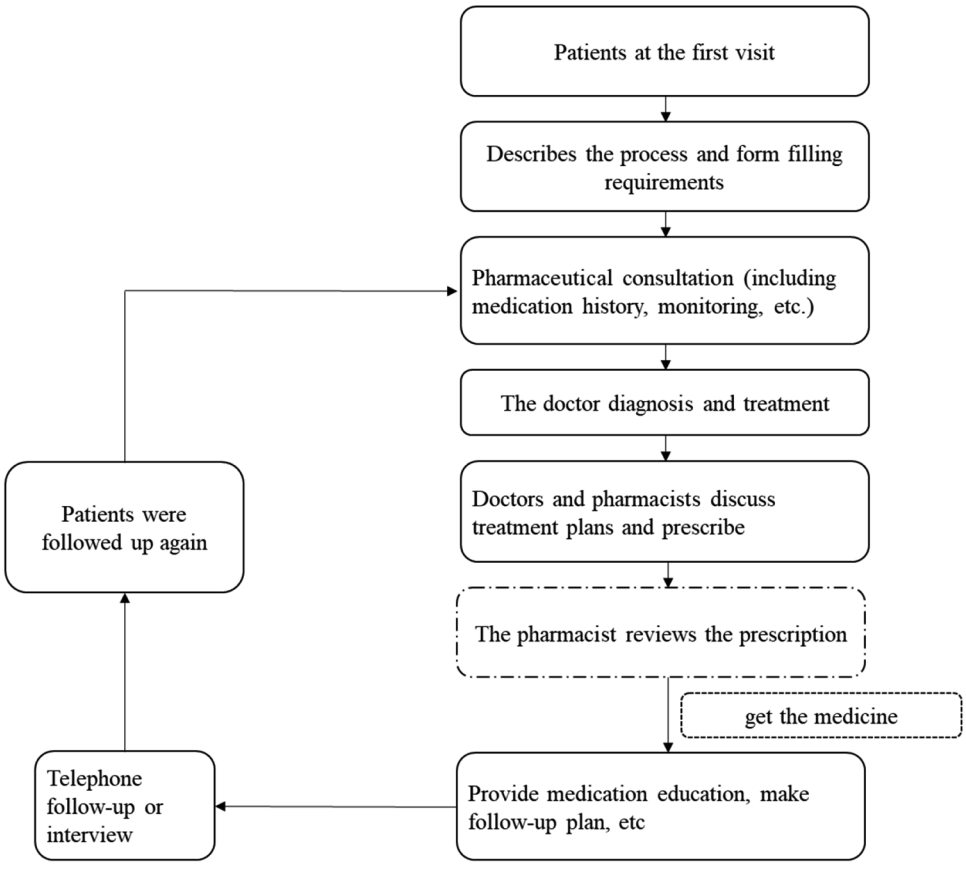

Fig. 1 The working mode of joint pharmacy clinic for outpatients

of which were male and 114 were female. Inclusion criteria were as follows: (1) According to the diagnostic criteria recommended in the guidelines for the prevention and treatment of type 2 diabetes in China (2017 Edition) [2], the patient was diagnosed as T2DM; (2) Patients visited the MMC more than 3 times, blood glucose control is poor, and hemoglobin A1c (HbA1c) > 7%; (3) The patients had basic reading and writing ability and participated in the study voluntarily. Exclusion criteria were as follows: (1) Patients were excluded if they had serious organic diseases, including coronary heart disease, stroke, tumor, and severe liver and kidney dysfunction; (2) There are communication barriers or failure to insist on the completion of follow-up or refusal to participate in this study.

Medication education

On the basis of standardized diagnosis and treatment in MMC, the intervention group was provided with medication education by the clinical pharmacists in pharmacy clinic. The content of education mainly includes three aspects: (1) The classification, indications, usage and dosage, mechanism of action, drug safety, pharmaceutical care, and other drug treatment knowledge of hypoglycemic drugs; (2) Patients' self-management knowledge, including the importance of compliance, diet, and sports-related knowledge; (3) Clinical pharmacists provide diabetes logs for patients, and cultivate their ability of self-management of blood glucose by recording drug use, blood glucose level monitored, diet and exercise, and special events (such as hypoglycemia).

Follow-up

Clinical pharmacists conducted follow-up by telephone or interview in pharmacy clinic for 3 months. Telephone follow-up was carried out every week (except for interview week), enquiring about medication, self-monitoring of blood glucose results, diet and exercise, adverse events, etc., and providing targeted suggestions to continuously improve the compliance of patients with the management plan. In addition to the corresponding examinations and drugs issued by doctors, pharmacists also provide the whole process pharmaceutical care with medication education as the core.

Data collection

Baseline information of patients was collected, including gender, age, body mass index, course of diabetes, and education level. The type of medication, the number of antidiabetic drugs, and the type of antidiabetic drugs were recorded. The Chinese version of 8-item Morisky Medication Compliance Questionnaire was used to evaluate the compliance [8, 9], with a total score of 8 points. The results were divided into three levels: I (8 points), II ($6 \leq$ total score < 8 points), and III (< 6 points). The higher the score, the higher the compliance. The main indexes of comprehensive management effects were HbA1c, fasting plasma glucose (FPG), low-density cholesterol (LDL-c), systolic blood pressure (SBP), and diastolic blood pressure (DBP).

Statistical analysis

Statistical analyses were carried out using the SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± standard deviation (SD). For the parameters of normal distribution, two-sample t-test was used for comparison between the two groups. Parameters with nonnormal distribution were analyzed by the Mann–Whitney U test or the Kruskal–Wallis test. χ^2 test was used to compare the differences of rates. A value of $p < 0.05$ was considered statistically significant.

Results

Clinical and demographic characteristics

The general data of subjects in the control group and intervention group included gender, age, body mass index, course of diabetes mellitus, and education level. At baseline, there was no significant difference between the control group and intervention group (p all > 0.05, Table 1).

Drug use and compliance

There was no significant difference between the control group and the intervention group in the total number of drugs, varieties of hypoglycemic drugs, types of

Table 1 Comparison of general data of diabetic patients between control group and intervention group

Group	Gender (male/female)	Age (year)	Body mass index (kg/m^2)	Course of diabetes (year)	Education level above junior high school (%)
Control	100(44/56)	50.21 ± 8.45	24.73 ± 3.34	10.7 ± 6.2	14(14.00%)
Intervention	104(46/58)	49.14 ± 12.21	25.15 ± 3.42	9.8 ± 5.5	13(12.50%)
p	0.914	0.661	0.632	0.398	0.821

Table 2 Comparison of drug use between the control group and the intervention group

Characteristic	Control group (n = 100)	Intervention group (n = 104)	*p*
Number of drug varieties	5.2 ± 2.1	4.6 ± 3.5	0.072
Number of antidiabetic drug varieties	3.2 ± 1.5	3.5 ± 1.8	0.231
Type of hypoglycemic program			
Non-insulin hypoglycemic drugs	44(44.00%)	42(40.38%)	0.530
Insulin alone	25(25.00%)	28(26.92%)	
Non-insulin hypoglycemic drugs + insulin	31(31.00%)	34(32.69%)	
Adjustment of hypoglycemic program			
Unadjusted	88(88.00%)	94(90.38%)	0.312
Drug dosage adjusted	12(12.00%)	10(9.62%)	

Table 3 Comparison of medication compliance between the control group and the intervention group

Group	Level I (n)	Level II (n)	Level III (n)	Total score ≥ 6 (n, %)
Control group				
Baseline	17	45	38	62 (62.00%)
After 3 months	19	45	36	64 (64.00%)
Intervention group				
Baseline	18	46	40	64 (61.54%)
After 3 months	50	41	13	91 (87.50%)* #

*Compared with baseline, $\chi^2 = 25.481$, $p < 0.05$.

#Compared with control group in the same time point, $\chi^2 = 28.224$, $p < 0.05$.

hypoglycemic programs, and drug adjustment during the follow-up period ($p > 0.05$, Table 2). At baseline, there was no significant difference in the medication compliance rate between the two groups ($p > 0.05$, Table 3). After 3 months, there was no significant change in the medication compliance rate of the control group compared with the baseline ($p > 0.05$), but the medication compliance rate of the intervention group intervened by clinical pharmacists was significantly increased compared with the baseline ($p < 0.05$). Comparing the two groups, the medication compliance rate was higher in the intervention group intervened by clinical pharmacists than the control group ($p < 0.05$) (Table 3).

Main clinical indicators of comprehensive management

At baseline, there was no significant difference in HbA1c, FPG, LDL-C, SBP, and DBP between the control group and the intervention group ($p > 0.05$). After 3 months, HbA1c, FPG, LDL-C, and DBP in the intervention group were significantly improved compared with the baseline state ($p < 0.05$), but there was no statistically significant difference in the control group ($p > 0.05$). Compared with the control group, the improvement of HbA1c, FPG, LDL-C, and DBP was more obvious in the intervention group intervened by clinical pharmacists (p all < 0.05) (Table 4).

Table 4 Comparison of main indicators of comprehensive management of diabetic patients before and after 3 months

Characteristic	Control group (n = 100)	Intervention group (n = 104)	*p*
HbA1c(%)			
Baseline	7.71 ± 0.79	8.13 ± 0.94	0.795
After 3 months	8.09 ± 0.89	6.70 ± 0.69*	< 0.001
FPG(mmol/l)			
Baseline	8.65 ± 0.95	8.87 ± 1.15	0.267
After 3 months	8.61 ± 1.00	6.91 ± 1.25*	< 0.001
LDL-c(mmol/l)			
Baseline	3.19 ± 1.18	3.12 ± 1.04	0.682
After 3 months	2.98 ± 1.11	2.46 ± 1.12*	< 0.001
SBP(mmHg)			
Baseline	143.23 ± 13.87	136.39 ± 16.60	0.216
After 3 months	138.15 ± 10.89	135.72 ± 12.46	0.416
DBP(mmHg)			
Baseline	78. 76 ± 9.62	77.2 ± 10.13	0.632
After 3 months	77.21 ± 9.43	72.25 ± 8.54*	< 0.001

HbA1c hemoglobin A1c, *FPG* fasting plasma glucose, *LDL-c* low-density lipoprotein-cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure.

*Compared with baseline, t = 11.320, 9.115, 2.541, and 3.251, $p < 0.05$.

Adverse events

During the follow-up, 4 patients (4%) in the control group had hypoglycemia, of which 2 cases were related to the increase of drug dosage; the other 2 cases were related to the failure to eat in time. The researcher determined that no other adverse events were found in the control group through the consultation and access to the self-monitoring of blood glucose records. In the intervention group, adverse events were found in 5 patients (4.81%), of which 4 had hypoglycemia. The occurrence of hypoglycemia was related to poor appetite or not eating in time. One patient complained of general asthenia and seemed to have muscle ache. The pharmacist suggested the patient checking the blood creatine kinase, and the result was 746 U/L. After discontinuing rosuvastatin calcium tablets, the symptoms had improved, the blood creatine kinase returned to normal, and muscle damage caused by rosuvastatin was considered. There was no significant difference in the incidence of adverse events between the two groups ($p = 0.825$).

Discussion

Since the former Ministry of Health of China launched the pilot training of clinical pharmacists in 2005, after more than 10 years of exploration and development, tens of thousands of clinical pharmacists with standardized training have been providing professional patient-centered pharmaceutical care, laying a talent foundation for the transformation and development of hospital pharmacy [10, 11]. However, at present, Chinese clinicians still have insufficient awareness of the value of clinical pharmacists. For example, there was no clinical pharmacist workstation at the beginning of the establishment of MMC, and there is an absence of clinical pharmacists in the integrated diagnosis and treatment of metabolic diseases in the outpatient department. Clinical pharmacists in our hospital have been taking the initiative to join the drug therapy team in the form of the doctor-pharmacist joint pharmacy clinic for outpatient, and evaluating the work in practice.

An interdisciplinary approach is a key to the success of controlling diabetes and its complications. In this study, a total of 220 T2DM patients were recruited and randomly divided into the control group and intervention group. Finally, 100 T2DM patients in the control group and 104 patients in the intervention group completed the follow-up, 3 patients in the control group were lost to follow-up and 7 patients dropped out, while 2 patients in the intervention group were lost to follow-up and 4 patients dropped out. The reason for the loss of follow-up was that the patient's phone could not be connected, and the withdrawal was due to the refusal to visit the pharmacy clinic of outpatients to check HbA1c and FPG of venous blood.

A total of 104 patients who had visited MMC for more than 3 consecutive times and received multiple drug treatments (4.6 kinds per person on average) and failed to reach the control standard of HbA1c interacted in doctor-pharmacist joint pharmacy clinic for outpatients. After 3 months, the improvement of medication compliance, HbA1c, FPG, LDL-C, and DBP was more obvious than that of the control group. It indicates that even in MMC with a good integrated diagnosis and treatment and a high comprehensive diabetes compliance rate (17.3%), doctor-pharmacist joint pharmacy clinic for outpatients can still improve medication compliance and the effect of comprehensive management. Many studies have shown that diabetic care intervention can significantly improve medication compliance, and medication education is an important aspect of comprehensive management of diabetes patients, which is related to clinical outcomes [5, 6, 12–14]. Pharmacists can improve medication compliance through diabetes care intervention, which has long-term benefit for diabetic patients [15, 16]. In clinical practice, we often encounter T2DM patients who have poor medication compliance, which leads to the near-term result that the comprehensive management is not up to standard, and the long-term consequence is that their quality of life is seriously declined. Maier et al. have found that guiding patients to use diabetes logs can improve their medication behavior and reduce the level of HbA1c [17]. Therefore, the education and follow-up of the doctor-pharmacist joint pharmacy clinic for outpatients included the guidance of diabetes log recording, which achieved good results. Another important content of our clinic is to help patients better understand the blood glucose management plan, improve medication compliance with the plan, and thus improve blood glucose control. In the practice of doctor-pharmacist joint pharmacy clinic for outpatients, a close cooperative relationship has been gradually established between pharmacists and patients, and the acceptance of pharmacists' suggestions has been increasing, especially patients with low compliance or poor comprehensive management. In addition, although there was no statistically significant difference in the incidence of adverse events between the two groups, the results of the study still suggested that the pharmaceutical care provided by the doctor-pharmacist joint pharmacy clinic for outpatients, including medical education and continuous follow-up, was conducive to the discovery and management of drug use risks and the improvement of drug treatment safety.

In developing countries such as China, the pharmaceutical care of clinical pharmacists is mostly focused on patient education and follow-up. In the process of patient management, it is very difficult to adjust the prescription problems such as the dosage of drug treatment. The fundamental

reason is that pharmacists do not have the prescribing power to issue clinical examination orders or adjust drug treatment programs. To explore and refer to advanced practice cases at home and abroad to carry out cooperative drug treatment management [18–21], it is feasible to sign "prescription agreement" with doctors to solve the problems of pharmacists' prescription right of drugs once issued by doctors at the initial diagnosis, common drugs with high safety, and for routine monitoring or inspection.

In the doctor-pharmacist joint pharmacy clinic for outpatients established in MMC, the problem of insufficient pharmacists is faced with the comprehensive development of pharmaceutical care and medical education. The pharmacist in the doctor-pharmacist joint pharmacy clinic for outpatients shall have the post training certificate of clinical pharmacist, or have been engaged in clinical pharmacy work for more than 2 years with senior professional title qualification. In China, medical institutions generally have the problem of insufficient number of pharmacists with corresponding conditions. Specialized clinical pharmacists shall also be responsible for the pharmaceutical care of patients in the ward. According to previous practice and studies, it is worth trying to promote the transformation of outpatient pharmacists to pharmaceutical clinic through a series of training, assessment, and practice, so that they can master the ability of drug education, telephone follow-up, and prescription audit [22, 23].

Conclusions

Establishing a joint pharmaceutical clinic based on MMC, which is in line with the expectations of patients and medical staff for pharmaceutical workers, and fully reflects the value of pharmacists in the management of patients with chronic diseases, is a beneficial work for patient management and the transformation and development of clinical pharmacy. A clinical pharmacist can improve the comprehensive management of diabetes mellitus by building doctor-pharmacist joint pharmacy clinic for outpatients.

Declarations

Ethics and consent This study was carried out in accordance with the recommendations of the Ethics Committee of the Affiliated Hospital of Jiangnan University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

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ORIGINAL ARTICLE

Therapeutic outcome of dapagliflozin on various parameters in non-alcoholic fatty liver disease (NAFLD) patients

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Abstract

Background Body weight reduction is the first-line recommendation in NAFLD patients. Dapagliflozin has a strong potential to reduce body weight in addition to its glycemic control effect

Objective To study the effect of dapagliflozin on various parameters in NAFLD type 2 diabetic patients.

Methods In this randomized controlled trial, 150 type 2 diabetic patients with NAFLD diagnosed on the basis of radiological findings and deranged liver enzymes were randomly allocated into two groups. Both groups were taking tab glimepiride as a standard treatment of type 2 diabetes. Group A was given tab dapagliflozin 5–10 mg while group B was given tab placebo in addition to lifestyle modifications for a period of 12 weeks. Body weight, BMI, HbA1c, and serum aminotransferases were analyzed pre- and post-treatment using SPSS 20.

Results After 12 weeks of treatment, glycemic control was improved in both study groups, i.e., dapagliflozin (FBS from 95 ± 14 to 80 ± 11 mg/dl with $p = 0.003$, HbAIc from 7.5 ± 4.5 to $6.4 \pm 5.4\%$ with $p = 0.002$) and placebo (FBS from 100 ± 9.5 to 90.5 ± 13 mg/dl with $p = 0.004$, HbA1c from 8.2 ± 3.5 to$7.6 \pm 4.2\%$ with $p = 0.006$). However, the dapagliflozin group showed a significant reduction in FBS and HbA1c as compared to placebo with p value 0.006 and 0.04 respectively. Body weight was significantly reduced in the dapagliflozin group (from 90 ± 13.5 to 84 ± 11.6) as compared to the placebo group (from 85 ± 17.8 to 85.5 ± 13.7) with $p = 0.002$. BMI also reduced in the dapagliflozin group (from 29.5 ± 2.5 to 26.5 ± 3.5) versus the placebo group (from 31.5 ± 3.0 to 29.5 ± 4.2) with $p = 0.002$. There was also significant reduction in ALT in the dapagliflozin group from 69 ± 15.5 to 52 ± 12.8 versus placebo from 68 ± 20.5 with $p = 0.04$ and AST from 74 ± 13 to 47 ± 13.5 versus placebo from 71 ± 12.5 to 65 ± 10.5 with $p = 0.02$

Conclusion Dapagliflozin has a strong potential to reverse NAFLD-associated changes in type 2 diabetic patients.

Keywords Dapagliflozin · Body weight · NAFLD · HbA1c · Aminotransferases

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent progressive liver disorders. Its global prevalence is about 20.24% in the general population and its comorbidity with T2DM is about 22.51. NAFLD prevalence is escalating at an alarming rate all across the globe due to its associated comorbidities such as obesity, metabolic syndrome, T2DM, hypertension, and dyslipidemia. NAFLD is usually a reversible disease; however, it can lead to significant morbidity

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and mortality in the form of non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis, hepatocellular carcinoma, and various cardiovascular disorders if cannot control appropriately [1, 2].

NAFLD has a strong association with T2DM. Its prevalence reaches up to 50% in patients with T2DM [3]. There is no any FDA-approved drug for NAFLD. In addition to lifestyle modification, anti-diabetic drugs have a potential role in the treatment of NAFLD. The most commonly studied anti-diabetics drugs that have beneficial effects in NAFLD patients are biguanide, thiazolidinediones, glucagon-like-peptide-1 (GLP-1), and dipeptidyl-peptidase inhibitor (DPP-4 inhibitors). Their potential roles have been clearly investigated in various clinical studies [4]. These drugs ameliorate all those pathgnomic mechanisms that have been implicated in NAFLD such as body weight, dyslipidemia, blood pressure, and inflammation. Moreover, these drugs improve insulin sensitivity and reduce insulin resistance which is the hallmark of NAFLD [5].

Dapagliflozin is one of the latest oral sodium-glucose co-transporter (SGLT-2) inhibitors anti-hyperglycemic agent that is usually prescribed in patients with T2DM both as mono as well as combination therapy. The anti-hyperglycemic effect of dapagliflozin is mediated by specifically binding to SGLT-2 in the renal proximal tubule, thus inhibiting glucose reabsorption. This leads to increase excretion of glucose in urine. The glycosuria mediated by SGLT-2 inhibitors leads to a change in body fluid composition results in weight loss and also a reduction in body fat [6]. In addition to glycemic control, dapagliflozin has very beneficial effect on body weight, blood pressure, insulin resistance, silent inflammation, oxidative stress, and hyperuricemia. SGLT-2 inhibitors also reduce major cardiovascular events and have reno-protective effects [7].

The purpose of choosing dapagliflozin in this study was due to its strong potential to reverse all those pathological changes that have been implicated in NAFLD. First, it controls blood sugar as NAFLD is prevalent in more than 50% of patients with type 2 diabetes. Second, it lowers body weight as body weight reduction usually the first-line non-pharmacological management in NAFLD patients. The reduction in blood weight and glucose by dapagliflozin ameliorates insulin resistance which is the main metabolic abnormalities in NAFLD patients. Third, it has weight and glucose-independent effects such as decrease lipogenesis and increase oxidation of free fatty acid. It also possesses anti-oxidative and anti-inflammatory properties and the role of drugs having these properties such as vitamin E and silymarin is already well established in NAFLD patients. Finally, it has beneficial effects on other risk factors of NAFLD such as blood pressure and lipid profiles [8, 9].

So, the present study was conducted to see the effect of dapagliflozin on glycemic control, body weight, and deranged liver enzymes in patients of NAFLD with concomitant diabetes.

Methods

The present study was conducted at medical outdoor unit 2 of Sheik Zayed Medical College/Hospital Rahim Yar khan. Initially, 450 overweight type 2 diabetic patients who were on sulphonylurea (glimepiride) were recruited over a period of 6 months. Out of these, 150 patients were enrolled in this double-blind randomized placebo-controlled trial based upon the following criteria. The diagnostic criteria of NAFLD were fatty liver changes (grades 1–3) on abdominal ultrasound, deranged liver enzymes, and absence of chronic alcohol consumption. The inclusion criteria were patients having T2DM with BMI > 28 and HbA1c < 8.5. They were having fatty liver changes on abdominal ultrasound and mild to moderate elevation of serum liver enzymes. Patients with a history of alcohol, smoking, uncontrolled diabetes (HbA1c > 9.0) pregnancy, lactation, thyroid disorders, renal dysfunction, cardiac problem, and chronic liver and decompensated liver disease in the form of hepatitis B and C were excluded from the study. Patients whose abdominal ultrasounds findings were extremely abnormal (mass, fibrosis, ascites, and cirrhosis) and aminotransaminase levels were severely high (ALT and AST greater than 15 times the upper limit of normal according to Johns Hopkins Diabetes Guide) suggest severe liver cell injury was also worked for acute viral hepatitis, hereditary, and autoimmune, and other disorders of liver were also excluded from the study. In addition, patients taking any anti-diabetic and other drugs that have beneficial effects in NAFLD such as biguanide, thiazolidinediones, GLP-1 analogue and DPP-4 inhibitors, vitamin E, silymarin, and anti-dyslipidemic agents were also excluded from the study. Ethical permission was got from the institutional review board (IRB) and study perspectives were clearly explained to all patients before taking informed consent.

The patients were randomly divided into two groups. The randomization was done by randomization bock of 1:1 by computer-generated software. The randomization number was assigned in chronological order to each patient. Randomization and allocation were concealed from the researchers and patients until the statistical analysis was completed. Patients in group A (67) were given tab dapagliflozin 5–10 mg while patients in group B (71) were given tab placebo in addition to lifestyle modifications for a period of 12 weeks. The dose was adjusted according to blood sugar level. Patients were advised to do a morning walk daily for 30 min and were advised hypo-caloric diet as a lifestyle modification of NAFLD.

An experienced radiologist assessed the fatty liver changes with a high-resolution ultrasound machine. The fatty liver grading from 1 to 3 was done on the basis of hepatic parenchyma echogenicity, hepato-renal contrast, blurring of the diaphragm, and intrahepatic vessel. The radiologist, researches, and patients were blinded to the treatment plan.

The study's primary endpoint was to assess changes in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while the secondary end point was to determine body weight, BMI, fasting blood sugar, and HbA1c from baseline by each drug.

Body mass index (BMI) was calculated by standardized formula as body weight (kg)/height (m^2). A 5 ml of the blood was collected through a cubital vein to analyze blood sugar, HbA1C, lipid profile, and liver enzymes following a standardized method.

Data analysis

Statistical package for social sciences (SPSS-20) was used to analyze data. Numeric values were expressed as mean ± standard deviation. A t-test was applied to see the baseline changes among the two groups. A paired t-test was applied to compare changes within group while Mann–Whitney U-test and t-test were applied to compare changes between groups from baseline to 12 weeks. A p-value < 0.05 was seemed to be statistically significant. A sample size of 65 ± 10 was calculated by anticipating more than 20% reduction in ALT in group B while more than 50% reduction in group A with a dropout rate of 10%.

Results

A total of 450 patients were screened, out of which 150 patients were enrolled in the study. They were divided into two groups, each containing 75 patients. The safety and tolerability profile of drugs in both groups was quite good and no major adverse effects were recorded over a period of 12 weeks. However, 4 patients in the control group and 3 patients in the dapagliflozin group developed repeated hypoglycemic attacks and left the study. Five patients complain of increased frequency of urine in the dapagliflozin group and refused to participate further in the study. So, a total of 138 patients completed the study, 67 in dapagliflozin and 71 in the placebo group. These all have been shown in the flowchart (Fig. 1). There was no significant statistical difference recorded in terms of body weight, BMI, fasting blood sugar, HbA1c, aminotransferases, and fatty liver grading at the start of the study in both study groups (Table 1).

After 12 weeks of treatment, glycemic control was improved in both study groups, i.e., dapagliflozin (FBS from 95 ± 14 to 80 ± 11 mg/dl with p = 0.003, HbAIc from 7.5 ± 4.5 to 6.4 ± 5.4% with p = 0.002) and placebo (FBS from 100 ± 9.5 to 90.5 ± 13 mg/dl with p = 0.004, HbA1c from 8.2 ± 3.5 to7.6 ± 4.2% with p = 0.006).However, dapagliflozin showed a significant reduction in FBS and

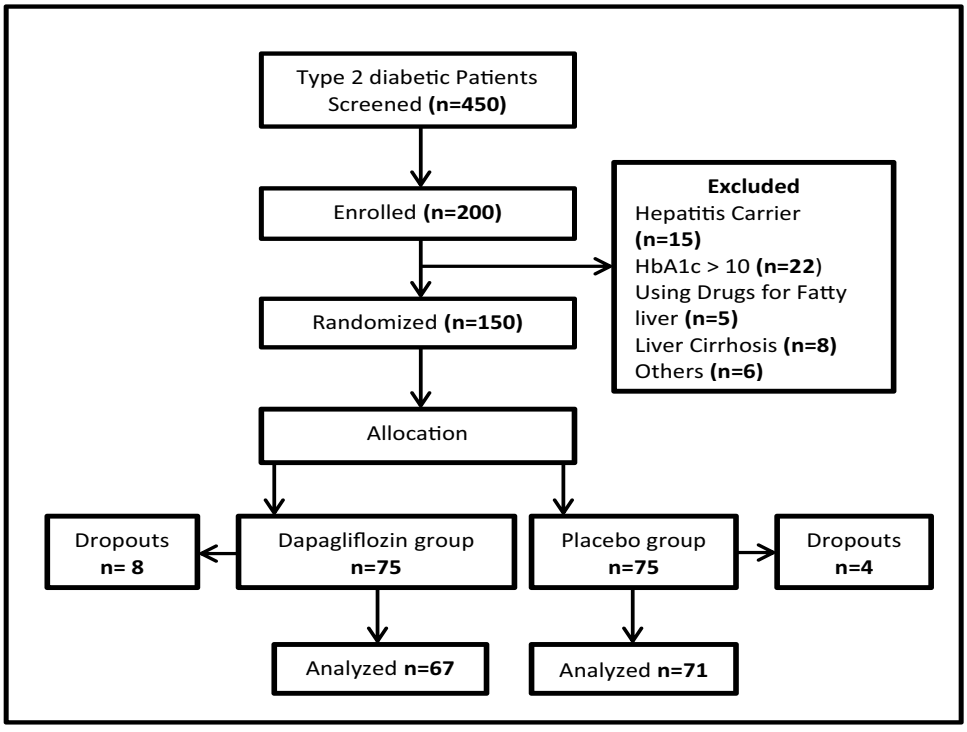

Fig. 1 Flowchart of the randomized placebo-controlled trial

Table 1 Baseline demographics parameters of study subjects (N = 150)

Baseline parameters	Dapagliflozin (n = 67	Placebo (n = 71)	*p*-value
Age (years)	29 ± 16	31 ± 14	0.22
Gender (M/F)	34/20	36/19	0.67
Body weight (kg)	90 ± 13.5	85 ± 17.8	0.073
BMI (body mass index kg/m^2)	29.5 ± 2.5	31.5 ± 3.0	0.04
Blood pressure systolic (mmhg)	110 ± 9.5	120 ± 7.5	0.42
Blood pressure diastolic (mmhg)	75 ± 6.5	85 ± 5	0.07
Blood sugar fasting (mg/dl)	95 ± 14	100 ± 9.5	0.68
HbA1c	7.5 ± 4.5	8.2 ± 3.5	0.86
Ultrasound fatty liver grading (1/2/3)	67 (18/41/8)	71 (14/46/11)	0.36

Values are presented ± standard deviation

t-test between two groups

HbA1c as compared to placebo with p value 0.006 and 0.04 respectively.

On the other hand, body weight was significantly reduced in the dapagliflozin group (from 90 ± 13.5 to 84 ± 11.6) as compared to the placebo group (from 85 ± 17.8 to 85.5 ± 13.7) with p = 0.002. BMI was also reduced in the dapagliflozin group (from 29.5 ± 2.5 to26.5 ± 3.5) versus placebo group (from 31.5 ± 3.0 to 29.5 ± 4.2) with p = 0.002. There was also significant reduction in ALT in the dapagliflozin group from 69 ± 15.5 to 52 ± 12.8 versus placebo from 68 ± 20.5 with p = 0.04 and AST from 74 ± 13 to 47 ± 13.5 versus placebo from 71 ± 12.5 to 65 ± 10.5 with p = 0.02 (Table 2). The difference in ALT and AST reduction between two groups was statistically significant, i.e., ALT – 17 ± 14.2 vs + 4 ± 17.4, $p = 0.001$ and AST – 27 ± 5.4 vs – 6 ± 16.5, $p = 0.002$ (Fig. 2).

Table 2 Comparison of changes at baseline and after treatment with Dapagliflozin

Parameters	Dapagliflozin (n-67)	Placebo (n-71)	*p* value*
Body weight (kg)			
Baseline	90 ± 13.5	85 ± 17.8	0.073
End point	84 ± 11.6	85.5 ± 13.7	0.002
Changes from baseline	− 6 ± 7.2	− 0.5 ± 8.6	0.005
BMI (kg/m^2)			
Baseline	29.5 ± 2.5	31.5 ± 3.0	0.04
End point	26.5 ± 3.5	29.5 ± 4.2	0.005
Changes from baseline	− 3 ± 2.8	− 1.5 ± 3.6	0.002
Blood sugar fasting (mg/dl)			
Baseline	95 ± 14	100 ± 9.5	0.68
End point	80 ± 11	90.5 ± 13	0.006
Changes from baseline	− 15 ± 16.6	− 9.5 ± 12.8	0.002
HbA1c			
Baseline	7.5 ± 4.5	8.2 ± 3.5	0.86
End point	6.4 ± 5.4	7.6 ± 4.2	0.04
Changes from baseline	− 3 ± 4.1	− 0.6 ± 5.8	0.001
ALT (IU/l)			
Baseline	69 ± 15.5	68 ± 20.5	0.48
End point	52 ± 12.8	72 ± 15.0	0.04
Changes from baseline	− 17 ± 14.2	+ 4 ± 17.4	0.001
AST (IU/l)			
Baseline	74 ± 13	71 ± 12.5	0.23
End point	47 ± 13.5	65 ± 10.5	0.02
Changes from baseline	− 27 ± 5.4	− 6 ± 16.5	0.002

*P value (comparison of changes of each variable between the two groups)

Discussion

In this study, we investigated the effect of dapagliflozin on body weight, BMI, glycemic control, and liver enzymes in patients of NAFLD with concomitant diabetes. Our results showed that dapagliflozin significantly lowered body weight, BMI, fasting blood sugar, and HbA1C level. This improvement in glycemic control and body weight was further accompanied by a reduction in serum aminotransferase levels.

The data about the effect of dapagliflozin on various parameters in NAFLD patients having T2DM in Pakistan is sparse and limited. So, the results were compared with international studies. A meta-analysis of various clinical studies revealed the well-documented potential role of SGLT-2 inhibitors on body weight reduction [10]. Our study showed that dapagliflozin significantly reduced body weight and BMI over a period of 12 weeks. Weight loss either by exercise or by the dietary plan is usually preferred by most clinicians because there is still no FDA-approved drug for the treatment of NAFLD so far. Studies showed that a 5–10% reduction in body weight significantly improved NAFLD-associated metabolic and radiological changes. Moreover, 10% weight loss significantly improved histological changes

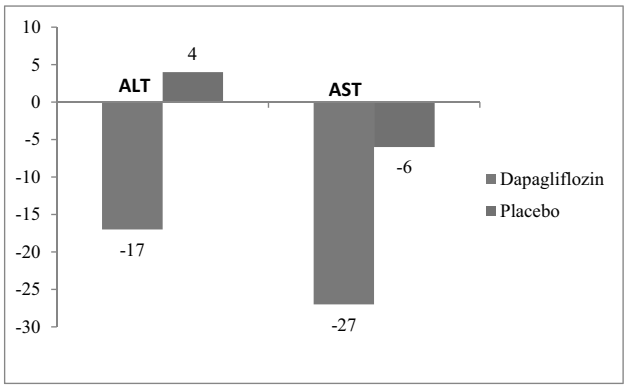

Fig. 2 Comparison of ALT and AST reduction between two groups. ALT and AST decreased significantly in the dapagliflozin group than in the placebo group. The difference in ALT and AST reduction between two groups was statistically significant, i.e., ALT − 17 ± 14.2 vs + 4 ± 17.4, $p = 0.001$ and AST − 27 ± 5.4 vs − 6 ± 16.5, $p = 0.002$

in patients with NASH which is an advanced form of fatty liver that ultimately progress to hepatic fibrosis, cirrhosis, and even hepatocellular cancer [11, 12].

The weight-reducing properties of various anti-diabetic drugs made them renders for the treatment of NAFLD and their role have been already well established in various clinical studies. Out of which biguanides, thiazolidinediones, DPP-4 inhibitors, and GLP-1 analogues reduced body weight through various known mechanisms [5]. The mechanism through which dapagliflozin reduce body weight involves changes in body fluid composition through osmotic diuresis. This weight reduction property of dapagliflozin is dose dependent. Our results were consistent with various studies that showed that dapagliflozin reduced body weight more effectively than any other class of anti-diabetic drugs [13].

Choi et al. [14] revealed that dapagliflozin has a more pronounced effect on body weight loss, glycemic control, and ALT reduction as compared to DPP-4 inhibitors when both used as a combination therapy with metformin. However, we analyzed dapagliflozin as a monotherapy in NAFLD patients and found similar results. In another study, dapagliflozin significantly reduced body weight in comparison with pioglitazone and sitagliptin. This body weight reduction was accompanied by improvement in liver inflammation and fibrosis. The duration of the study was 76 weeks in comparison with our study. Moreover, we did not perform liver fibrosis index, i.e., aspartate aminotransferase to platelets ratio (APRI) index [15].

In a randomized control trial over a period of 24 weeks, dapagliflozin not only reduced body weight and visceral fat but also significantly improved hepatic steatosis and ameliorates hepatic fibrosis in type 2 diabetic NAFLD patients. They used transient elastography for the assessment of liver steatosis and fibrosis [16]. On the contrary, we used only ultrasonogrpahic assessment of fatty liver changes at the start of the study.

Our results were almost similar to studies conducted by Itant et al. [17] and Seko et al. [18] in which canagliflozin another SGLT-2 inhibitor significantly reduced body weight, blood sugar, HbA1c, triglycerides, and liver enzymes in T2DM patients with NAFLD. However, in our study, we did not analyze serum lipid profile as they were not deranged. Similarly, a study analyzed by Scheen et al. [19] concluded that SGLT-2 inhibitors in comparison with metformin, glimepiride, and DPP-4 inhibitors significantly reduced liver enzyme levels beyond similar glycemic control effects. A study was conducted in Japan having biopsy-proven NAFLD in T2DM patients. In this study, both SGLT-2 and DPP-4 inhibitors significantly reduced HbA1c, body weight, and transaminase level similar to our study [20].

Aso et al. [21] pointed out that dapagliflozin significantly decreased serum DPP-4 enzyme level. The role of the DPP-4 inhibitors in the treatment of NAFLD is already well established. SGLT-2 also decreased subcutaneous adipose tissue, visceral adipose tissue, and liver enzymes in this randomized control trial over a period of 24 weeks. This reduction of the DPP-4 enzyme by SGLT-2 may be a good therapeutic option in patients of NAFLD and NASH in future.

A study conducted by Tobita et al. [22] on NASH patients which is the more advanced form of NAFLD revealed that dapagliflozin significantly reduced body mass index (BMI), waist circumference, body fat, blood sugar, HbA1c, liver enzymes, and insulin concentration. Dapagliflozin also significantly increased the level of adiponectin. Adiponectin level usually decreased in obese people and dapagliflozin has a potential effect on this enzyme to reduce body weight.

A systematic view of 94 randomized control trials and 120 observational studies over a period of 12 weeks concluded that SGLT-2 inhibitors significantly improved liver enzymes, liver fat content, and liver fibrosis indices in patients of NAFLD with concomitant diabetes. Moreover, SGLT-2 inhibitors have pronounced effects on body weight, HbA1c, dyslipidemia, and insulin resistance with good safety and tolerability profile [23].

Many studies have postulated that SGLT-2 inhibitors caused a significant improvement in hepatic steatosis, liver fat, visceral fat, and liver fibrosis measured by fibroScan, MRI-derived hepatic fat fraction (HFF), MRI-derived proton density fat fraction (PDFF), and liver biopsy in addition to improvement in other parameters. However, we did not perform these tests because of high cost and invasiveness. Moreover, some tests are also not available at our hospital.

Strength and limitation of the study

The main limitation of our study is that we performed ultrasound at the start of the study only for the diagnosis of NAFLD. It was much better that we also performed it at the end of the study in order to see the radiological improvement of NAFLD. We did not perform CT-scan, fibro scan, MRI-derived hepatic fat fraction (HFF), and other radiological tests due to high cost.

The main strength of our study was that we ruled out all drugs such as biguanides, that had a beneficial effect in NAFLD patients.

Conclusion

Dapagliflozin has a strong potential to reverse NAFLD-associated metabolic and biochemical changes in type 2 diabetic patients.

Recommendation

However, for quality evidence, it is necessary to compare SGLT-2 inhibitors randomized control trial with standardized treatment of NAFLD. Moreover, a large sample size and long follow-up studies are needed in the future. A similar study should be conducted in non-diabetics to investigate dapagliflozin-independent effects as treatment of NAFLD.

Data availability Can be provided on request.

Declarations

Ethics approval Ethical approval is obtained from the Institutional Review Board of Sheikh Zayed Medical College, Rahim Yar Khan via letter no:1351/IRB/SZMC/SZH.

Consent to participate Written informed consent was obtained.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Factors associated with influenza vaccine coverage among patients with diabetes: Korea National Health and Nutrition Examination Survey 2016–2018

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Abstract

Background Although the influenza vaccine has been proven to be effective, this common disease has high morbidity and mortality rates. Moreover, adults with diabetes are at a high risk of influenza-mediated morbidity and mortality.

Purpose of the study With the increasing prevalence of diabetes, influenza is more lethal in diabetics; thus, we aimed to investigate the factors associated with influenza vaccination coverage in patients with diabetes.

Methods Cross-sectional data were obtained from the Korea National Health and Nutrition Examination Survey (2016–2018). We retrospectively analyzed whether sociodemographic, health-related, and medical factors are associated with influenza vaccination coverage in patients with diabetes. We performed a complex sample logistic regression analysis and estimated the odds ratios (ORs) by adjusting for statistically significant factors.

Results The number of survey subjects was 18,553. The vaccine coverage rate among patients with diabetes was 60.6%. In the univariate analysis, sex, educational level, smoking, exercise, drinking, marital status, private health insurance, activity limit, economic activity, age, and EuroQol-5 Dimension scores, which were used to assess health-related quality of life, were associated with vaccination coverage. In the multivariate analysis, only age and economic activity were associated with vaccination coverage. The vaccination coverage rate was higher for people who did not undertake economic activities and who were older (OR 1.512 (1.087–2.105), OR 2.212 (1.822–2.686), respectively, $p < 0.001$).

Conclusion National interventions involving public health centers are necessary to encourage influenza immunization for patients with diabetes, especially younger patients and those who work or undertake economic activities.

Keywords Diabetes · Influenza vaccination coverage · South Korea

Introduction

Despite the proven effectiveness of the influenza vaccine, this common disease still has high mortality rates [1]. Vaccination is the most cost-effective way of reducing the risk of influenza and its complications, especially in older adults and patients with chronic diseases [1–3]. Seasonal influenza pandemics are disastrous for public health, especially during winter [1]. Further, adults with diabetes are at a high risk of influenza-mediated morbidity and mortality [4, 5].

A systematic review of 15 studies published between January 2000 and March 2017 [6], provided data on the immunogenicity, safety, effectiveness, and cost effectiveness of influenza vaccination in diabetics. The immunogenicity of influenza vaccination in diabetic patients after vaccination is comparable to that of healthy participants. Vaccination against influenza is reported to reduce the risk of hospitalization and fatality in diabetics, especially those over the age of 65 years. As such, the need for and value of annual influenza vaccination for diabetics to alleviate serious complications, such as hospitalization and death, is high. In another study, data from the Health and Nutrition Examination Survey III–VI (2005–2015) were analyzed to identify the factors associated with influenza vaccine coverage among diabetic patients in Korea [7]. The study found that socioeconomic and health-related factors are associated with influenza vaccination among diabetic patients.

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In a Canadian study, patients with diabetes, even those aged under 65 years, were found to be susceptible to influenza and had higher rates of influenza-attributable all-cause hospitalization [4]. The reason for the increased vulnerability to influenza in patients with diabetes is their impaired immune response; they have abnormal CD4/CD8 ratios, and their natural killer cells and monocytes do not function well [8, 9].

For effective prevention against influenza, the World Health Organization (WHO) recommends a vaccination coverage rate (VCR) of at least 75% in vulnerable populations [10]. However, recent data demonstrate that the recommended influenza VCR is not being achieved in most countries [11–13].

In 2011, 366 million patients worldwide were recorded as having diabetes, and according to the International Diabetes Federation, this number is expected to increase by 552 million by 2030 [14]. As the prevalence of diabetes increases and people with diabetes remain vulnerable to infection [15, 16], it is important to examine the influenza VCR in this population. In South Korea, national support for the influenza VCR is very high, and it contributes to various indicators, including influenza-related outpatient consultations, hospitalization and excess mortality, quality-adjusted life years, and the incremental cost-effectiveness ratio [17].

While several studies on the influenza VCR have been conducted in South Korea, these have focused only on chronic obstructive pulmonary disease [18], other chronic diseases [19, 20], or older adults [21–23]. Thus, the aim of this analysis was to evaluate the socioeconomic, personal, and environmental factors associated with the influenza VCR among people with diabetes, who are very vulnerable to infection.

Materials and methods

Data source

The present analysis was conducted using cross-sectional data obtained from the 2016–2018 Korea National Health and Nutrition Examination Survey (KNHANES). The KNHANES is annually conducted to assess the health and nutritional status of the non-institutionalized civilian population in South Korea. These investigations, which include a health interview survey, a health examination survey, and a nutrition survey, are conducted by trained investigators. In the present analysis, a clustered and stratified random sampling method based on national census data was used. All factors included in the analysis were assessed using the KNHANES methodology to ensure objectivity of data. Participants below the age of 19 years, as well as those whose survey items were missing, were excluded from the analysis.

Further, diabetes was the only disease included in the survey; all other chronic diseases, such as cardiovascular disease, respiratory disease, liver disease, and cancer, were excluded.

Ethical considerations

The analysis was approved by the Clinical Trial Screening Committee of W Hospital, and the requirement of informed consent was waived (institutional review board approval number: 2020–03-013). Detailed information on the KNHANES is available for reference on the website [24]. The KNHANES allows the public use of anonymous data once researchers sign and submit an agreement to use the data for research purposes only. The present analysis complies with the principles laid down in the amended Declaration of Helsinki.

Sociodemographic factors

The following sociodemographic factors were considered: age, gender, educational level, economic activity, marital status, and cohabitation status. Educational level was classified into four groups: elementary school graduates, middle school graduates, high school graduates, and university graduates. Marital status was divided into two groups: married and unmarried. Cohabitation status was divided into four groups: cohabiting, separated, bereaved, and divorced. Non-employment was divided into two groups: unemployed and non-economically active.

Health behavior and medical factors

Subjective health status and disease were investigated as medical factors. Subjective health status was classified into five groups: very good, good, average, bad, and very bad. The participants' usually perceived level of stress was investigated through the following statements:(1) "I feel a great deal of stress," (2) "I feel much stress," (3) "I feel a little stress," (4) "I hardly feel stress," (5) "Not applicable," and (6) "Unknown."

Choosing statement 1 or 2 indicated that participants experienced much stress, while choosing statement 3 or 4 indicated that they experienced less stress. A stress-sensitive participant was marked 1, and a less stress-sensitive participant was marked 0. The average value was recorded as the stress recognition rate.

The EuroQol-5 Dimension (EQ-5D) is a comprehensive index used to assess health-related quality of life across

five dimensions: exercise ability, self-management, daily activities, pain/discomfort, and anxiety/depression. Participants select one of three levels: "No problem at all," "Some problems," and "There are many problems" for the items on the five dimensions. A scoring conversion system was used to convert responses into a number between 1 (completely healthy) and − 1 (not healthy at all) [25]. This was calculated using the estimated quality weight for the Korean population, which yielded the EQ-5D index. The following formula was used:

$$\begin{aligned}\text{EQ} - \text{5D index} = 1 - (&0.0081 + 0.1140 * \text{M2} + 0.6274 * \text{M3} \\ &+ 0.0572 * \text{SC2} + 0.2073 * \text{SC3} + 0.0615 * \text{UA2} \\ &+ 0.2812 * \text{UA3} + 0.0581 * \text{PD2} + 0.2353 * \text{PD3} \\ &+ 0.0675 * \text{AD2} + 0.2351 * \text{AD3})\end{aligned}$$

Regarding smoking status, participants were classified as either smokers or non-smokers. People who smoked at the time of data collection or had smoked more than 100 cigarettes throughout their lives were defined as smokers. Alcohol consumption was investigated by calculating the amount of alcohol consumed by a participant per week in grams. The International Physical Activity Questionnaire was used to measure the extent of physical exercise; "regular exercise" was defined as exercising at least five times a week for 30 min per session or participation in vigorous physical activity three times a week for more than 20 min per session.

The participants' blood samples were randomly collected once after an 8-h fast and subjected to laboratory testing. The samples were immediately processed, refrigerated, and transported to the central laboratory (Neodin Medical Institute, Seoul, South Korea).

Statistical analysis

SPSS for Windows version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and statistical significance was set at $p < 0.05$. Owing to the complexity of KNHANES data, a complex sample analysis was conducted considering weights. Weights were applied according to the Centers for Disease Control and Prevention's guidelines for using KNHANES raw data.

For the overall results, a frequency analysis was performed using the Complex Samples Frequencies procedure. The complex sample Rao-Scott adjusted chi-square test and complex sample generalized linear model were used to compare differences in the general characteristics of patients with and without diabetes. A complex sample logistic regression test was used to analyze the factors associated with influenza vaccination among patients with diabetes.

Results

General characteristics and diabetes prevalence (Table 1)

There were two groups—patients with and without diabetes. Among patients with diabetes, there were more people with an educational level of primary school or lower than those who had a college degree or higher (36 vs. 15%). Further, in this group, 18.3% were smokers, 33.4% engaged in aerobic exercise, and 44.3% drank monthly. The average stress perception rate was 23.5%, 96.6% were married, 72.1% were living with someone, 58.6% had private health insurance, and 16% performed limited physical activity. When evaluating their subjective health, the proportions of participants with diabetes who answered "very good" (1.4%) and "good" (10.8%) were low. Further, 46.9% were not employed and had lower EQ-5D scores than people not living with diabetes (0.90 ± 0.004 vs. 0.96 ± 0.001; $p < 0.0001$).

People with diabetes had higher influenza VCRs compared with those who did not have diabetes (60.6 vs. 34.1%). People with diabetes were rarely inoculated twice a year and were more likely to be vaccinated in hospitals or clinics than in public health centers (62.8 vs. 34.9%; $p < 0.0001$).

Factors associated with influenza vaccination among people with diabetes (Table 2)

A single analysis of factors associated with influenza vaccination among people with diabetes revealed that women had higher VCRs than men. Further, those with an educational level of primary school or lower had higher VCRs than those who had obtained a college degree or higher odds ratio [(OR) 1.928 (1.516–2.452), OR 4.215 (2.905–6.116), respectively; $p < 0.001$].

VCRs were higher among non-smokers, those who did not engage in aerobic exercise, and those who were not monthly drinkers (OR 2.437 (1.766–3.363), OR 1.339 (1.034–1.734), OR 2.180 (1.689–2.814), respectively; $p < 0.001$).

VCRs were low among unmarried participants and high among non-private health subscribers (OR 0.184 (0.081–0.418), OR 2.984 (2.323–3.835), respectively; $p < 0.001$). VCRs were also higher among those with limited physical activity and those who were not employed

Table 1 General characteristics and diabetes prevalence

		$N = 18,553$	Normal	Diabetes	p value
Gender	Men	8127 (49.8)	7240 (49.6)	885 (52.3)	0.058
	Women	10,426 (50.2)	9496 (50.4)	924 (47.7)	
Educational level	≤Elementary school	3656 (13.9)	2907 (12.1)	749 (36)	< 0.0001
	Middle school	1782 (8.3)	1490 (7.7)	292 (15.7)	
	High school	5617 (33.2)	5184 (33.7)	433 (27.4)	
	≥College	6530 (39.1)	6296 (41.1)	234 (15)	
Smoking		3338 (21.3)	3044 (21.6)	293 (18.3)	< 0.0001
Exercise		7554 (43.8)	7008 (44.6)	546 (33.4)	< 0.0001
Drinking		9888 (58.1)	9158 (59.2)	729 (44.3)	< 0.0001
Stress recognition		4903 (28.1)	4503 (28.5)	398 (23.5)	< 0.0001
Marital status	Married	15,477 (76.8)	13,708 (75.1)	1761 (96.6)	< 0.0001
	Single	3076 (23.2)	3028(24.9)	48(3.4)	
Cohabitation status	Cohabiting	12,837 (65.6)	11,560 (65.1)	1271 (72.1)	< 0.0001
	Separated	116 (0.6)	100 (0.5)	16 (0.9)	
	Bereaved	1705 (6.6)	1333 (5.6)	371 (18.4)	
	Divorced	812 (3.9)	708 (3.8)	103 (5.3)	
Private health insurance		14,172 (80.7)	13,227 (82.6)	944 (58.6)	< 0.0001
Activity limit		1514 (6.5)	1211 (5.8)	303 (16)	< 0.0001
Subjective health	Very good	799 (4.5)	774 (4.7)	25 (1.4)	< 0.0001
	Good	4164 (23.9)	3962 (25)	201 (10.8)	
	Average	9203 (49.6)	8402 (50)	801 (45.5)	
	Bad	2769 (14)	2290 (12.9)	479 (26.7)	
	Very bad	765 (3.1)	9 (16.1)	216 (10.7)	
Economic activity	Employed	10,677 (61.1)	9906 (62.3)	771 (47.3)	< 0.0001
	Non-employed	6918 (33.5)	5980 (32.4)	938 (46.9)	
Influenza vaccination	Yes	7750 (35.4)	6557 (33.3)	1193 (60.6)	< 0.0001
	No	9900 (59.5)	9373 (61.6)	527 (34.1)	
Frequency of vaccination	1	7675 (35.1)	6493 (33)	1182 (60.1)	< 0.0001
	2	75 (0.3)	64 (0.3)	11 (0.5)	
Place of vaccination	Public health center	2239 (24.5)	1796 (23)	443 (34.9)	< 0.0001
	Hospital,	5210 (70.4)	4484 (71.6)	726 (62.8)	
	Others	295 (5.0)	272 (5.4)	23 (2.2)	
Age, years		40.87 ± 0.27	45.89 ± 0.23	63.60 ± 0.36	< 0.0001
EQ-5D		0.95 ± 0.001	0.96 ± 0.001	0.90 ± 0.004	< 0.0001
HbA1c		5.61 ± 0.008	5.51 ± 0.01	7.21 ± 0.04	< 0.0001

Smoking: the percentage of people who had smoked more than five packets of cigarettes (100 cigarettes) in their lifetime or who smoked at the time of the survey; alcohol: percentage of participants who had an alcohol intake of more than once a month in the last year; non-employed: unemployed and non-economically active population; stress: percentage of stress in everyday life; EQ-5D: EuroQol-5 Dimension; HbA1c: hemoglobin A1c

Values are presented as number (%) or mean ± standard deviation

[a]The p value was calculated using the complex sample Rao-Scott adjusted chi-square test and complex sample generalized linear model t-test

(OR 1.471 (1.048–2.065), OR 3.236 (2.473–4.235), respectively; $p < 0.001$).

The VCR increased as participants' age increased by 10 years, and as the EQ-5D score decreased by 1 (OR 2.586 (2.208–3.028), OR 10.939 (3.961–30.207), respectively; $p < 0.001$).

A multivariate analysis of the factors associated with influenza vaccination among people with diabetes demonstrated that VCRs were higher among those who were not employed, and that VCRs increased with age (OR 1.512 (1.087–2.105), OR 2.212 (1.822–2.686), respectively; $p < 0.001$).

Table 2 Factors associated with influenza vaccination among people with diabetes

		Univariate OR (95% CI)	Multivariate OR (95% CI)
Gender	Men	1	1
	Women	1.928 (1.516–2.452)	1.095 (0.79–1.519)
Educational level	≤Elementary	4.215 (2.905–6.116)	1.266 (0.785–2.042)
	Middle	2.406 (1.548–3.739)	1.462 (0.88–2.429)
	High	1.409 (0.947–2.096)	1.225 (0.766–1.961)
	≥College	1	1
Smoking	No	2.437 (1.766–3.363)	1.319 (0.902–1.930)
Exercise	No	1.339 (1.034–1.734)	0.822 (0.607–1.112)
Drinking	No	2.180 (1.689–2.814)	1.134 (0.826–1.558)
Marital status	Married	1	1
	Single	0.184 (0.081–0.418)	0.566 (0.193–1.659)
Private health insurance	No	2.984 (2.323–3.835)	1.061 (0.774–1.456)
Activity limit	Yes	1.471 (1.048–2.065)	0.821 (0.531–1.271)
Economic activity	Employed	1	1
	Non-employed	3.236 (2.473–4.235)	1.512 (1.087–2.105)
Age	10-year increase	2.586 (2.208–3.028)	2.212 (1.822–2.686)
EQ-5D	-1	10.939 (3.961–30.207)	1.08 (0.365–3.193)

OR: odds ratio, CI: confidence interval.

Smoking: the percentage of people who had smoked more than five packets of cigarettes (100 cigarettes) in their lifetime or who smoked at the time of the survey; alcohol: percentage of participants who had an alcohol intake of more than once a month in the last year; non-employed: unemployed and non-economically active population; EQ-5D, EuroQol-5 Dimension

Adjusted for gender, age, educational level, smoking, exercise, drinking, marital status, private health insurance, activity limit, economic activity, age, EQ-5D score

[a] OR and 95% CI were calculated using a complex sample logistic regression test

Discussion

As previously discussed, people with diabetes are especially vulnerable to all infectious diseases, including influenza. Recently, people with diabetes were reported to have the second-highest mortality risk from COVID-19. Before highlighting the need for vaccination, however, it is necessary to evaluate the factors associated with the influenza VCR among people with diabetes. Previous South Korean studies that investigated the influenza VCR and associated factors have focused either on the general population [26, 27] or those with other chronic diseases [19, 20].

As people with diabetes are more susceptible to influenza and have a greater risk of medical complications from infection, the WHO and several National Immunization Technical Advisory Groups recommend annual influenza vaccination [28, 29].

A systematic review and meta-analysis of people with diabetes demonstrated that the effectiveness of the influenza vaccine differs slightly according to age [30]. Overall, the effectiveness of the influenza vaccine among working-age people (18–64 years) was 58%. Although hospitalization due to influenza or pneumonia did not affect the overall mortality rate, it was found that vaccination for influenza decreased the hospitalization rate among people with diabetes of working age. In addition, vaccination for influenza among older adults (over 65 years old) has been demonstrated to reduce mortality rates from all causes, hospitalization rates from all causes, and hospitalization rates from influenza or pneumonia [30].

The findings mentioned above prove that the influenza vaccine is important, regardless of age and comorbid diseases. Thus, efforts to increase the influenza VCR are required. VCRs vary from country to country, and the factors affecting the rate within each country are different [31]. Therefore, in this analysis, we sought to identify the factors associated with the influenza VCR in South Korea.

According to the most recent data, the influenza VCR among people with diabetes in South Korea was 60.6%. In a recent analysis, the goal was to increase the influenza VCR to 90% among people over 65, and 60% among high-risk groups aged 18–64 in the USA [32]. In comparison with these numbers, the VCR in South Korea has met the target rate. The high VCR can be attributed to the South Korean medical system. In South Korea, the National Health Insurance Service is mandatory for everyone. Under this program, people receive deductions for a significant portion of medical costs. They also receive benefits when utilizing health

management services. The cost of influenza vaccination varies slightly depending on the type of vaccine and hospital but does not exceed $50. As per the national policy, people aged above 65 receive free immunization.

Although the VCR across risk groups and countries continues to increase, few countries are close to achieving the VCR target set by the WHO [28]. In the univariate analysis, many variables, such as gender, educational level, smoking, exercise, drinking, marital status, private health insurance, activity limit, economic activity, age, and EQ-5D score, were found to be associated with the VCR. However, the multivariate analysis revealed that only age and economic activity were associated with the VCR. Our findings demonstrated that there are only two individual patient factors (not employed and old age) that are associated with VCRs. In a previous Korean study of 32,268 individuals who participated in the KNHANES III–VI (2005–2015), the influenza VCR among diabetic patients was 50.0%, which was lower than our results. For the non-diabetes group, the VCR was 38.2%, which was higher than our results. Influenza VCR in diabetic patients was associated with socioeconomic (old age, female sex, high family income, medical assistance insurance) and health-related factors (dangerous drinking, obesity, lack of recent health screening) [7].

A study of 10 countries in Africa, Asia–Pacific, Eastern Europe, Latin America, and the Middle East demonstrated that the influenza VCR is not affected by patient factors [33]. This is similar to our findings and suggests that national programs are necessary to increase the influenza VCR [33]. Indeed, the UK has achieved its target VCR, and this is the result of active national support and health programs [34].

Although the rates were calculated in different years, a study conducted in five European countries demonstrated that the influenza VCRs among patients with chronic diseases, such as diabetes, were as follows: 59.4% in the UK, 29.8% in Germany, 36.7% in Italy, 34.4% in France, and 37.1% in Spain [35].

In a large-scale study in European countries conducted after the previously mentioned study, an increase in VCRs was observed [34]. However, they still did not reach the rate recommended by the WHO, indicating that management at the national level is necessary [34].

Contradictory to our results, some studies have suggested that several factors affect VCRs. A study in Singapore revealed that high income and high educational levels were associated with high VCR [36]. A study in Spain demonstrated that the following factors affect the influenza VCR: age, urban residence, income, marriage, health awareness, and caregivers [37]. A study of older adults in Brazil revealed that the factors that increase the influenza VCR include old age, being male, high income, high educational level, non-smoking, and solicitation [38]. In Canada, research demonstrated that higher education, higher income, smoking, increased levels of drinking, poor health perception, exercise, and city dwelling increased the influenza VCR [39]. A nationwide study in Spain revealed that old age, previous vaccination, chronic disease, and being female increased the influenza VCR among the vulnerable population [40]. In France, it was demonstrated that VCRs were higher in families with infants, higher educational levels, professional occupations, and previous influenza vaccination [40].

Influenza, which can be prevented through vaccination, causes significant economic losses. In the USA, the total annual cost related to influenza is €10,000–17,000 million [34]. French research estimates that the total cost related to influenza is over €1796 million per year [34]. These economic losses highlight the importance of increasing the influenza VCR.

Based on the most recent data, we analyzed the factors associated with the influenza VCR among people with diabetes in South Korea. However, it does have limitations. First, owing to the use of cross-sectional data, causality cannot be established. Second, not all factors that could be associated with vaccination history were considered. According to Korea's national policy, people over the age of 65 years are eligible for free vaccinations. Therefore, it is possible that the results of this analysis are biased. However, the reason why we included individuals aged 65 years and older and examined their employment status was to analyze the factors that affect the overall VCR in Korea, including the special policy background of Korea.

Conclusions

This study demonstrated that the influenza VCR is low in younger and employed people, possibly owing to time constraints. Overall, the influenza VCR is rarely associated with personal factors in people with diabetes. The results suggest that owing to the governmental healthcare system's major impact on the VCR and based on the fact that only two personal factors (not employed and old age) were associated with the influenza VCR, government intervention is necessary to increase the influenza VCR. Thus, it would be helpful to develop a national program that connects companies with public health centers with the joint goal of improving VCRs.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval The study was approved by the Clinical Trial Screening Committee of Wonkwang University Hospital (approval number: 2020–03-013).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest None.

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ORIGINAL ARTICLE

Association between neutrophil–lymphocyte ratio on arterial stiffness in type-2 diabetes mellitus patients: a part of DiORS Study

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Abstract

Introduction Type-2 diabetes mellitus (T2DM) enhances the risk of atherosclerosis and cardiovascular diseases, which are the primary cause of death among T2DM patients. Neutrophil–lymphocyte ratio (NLR) is a widely available, easy-to-use, and reproducible inflammatory marker. Brachial-ankle pulse wave velocity (baPWV) serves as the indicator for early atherosclerosis changes. The exact mechanism of association between the high NLR and diabetes complications is still unclear, and the most significant mechanism may be related to inflammation. Since an inflammatory marker in clinical practice is limited, a simple, easy-to-use, and widely available marker is needed. The aim was to analyze the association between NLR and arterial stiffness in T2DM patients.

Method This study is part of the Diabetic Ocular Renal Surabaya Study (DiORS Study). Participants were measured for their NLR count by dividing absolute neutrophil count with absolute lymphocyte count in peripheral blood and measuring of arterial stiffness with baPWV. The statistical analysis in use included independent t-test, Mann–Whitney test, Pearson correlation test, or Spearman correlation test. The results of the statistical analysis were significant if $p < 0.05$.

Result The participants' mean age was 54.33 ± 11.34 years, with the duration of diabetes for 7.34 ± 6.80 years. The mean of BMI was 25.47 ± 4.10 kg/m^2, most patients were overweight and obese. The mean of HbA1c was 8.14 ± 1.59% and only 24% participants with good glycemic control. The mean of NLR was 2.69 ± 1.23, with a range of 0.95–6.24, while 84.7% of participants with a high count of NLR (NLR > 1.65). The mean of baPWV was 15.19 ± 2.72 m/s with a range of 10.20–23.30 m/s, and 75.0% of them saw an increased arterial stiffness (baPWV > 13.5 m/s). Association analysis between NLR count and arterial stiffness shows significant results ($r = 0.235$; $p < 0.047$).

Conclusions There is a significant association between NLR with arterial stiffness and the higher NLR count, the more stiffening of the arteries experienced by the participants.

Keywords Arterial stiffness · Brachial-ankle pulse wave velocity · Neutrophil–lymphocyte ratio · Type-2 diabetes mellitus

Introduction

Type-2 diabetes mellitus (T2DM) is the leading health issue with a growing prevalence around the world. Globally, the figures for diabetes cases on 20–79-year-old people in 2019 were estimated at 463 million cases or 9.3% of the world's population and will increase to 578 million in 2030 (10.2%). Indonesia belongs to the top ten countries with the most estimated diabetes cases, collecting around 10.7 million cases in 2019 and is bound to leap to 13.7 million in 2030, then 16.6 million in 2045 [1]. The prevalence of diabetes complications, as a multinational study indicated, recorded 27.2% of macrovascular complications and 53.5% of microvascular complications. In comparison, renal complications accounted for 27.9%, eye

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disease 26.3%, diabetic foot 5.4%, and neuropathy 38.4% [2].

Diabetic macroangiopathy, atherosclerosis due to diabetes can cause cerebrovascular diseases, ischemic heart diseases, peripheral arterial diseases, and other vascular diseases, which are the significant causes of death in diabetic patients and the reducer the quality of life [3]. The latest studies show that arterial inflammation plays a vital role in the pathogenesis of atherosclerosis. The high level of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), IL-18, and tumor necrosis factor-α (TNF-α) are connected to the morbidity and mortality of cardiovascular diseases. These inflammatory markers are related to asymptomatic atherosclerosis but are challenging to conduct in daily clinical practices. Therefore, simple and easy markers are needed. White blood cells (WBC) count is one of the inflammatory biomarkers in clinical practice. Nowaday, WBCs are useful predictors for certain diseases in addition to being an infection marker [4].

Neutrophil–lymphocyte ratio (NLR) is an independent prognostic factor of coronary heart disease and can predict mortality on cardiovascular diseases [5]. Differences are found between NLR counts on diabetes patients with and without complications. An increase in NLR counts is related to a microvascular complication on elderly diabetic patients [6]. NLR is associated with atherosclerosis, which is measured by brachial-ankle pulse wave velocity (baPWV) and coronary calcium score (CCS). Having a high rate of NLR is connected to arterial stiffness and CSS [4].

One of the clinical implication of stiffness in the artery is to predict the increasing risk of cardiovascular disease. Inflammation takes the primary role in stiffness in a large artery, which connects to atherosclerosis, arteriosclerosis, and endothelial dysfunction. The last mentioned can be measured with non-invasive measurement of pulse wave velocity (PWV), which serves as the parameter for arterial stiffness [7]. PWV is a predictor of cardiovascular cases in the general population with hypertension, diabetes mellitus, and end-stage renal disease. Carotid-femoral PWV test is known to be a conventional method. The baPWV tool is currently a more accessible device to use compared to other non-invasive automatic devices. This method can be used to measure PWV in studies with a large volume of samples. BaPWV is related to carotid artery intima-media thickness, which marks the severity of atherosclerosis [8]. The Rotterdam study reported that arterial stiffness is closely related to atherosclerosis in various vascular branches [9]. Pulse wave velocity is the measure of the speed of the pulse wave between two distant places in the arterial system. Since the PWV correlates with distensibility and arterial stiffness, the hardening of the artery walls will cause a high rate of PWV [10]. The latter is then related to cardiovascular diseases and can be considered the marker connecting hyperglycemia and vascular complications [11].

NLR and baPWV increase in T2DM. Early detection of abnormal NLR counts could help find subclinical atherosclerosis in T2DM patients [12]. NLR is a readily available, easy-to-use, and reproducible inflammatory marker. It also can add cardiovascular risk stratification alongside the current risk score [5]. Strong evidence shows that inflammation plays a considerable role in arterial stiffness, and an inflammatory marker can be used as an additional examination to assess cardiovascular risk in clinical practice. The combination of measurement of arterial stiffness and inflammatory marker can complement the non-invasive cardiovascular risk assessment, thus being able to detect high-risk patients and give them preventive care or more regular medical check [13].

The exact mechanism of association between the high NLR and diabetes complications is related to inflammation [14–16]. In Indonesian clinical practice, inflammatory markers such as fibrinogen, CRP, IL-18, and TNF-α were limited. Therefore, more simple and easy markers were needed. This research was conducted to study NLR as a marker of systemic inflammation and its correlation to arterial stiffness, which is a marker of subclinical atherosclerosis in T2DM.

Methods

Participant

Those who participated in this study were T2DM patients who met the inclusion and exclusion criteria. Inclusion criteria include those who had been diagnosed with T2DM and were over 18 years of age. Exclusion criteria include signs of infection (swelling, pain, redness at the site of infection), complaints of fever, abnormal markers of complete blood count tests (WBC count > 12,000/mm^3) or increased erythrocyte sedimentation rate (ESR), active smoker, diagnosed with cancer/malignancy, diagnosed with chronic kidney disease (CKD) stage 5 with hemodialysis, and CKD with kidney transplantation. Participants who are willing to be included in this study were obligated to fill in the consent form.

Design

This study is a cross-sectional design conducted in the Endocrinology and Diabetes outpatient clinic at the Dr. Soetomo General Academic Hospital in Surabaya, Indonesia, from July to December 2019. The total number of study participants was 72 patients who met the inclusion and exclusion criteria. The sampling technique in this study is purposive sampling, based on the sample size formula for the correlation

coefficient of one sample [17], in which the minimum sampling was $n=69$ (r=0.38) as the equation below [12].

$$n = \left[\frac{Z\alpha+Z\beta}{0.5ln\frac{1+r}{1-r}}\right]^2 + 3$$

$$n = \left[\frac{1.95+1.282}{0.5ln\frac{1+0.38}{1-0.38}}\right]^2 + 3$$

$$n = 69$$

This research was part of the Diabetic Ocular Renal Surabaya Study (DiORS Study). Participants were measured for their characteristics, NLR count, and arterial stiffness. Participants' characteristics include age, sex, body mass index (BMI), duration of diabetes, HbA1c levels, blood pressure, lipid profiles (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides), glomerular filtration rate estimation (eGFR) (which measured using the MDRD equation formula), and hematological parameters (hemoglobin, WBC, and platelets count). This study applied a procedure in which patients' data was collected through anamnesis and physical examination according to the required characteristic data. Their venous blood was collected around ± 3 mL, then stored in the EDTA tube. The blood was taken to Dr Soetomo General Academic Hospital Laboratory in Surabaya, Indonesia, to be thoroughly tested using the flow cytometry method with Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan). In a Hematology Analyzer Sysmex XN-1000, there are two kinds of reagent for carrying out the quality control, being XN Check and XN Check BF. Besides doing quality control, calibration using XN CAL dan XN CAL PF of the instrument has been done periodically. Arterial stiffness was examined with baPWV by using VeraSera VS-1000 (Fukuda Denshi, Tokyo, Japan).

NLR is the result of neutrophil count divided by lymphocyte count. Neutrophils are the most numerous type of leukocyte cells, which account for about 50–70% among other leukocyte cells. Absolute neutrophil count (ANC) can be calculated from the type count results by adding up the percentage of segments and stems, then multiplying it with the total leukocyte number. Lymphocytes are the second most leukocyte type after neutrophils (20–40% of total leukocytes). Lymphocyte's rate is calculated from multiplying lymphocyte percentage with the total number of leukocytes. NLR is normal if < 1.65 [18].

In this study, arterial stiffness is obtained when the pulse wave delivery is faster than normal, indicating vascular stiffness. Arterial condition is normal if PWV < 13.5 m/s [9].

Statistical analysis

Results of measurement were presented in the distribution of frequency and average value based on the variable. Statistical analysis was carried out using the IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). The data of measurement results were tested normality using Kolmogorov–Smirnov test, in which the data distribution was not normal. The statistical test used to compare the NLR and baPWV values was the Mann–Whitney test (nonparametric). Meanwhile, the statistical test used for the association between neutrophil–lymphocyte ratio on arterial stiffness is the rank Spearman correlation test (nonparametric). The results of the statistical examination are significant if $p<0.05$.

Result

Participant characteristics

Characteristics of the participants based on age were 54.33 ± 11.34 (18–76) years old. The mean of BMI was 25.47 ± 4.10 (15.6–36.6) kg/m^2. Based on the Asian BMI category, the results showed a normal BMI (18.5–22.9 kg/m^2) in 18 participants (25.0%), overweight (23.0–24.9 kg/m^2) in 20 participants (27.8%), obesity I (25.0–29.9 kg/m^2) in 23 participants (31.9%), and obesity II (≥ 30.0 kg/m^2) in 11 participants (15.3%; Tables 1 and 2).

Neutrophil–lymphocyte ratio

The mean of NLR participants was 2.69 ± 1.23 (0.95–6.24). Most of them (84.7%) or 61 participants had high NLR count (> 1.65), and the other 11 participants (15.3%) in the normal category (≤ 1.65). Analysis of NLR by sex, HbA1c levels, and systolic and diastolic blood pressure factor resulted in a mean NLR count for male and female which were 2.73 ± 1.03 (1.21–5.62) and 2.66 ± 1.36 (0.95–6.24)

Table 1 Distribution of participants' characteristics frequency

Characteristics ($n=72$)	n (%)
Sex, n (%)	
Male	29 (40.3)
Female	43 (59.7)
Body mass index, n (%)	
Normal	18 (25.0)
Overweight	20 (27.8)
Obesity I	23 (31.9)
Obesity II	11 (15.3)
T2DM, n (%)	
Controlled	17 (24.0)
Uncontrolled	55 (76.0)
Hypertension, n (%)	
Yes	30 (41.7)
No	42 (58.3)

Abbreviations: *T2DM* type-2 diabetes mellitus

Table 2 Average of participants' characteristics

Characteristics ($n=72$)	Mean ± SD
Age, years	54.33 ± 11.34
Body mass index, kg/m^2	25.47 ± 4.10
T2DM	
Duration, years	7.34 ± 6.80
HbA1c, %	8.14 ± 1.59
Hypertension	
Systolic, mmHg	139.29 ± 20.30
Diastolic, mmHg	85.18 ± 14.50
Lipid Profile	
Total cholesterol, mg/dL (normal < 200 mg/dL)	206.86 ± 53.30
LDL, mg/dL (normal < 100 mg/dL)	128.44 ± 44.87
HDL, mg/dL (normal ≥ 40 mg/dL)	54.65 ± 20.75
Triglyceride, mg/dL (normal < 150 mg/dL)	157.36 ± 101.85
eGFR, mL/min/1.73 m^2 (normal ≥ 90 mL/min/1.73 m^2)	69.90 ± 34.65
Hematology	
Hemoglobin, g/dL (normal range 11.5–15 g/dL)	12.86 ± 1.75
Platelets, × 10^9/L (normal range 125–350)	302,277.78 ± 111,421.07
WBC, × 10^9/L (normal range 3.5–9.8)	8.16 ± 1.64

Abbreviations: *T2DM* type-2 diabetes mellitus, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *eGFR* estimated glomerular filtration rate, *WBC* white blood cell

respectively ($p=0.358$). The mean NLR count based on HbA1c value for patients with controlled glycemic (HbA1c < 7%) and uncontrolled glycemic (HbA1c ≥ 7%) was 2.62 ± 0.99 (1.11–5.13) and 2.71 ± 1.30 (0.95–6.24) respectively ($p=0.816$). In accordance with the systolic blood pressure (SBP) level, the participants' mean NLR count in normotensive (SBP < 140 mmHg) and hypertensive (SBP ≥ 140 mmHg) category was 2.48 ± 1.07 (0.95–6.15) and 2.94 ± 1.36 (1.00–6.24) respectively ($p=0.106$). Meanwhile, based on diastolic blood pressure (DBP) level, the participants' mean NLR count in normotensive (DBP < 90 mmHg) and hypertensive (DBP ≥ 90 mmHg) category was 2.64 ± 1.19 (0.95–6.24) and 2.79 ± 1.32 (1.00–6.24) respectively ($p=0.603$; Table 3).

Table 3 Neutrophil–lymphocyte ratio and brachial-ankle pulse wave velocity values

Characteristics ($n=72$)	NLR		baPWV	
	Mean ± SD	*p*	Mean ± SD	*p*
Sex				
Male	2.73 ± 1.03	0.358	15.51 ± 2.96	0.650
Female	2.66 ± 1.36		14.97 ± 2.57	
HbA1c				
Controlled	2.62 ± 0.99	0.819	14.62 ± 1.28	0.181
Uncontrolled	2.71 ± 1.30		15.35 ± 3.03	
Systolic hypertension				
Yes	2.48 ± 1.07	0.106	14.21 ± 2.41	0.000**
No	2.94 ± 1.36		16.35 ± 2.65	
Diastolic hypertension				
Yes	2.64 ± 1.19	0.603	14.50 ± 2.43	0.003*
No	2.79 ± 1.32		16.48 ± 2.83	

Statistical analysis using Mann–Whitney test *significant $p<0.05$; **significant $p<0.001$

Abbreviations: *NLR* neutrophil–lymphocyte ratio, *baPWV* brachial-ankle pulse wave velocity

Arterial stiffness measured with baPWV

The mean values of the participants' baPWV were 15.19 ± 2.72 m/s (10.20–23.30 m/s). Most of them, or 54 participants (75.0%), saw an increase in their baPWV level, which is a marker of arterial stiffness or subclinical atherosclerosis, while the remaining 18 participants (25.0%) showed normal baPWV. According to sex, the mean baPWV value of male and female participants were 15.51 ± 2.96 m/s (11.05–22.85 m/s) and 14.97 ± 2.57 m/s (10.20–23.30 m/s) respectively ($p=0.650$). Based on HbA1c level, it was found that the mean baPWV values of the participants with controlled glycemic and uncontrolled glycemic were 14.66 ± 1.28 m/s (11.60–17.20 m/s) and 15.35 ± 3.03 m/s (10.20–23.30 m/s) respectively ($p=0.362$). In accordance with the SBP, the participants' mean baPWV values in normotensive and hypertensive category were 14.21 ± 2.41 m/s (10.20–22.10 m/s) and 16.35 ± 2.65 m/s (12.00–23.30 m/s) respectively ($p<0.001$). Meanwhile, based on DBP level, the participants' mean baPWV values in normotensive and hypertensive category were 14.50 ± 2.43 m/s (10.20–22.50 m/s) and 16.48 ± 2.83 m/s (10.60–23.30 m/s) respectively ($p=0.003$; Table 3).

Association between neutrophil–lymphocyte ratio and arterial stiffness

Analysis result of the association between participants' NLR count and baPWV level showed $p = 0.047$ with $r = 0.235$. The result showed that there was a significant association between the participants' NLR count and baPWV value. The higher NLR count, the more arterial stiffness were experienced by the participants (Fig. 1).

Discussion

NLR is a biomarker of the inflammatory process involved in atherosclerosis that is effective, simple, and inexpensive to use in diagnosis and as a prognosis of coronary artery disease (CAD) [19]. In this research, it was found that the mean NLR of T2DM patients is 2.69. A study on healthy participants shows a lower mean of NLR at 2.1 [4]. Meanwhile, a study of acute coronary syndrome patients of low baPWV group shows NLR rate of 3.1, lower than that of high baPWV group with a 4.0 rate [20].

A study in the USA, comparing mean NLR in different races, indicated that all races, except non-Hispanic black patients, have mean NLR more than 2 [21]. The Asian population shows lower results of the normal NLR rate compared to those in the western country. The normal value of NLR in the Indonesian population is not available. The normal cutoff point of NLR in this study was referred from a study from South Korea, which has similar races with Indonesia. A study involving 12,160 healthy subjects in South Korea resulted in an average NLR rate of 1.65 (0.107–3.193) [18].

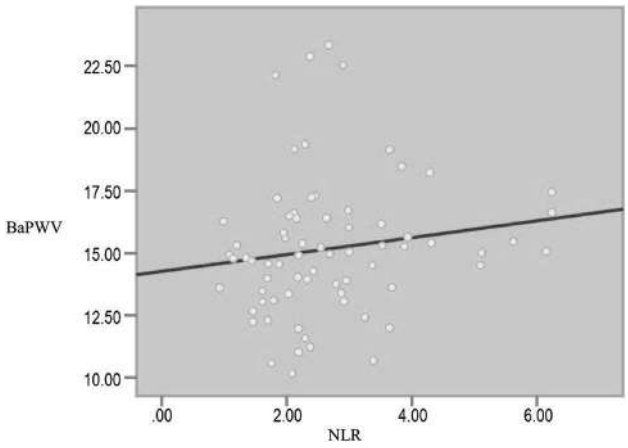

Fig. 1 Our study displays the association between NLR and baPVW in 72 participants. Spearman correlation shows positive significant correlation between NLR count and baPWV value ($p = 0.047$; $r = 0.235$). baPWV was a tool to detect arterial stiffness. This linear association indicates that increased NLR was associated with higher risk of arterial stiffness

A similar result was also shown in a study with adult, non-geriatric, healthy, and non-smoking populations, which showed a mean value of normal NLR of 1.65 (0.78–3.53) [22]. This study, with participants of T2DM patients, showed that most participants have high NLR count. Another study obtained an average NLR of 2.15 in which subjects with diabetes, cardiovascular diseases, and smoking habit have higher NLR than those without [21].

The results of this study do not show a significant difference in the NLR rate between male and female participants. Based on systolic blood pressure value, the average NLR of T2DM patients with normotension does not show a significant gap compared to the average of those with hypertension. Similarly, NLR numbers on controlled glycemic and uncontrolled glycemic show no noticeable differences. Former studies gained NLR count that had a significant correlation with glycemic control, in which the NLR count on HbA1c < 7% was at 2.0 ± 0.5, on HbA1c 7–9% at 2.7 ± 1.0, and on HbA1c > 9% at 4.3 ± 2.8. The elevated NLR number was related to poor glycemic control, which was marked by high HbA1c value on T2DM patients [23].

The Rotterdam study results showed that PWV numbers are normal if < 13.5 m/s [9]. While in this study, results show that the average baPWV value of T2DM patients is high. A study in Brazil recorded an average PWV value on DM patients that was higher than that of non-DM patients, namely 11.6 vs 9.3 m/s. The baPWV numbers of the healthy population in South Korea were found to be lower than those of the DM patients in this study, with 13.985 m/s [4].

This study results show that most participants saw an increased rate of baPWV. A study conducted by TODAY (The Treatment Options for Type 2 Diabetes in Adolescents and Youth) on 453 type-2 DM patients found arterial stiffness on more than 50% of its subjects [23]. In several studies, hyperglycemia and hyperinsulinemia contribute directly or indirectly to stiffness in the arteries, through the accumulation of advanced glycation end-product/AGE, endothelial dysfunction, and changes in the activity of vasoactive substances. Additionally, the increase in plasma glucose results in an increase in oxidative stress and activation of vascular inflammation, which directly causes arterial stiffness [24]. This study shows that HbA1c ≥ 7 group has a higher average rate of baPWV than the HbA1c < 7% group. However, this result is statistically not significant. In addition to glycemic control, other factors that play a role in arterial stiffness are age, race-ethnicity, gender, hypertension, dyslipidemia, and BMI [25]. Of all the factors above, age and hypertension are the most relevant factors [26]. Studies on type 1 diabetes mellitus indicated that PWV is significantly related to age, duration of diabetes, SBP, DBP, and eGFR, but are not related to total cholesterol and BMI [27].

In this study, it is found that baPWV in men is higher than in women, which statistically is not significant. Systolic and

diastolic blood pressure parameters found that the baPWV value is higher in the hypertension group compared to normotension, which is statistically significant. PWV can predict changes in systolic blood pressure and the occurrence of hypertension in the future [28].

NLR is an inflammatory marker that is easy to use and is also used as an indicator of risk for cardiovascular disease. BaPWV is an indicator of early atherosclerosis. Atherosclerosis is a disease triggered by chronic inflammation, which plays a role in its formation. In this study, the association between NLR values and arterial stiffness was measured by baPWV, which in line with other studies where there is an association between NLR values and arterial stiffness measured by baPW ($r = 0.403$; $p = 0.005$). This study, however, was conducted in a population of CAD patients [20]. Another study in CKD patients with peritoneal dialysis found high NLR values in the high baPWV group. In multiple linear regression analysis, NLR is found to be an independent factor in the increase of baPWV [29].

NLR is a predominant factor in increasing baPWV, which is part of arterial stiffness. Some studies suggest that vascular dysfunction, caused by inflammation, is a pathomechanism of arterial stiffness. Neutrophils attach to vascular endothelium that triggers microvascular and inflammatory dysfunction, vascular endothelial dysfunction, and increased expression of proinflammatory cytokines. These events lead to increased vascular inflammation and proliferation of smooth muscle, which causes vascular stiffness [12]. NLR and baPWV both increase in T2DM patients. There is a significant association between NLR and baPWV in patients with T2DM and diabetic retinopathy patients. In multiple linear regression analysis, NLR is found to be an independent and significant determinant of the increase in baPWV [30].

Advanced glycation end products (AGE) is produced from enzymatic processes of protein glycation, forming irreversible cross-links in stable tissue proteins such as collagen. When cross-links occur, it produces collagen that is rigid and inhibits replacement. AGE also influences endothelial function through cooling nitric oxide and increases reactive organ species (ROS) generation. The current study shows that AGE stimulates stress signals and inflammatory responses such as nuclear factor-ƙβ (NF-Ƙβ), ROS synthesis, cytokines, growth factors, and intercellular adhesion molecule (ICAM). These responses will result in arterial stiffness through matrix metalloproteinases (MMP) activity, endothelial dysfunction, increased smooth muscle tone, disrupting response endothelium against injury, influencing angiogenesis, and encouraging the process of atherosclerosis [31].

The limitations of our study include the limited number of samples and the short follow-up time of the participants; our findings are even better when such comparators (healthy patients or control group) are available and need to include its clinical significance. Our recommendation for further studies is to conduct over a longer period of time in order to acquire larger sample size in order to achieve more accurate correlation.

Conclusion

T2DM enhances the risk of atherosclerosis and cardiovascular diseases, which is the primary cause of death. NLR is an inflammatory marker that is readily available, easy-to-use, and reproducible. BaPWV is an indicator of changes in early atherosclerosis. Early detection of abnormal levels of NLR can help look for subclinical atherosclerosis in T2DM patients. These patient's NLR were mostly in the high category. There was no significant association between NLR with sex, HbA1c, systolic, and diastolic blood pressure. Besides, the measurement of arterial stiffness with baPWV were mostly found in the abnormal category. Arterial stiffness has a significant association with systolic and diastolic blood pressure. In contrast, gender and HbA1c show no significant association. There was a significant association between NLR and arterial stiffness, and the higher the NLR value, the more arterial stiffness experienced by participants.

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Author contribution All authors contributed toward data analysis, drafted and revised the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committee of Dr Soetomo General Academic Hospital, Surabaya, Indonesia (1311/KEPK/V/2019).

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Rapidly progressive diabetic kidney disease: South Asian experience

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Abstract

Objective There is limited discussion in the literature on clinical and pathological features of the rapidly progressive variant of diabetic nephropathy (DN). We aim to highlight the clinic-pathologic characteristics of biopsy proven DN in patients with type 2 diabetes (T2DM) and a rapid decline in glomerular filtration rate (GFR).

Methods We conducted a cross sectional study of patients with rapidly declining GFR and biopsy proven isolated DN from 2012 to 2018. Data on clinical details, laboratory, and histologic findings was collected.

Results A total of 46 patients were included; mean age was 49 ± 12.7 years with a predominantly male preponderance. Majority of the patients (82%) had hypertension and more than 40% required more than one antihypertensive medications. About half of the patients (47%) were on renin-angiotensin-system (RAS) inhibitors, and 70% were either overweight or obese. Almost half of the patients had HbA1c values greater than 7% and about 71% were on insulin. Mean urinary protein was 4.5 ± 2.6 g. Nodular and global glomerulosclerosis were the most common histologic findings, while 26.1% patients had crescents. During follow-up, 30% had one or more hospitalizations with congestive heart failure and 30% progressed to end-stage kidney disease (ESKD). Mean time to ESKD was 9.69 ± 17.87 months.

Conclusion Younger age, higher body mass index, coexisting hypertension, overt proteinuria, and suboptimal glycemic and blood pressure control with underutilization of RAS inhibitors were prevalent in rapid decliners in DKD. Rapidly progressive diabetic kidney disease is a globally under-recognized entity, and this is the first experience shared from the South Asian region.

Keywords Diabetes · Diabetic kidney disease · Diabetic nephropathy · End-stage kidney disease

Introduction

Diabetes is the leading cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide. A diagnosis of diabetic kidney disease (DKD) is typically made on clinical grounds and kidney biopsy is rarely required to confirm diagnosis. Kidney biopsy is considered in cases where an alternative diagnosis (e.g., glomerulonephritis or tubule-interstitial nephritis) is suspected. Triggers for biopsy usually include either short history of diabetes, abnormal serology, absence of diabetic retinopathy (DR), or features of a systemic illness, which could be responsible for the proteinuria or decline in glomerular filtration rate (GFR). Typically, DKD has a slowly progressive and indolent course. A rapid decline in GFR [defined as > 5 mL/min per $1.73m^2$ per year, by Kidney Disease: Improving Global Outcomes] creates suspicion of either complete or partial presence of non-diabetic kidney disease [1]. However, it has been observed that a certain percentage of incident ESKD and CKD patients with histologically proven isolated pure diabetic nephropathy (DN) may also experience a rapid decline of renal function [2, 3].

There is limited discussion in the literature on clinical and pathological features of the rapidly progressive variant of DN globally and none from our part of the world. Data on clinical features of this variant of DN would provide a contemporary information that will help in designing future interventional studies. The aim of the present review is to describe the

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clinical and pathologic characteristics of patients with type 2 diabetes (T2DM) and histologically proven isolated DN, who experience a rapid decline in kidney function.

Materials and methods

We conducted an observational cross sectional study of medical records of patients with T2DM who had biopsy proven isolated DN from January 2012 through December 2018 at our institute, the Aga Khan University Hospital. All patients (> 18 years of age) with T2DM who had undergone kidney biopsy due to rapidly declining GFR during the above time period and found to have isolated DN on histopathology were included. Patients, with incomplete medical records and those with non-diabetic lesions with or without DN, were excluded.

The biopsy material was processed for light microscopy and immunofluorescence. Electron microscopy was not available at our center. DN was diagnosed by an experienced renal pathologist by the presence of mesangial expansion and diffuse inter-capillary glomerulosclerosis, with or without the nodular Kimmelstiel–Wilson formation, basement membrane thickening, fibrin caps, or capsular drops [4].

Clinical details including age, gender, duration of T2DM, body mass index (BMI), presence of documented DR, other comorbidities, smoking, antihypertensive use, anti-diabetic drugs at the time of biopsy, and family history of T2DM and of CKD were recorded from the case records. The laboratory profile included serum creatinine, urinalysis, degree of proteinuria either by 24-h urine collection or by spot ratios (whichever was available), HbA1c, estimated GFR (eGFR) before and at the time of biopsy as well as within 6–12 months' follow-up after biopsy. Data on progression to ESKD and major adverse cardiovascular events (MACE) such as stroke, congestive heart failure (CHF), and myocardial infarction (MI) during the follow-up period was also recorded.

The study protocol conformed to the Declaration of Helsinki and approved by the Aga Khan University Hospital's Ethics Review Committee.

Data was analyzed using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used examine the normality of the data. Results were expressed as mean ± SD for continuous variables wherever normal distribution could be assumed. Categorical variables were presented as percentages.

Results

One hundred patients with diabetes were biopsied in the study period due to a rapid decline in kidney function. Among them 46 were identified to have isolated DN. Table 1 shows baseline clinical and laboratory parameters of the study population.

Mean age was 49 ± 12.7 years, with almost three-quarter of subjects above the age of 40 years. More than two-thirds (69.6%) were males. Vast majority (82%) had concomitant hypertension, with 40% taking more than one antihypertensive drugs. At the time of biopsy, calcium channel blockers were the most commonly used class of medications followed by renin-angiotensin-system (RAS) inhibitors, beta-blockers, and diuretics. Almost half of them were on statins and aspirin, and 13% admitted to use of non-steroidal anti-inflammatory drugs (NSAIDs) in the past. Only one patient reported use of herbal products. There were no active smokers, and only 3 patients had previous history of smoking.

Almost half of the patients had HbA1c values greater than 7% in 6–12 months preceding biopsy. Insulin was the most commonly used antidiabetic agent (71%), either alone or in combination with other agents; almost a third of patients reported using two or more than two antidiabetic agents at the time of biopsy.

Microscopic hematuria was present in 32% patients. Mean urinary protein was 4.5 ± 2.6 g at the time of biopsy. Almost one-third of patients had DR; in others, a documentary evidence on the same was lacking. About 5.6% patients reported an episode of acute kidney injury in 1–5 years prior to biopsy.

Figure 1 shows the histological findings of the study cohort.

Figure 2 shows the trend of eGFR decline over time in the study population. Patients had a mean eGFR of 41.6 ± 32.0 ml/min/1.73 m^2 in 3–12 months preceding biopsy. At the time of biopsy, it was 28.8 ± 27.60 ml/min/1.73 m^2 and that declined further to 24.2 ± 26.6 at 12 months' post biopsy. Mean eGFR at last follow-up visit was 17.5 ± 19.3 ml/min/1.73 m^2. Mean follow-up period from biopsy to last follow-up was 17.7 ± 18.9 months.

Over half of the patients had MACE during the follow-up period, and 46.7% had one or more hospitalizations with CHF. Of the total patients, long-term follow-up data was available for 38 patients (82%), and among those, 36.8% progressed to ESKD over the follow-up period. Mean time duration to ESKD was 9.69 ± 17.87 months. Patients' outcomes in the study cohort are shown in Fig. 3.

Discussion

Typically, DKD begins with hyper-filtration, followed by development of albuminuria. Decline in GFR is gradual and occurs after development of moderate, typically non-nephrotic range albuminuria, over years. However, contemporary evidence has shown that clinical outcomes in DKD especially in T2DM are extremely variable, and there has

Table 1 Baseline characteristics of patients

Patient characteristics	N(%) / mean ± SD
Age (years)	49 ± 12.7
< 40 years	12 (26.1)
≥ 40 years	34 (73.9)
Gender	
Male	32 (69.6)
Female	14 (30.4)
BMI (kg/m2) (n = 40)	
18.5–24.9 (Normal)	12 (30)
25–29.9 (overweight)	14 (35)
≥ 30 (obese)	14 (35)
Smoking status	
Non-smoker	43 (93.5)
Former smoker	3 (6.5)
Comorbid conditions	
Hypertension	38 (82.6)
Ischemic heart disease	8 (17.4)
Peripheral vascular disease	2 (4.3)
Liver disease	9 (19.6)
Family history of DM	20 (43.5)
Duration of DM	
< 5 years	10 (21.7)
≥ 5 years	31 (67.4)
Not known	5 (10.9)
Antihypertensive treatment	
Beta blockers	19 (41.3)
RAS blockers	22 (47.8)
Diuretics	21 (45.6)
CCBs	26 (56.5)
Alpha blockers	10 (21.7)
Anti-diabetic treatment	
Metformin	1 (2.8)
SU	4 (11.4)
DPP4i	0
Insulin	25 (71.4)
SGLT2i	0
TZD	0
> 2 OAD	11 (31.43)
Statin	21 (45.7)
Aspirin	22 (47.8)
HbA1c (%) n = 32	
< 7	12 (37.5)
≥ 7	20 (62.5)
BP control (pre-biopsy)	
< 140/90 mmHg	24 (53.3)
≥ 140/90 mmHg	21 (46.7)
Diabetic retinopathy n = 32	15 (46.9)
Proteinuria (g/day) n = 41	4.5 ± 2.6
Microscopic hematuria n = 42	15 (35.7)
AKI (in ≤ 5 years) n = 36	2 (5.6)
NSAID use	6 (13)
Herbal medications	1 (2.2)
CKD stage	
G1	2 (4.4)
G2	5 (10.7)
G3	9 (20)
G4	15 (33.3)
G5	11 (24.4)
G5D	4 (8.9)

BMI body mass index; *DM* diabetes mellitus; *RAS* renin-angiotensin system; *CCB* calcium channel blocker; *SU* sulfonylurea; *DPP4* dipeptidyl peptidase-4 inhibitor; *SGLT2* sodium-glucose transporter 2 inhibitor; *TZD* thiazolidinedione; *OAD* oral antidiabetics, *HbA1c* glycated hemoglobin; *BP* blood pressure; *AKI* acute kidney injury; *NSAID* non-steroidal anti-inflammatory drug; *CKD* chronic kidney disease; G1–5D grades

been an evolution in what was once thought to be a single and unidirectional natural history of DKD [5]. Recently there has been a growing interest in rate of decline of renal functions and its implications in DKD [6].

Rapidly progressive diabetic kidney disease (RPDKD) is a new subset of DKD that has gained much attention in the last decade [7]. It is a variant of DN that was initially identified in Pima Indians in the 1990s. This small population was observed to have a higher incidence of type 2 diabetic nephropathy and an accelerated progression of disease leading to 20 times greater incidence of diabetes associated ESKD [8]. Lately, rapid decline in kidney functions in diabetic patients has also been observed in other ethnicities. Lim et al. reported three cases from Malaysia showing rapid deterioration of kidney function at a rate of 46–60 ml/min/year [2]. Joslin Kidney studies highlighted significant prevalence of fast declining pattern of kidney functions in patients with DN. Joslin Clinic cohort comprised of 5000 patients mainly Caucasians and included equal number of type 1 diabetes mellitus and T2DM cases. Among the four subgroups of "very fast-," "fast-," "moderate-," and "non-rapid-" decliners, very fast and fast decliners constituted 50% of the cohort [7]. Peter and colleagues, in a subgroup study from longitudinal observational Fremantle Diabetes Study Phase II (FDS2) in Australian population, reported 10.1% incidence of rapidly progressive DN [9]. Similarly, rapidly declining GFR was also observed in Japanese [10, 11], Chinese [12, 13], and Thai [14] diabetic population. Furthermore, data from epidemiological surveys show higher prevalence of rapidly progressive DN in migrant South Asian population [15, 16]. As a newly emerged pattern of DN, literature on rapid decliners is sparse and diverse due to application of different criteria to determine GFR as well as evaluate rate of its decline. Moreover, data on incidence and

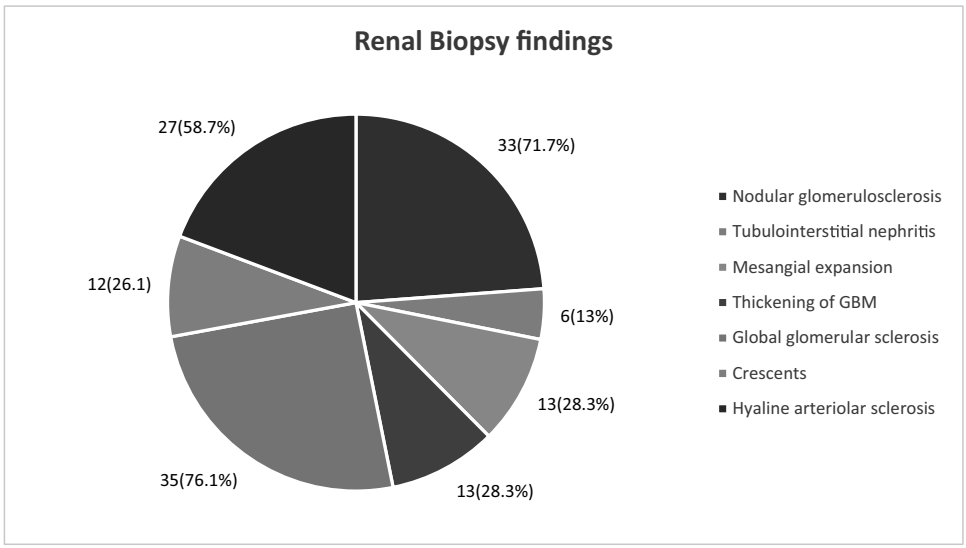

Fig. 1 Renal biopsy findings

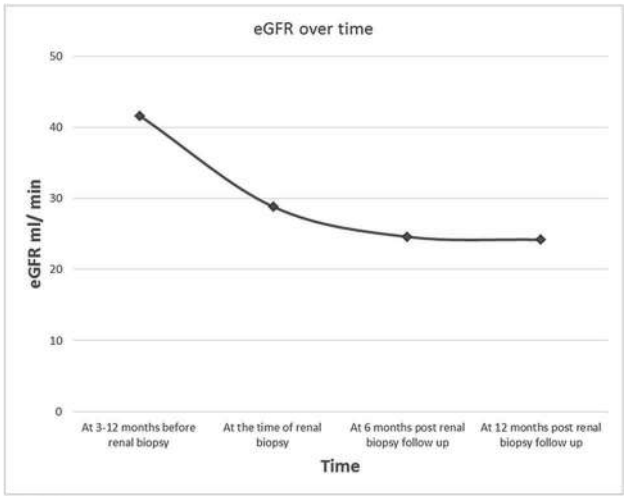

Fig. 2 Trend of estimated glomerular filtration rate (eGFR) decline

nature of DN in South Asian countries is lacking. Previously we reported isolated DN in 33.8% patients who underwent kidney biopsy due to rapidly deteriorating renal function with or without proteinuria [17]. In other studies, from the region, aimed to determine the spectrum of kidney biopsy performed on patients with T2DM for clinical suspicion of non-diabetic renal disease, 25–42% patients were found to have isolated DN [18–20]. In the current study, we present characteristics of 46 patients identified with rapidly declining kidney functions and isolated DN on kidney biopsy that may help understand the pattern of DKD in South Asian population.

Identification of accelerated variant of DKD imposes search of promoters and predictors of rapid GFR decline that may facilitate timely intervention to halt progression. Older age was found to be associated with fast deterioration of kidney function in a Japanese cohort of T2DM. Mean

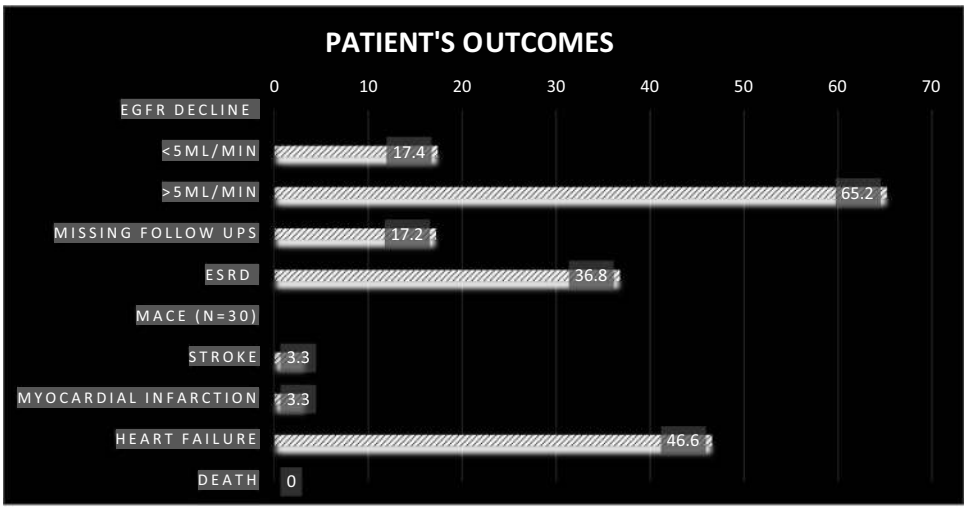

Fig. 3 Patients' outcomes eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; MACE, major adverse cardiovascular event

age of Japanese Diabetes Complication Study patients was 59 years [10]. Zoppini et al. also found independent relation of advanced age with rate of annual eGFR decline [21]. Parallel observation was made in FDS2 rapid decliner subgroup. In contrast, the mean age of patients in our study cohort was 49 years. This could be due to the fact that T2DM onset occurs at a much earlier age in South Asians than in other ethnic populations and that the peak prevalence of T2DM would be at a much younger age in South Asians compared with Chinese and Japanese subjects [22, 23].

Longer duration and poor control of T2DM are well identified risk factors for fast progression of DN [9, 21, 24, 25]. Chronic hyperglycemia and higher HbA1c were observed as independent predictors of accelerated progression of DKD in Caucasians and Japanese [10, 21]. Association of higher HbA1c with eGFR decline was also reported by Joslin Kidney studies [7]. This relation is further highlighted by a sub-study from ADVANCE trial that showed tighter glycemic control caused 65% reduction in progression of DKD to ESKD [26]. In the present study, 62.5% patients had HbA1c > 7%; implying less optimum glycemic control could have been a promoter of rapid progression of disease. Moreover, 71.4% patients in this study were on insulin that has also been identified as a risk factor by Zoppini et al. [21].

Hypertension is a strong predictor of progression of DKD [27], and optimization of blood pressure (BP) has been shown to reduce DKD progression in several trials [28, 29]. Hypertension and high systolic BP is associated with rapid decline in GFR in T2DM [6, 14, 21]. In the present study, hypertension was the most prevalent comorbid disease seen in 82.6% of patients. BP control in general was suboptimal, and almost half of these patients had systolic BP more than 140 mmHg. Most commonly used antihypertensive was calcium channel blocker followed by RAS blockers and diuretics. BP control with RAS blockers (angiotensin converting enzyme inhibitors and angiotensin II receptor blockers) has been the mainstay of treatment for DKD for decades because they may slow kidney disease progression more effectively than other antihypertensive drugs. Several high quality randomized clinical trials demonstrated the beneficial effects of RAS blockers beyond BP control particularly in terms of reduction in risk of hard renal outcomes such as worsening or progression of albuminuria, decline in GFR, and development of ESKD [30, 31]. Significant under-utilization of RAS blockers (47.8% patients only) along with suboptimal BP control especially in setting of significant proteinuria could have been a plausible explanation for a faster GFR decline and rapid progression of DKD seen in our patients. We could not ascertain the reasons for a lesser use of RAS blocker as antihypertensive therapy of choice given the cross-sectional nature of the study.

Lately, sodium-glucose cotransporter-2 (SGLT2) inhibitors have revolutionized the therapeutic approach of patients with T2DM who are at high risk of progressive worsening of kidney function, atherosclerotic cardiovascular disease, and heart failure. Several trials have demonstrated their cardiovascular and reno-protective effects, independent of the glycemic control [32–34]. The CREDENCE study primarily assessed kidney outcomes of canagliflozin and had to be terminated early due to clear benefits towards the composite endpoint of ESKD, doubling of the creatinine level, or death from renal cause in patients with T2DM and moderate to severely increased albuminuria [35].

Presence of retinopathy in diabetic patients is also considered a marker of progressive kidney disease [24, 36], cardiovascular events, atherosclerosis, and all-cause mortality [36]. Moriya et al. assessed the association of diabetic retinopathy (DR) with rate of kidney function decline in JDCS cohort of T2DM patients. They observed a faster trend of GFR decline in patients with DR, and it was 2–3 times higher with coexisting micro albuminuria [36]. Similar findings were reported in Chinese [12] and Caucasians [37] patients with T2DM. We observed DR in a little less than half of the patients who underwent ophthalmologic examination. However, this could be an erroneous estimate due to missing documentation of retinopathy in patient records, a limitation of retrospective review.

Mean proteinuria in our patients was 4.5 g/day, which was similar to findings presented by Lim et al. in their case report [2]. Proteinuria is an independent risk factor for acceleration of disease and has been suggested as a screening tool for early recognition of rapid decline in kidney function [38]. Go and colleagues studied a large cohort of 36,195 patients for predictors of fast progression of CKD with or without diabetes. Proteinuria was found as a strong predictor for rate of progression of CKD in diabetic as well as non-diabetics [6]. Similarly, 17–21% of proteinuric patients in Joslin cohort exhibited fast decline of renal function [7].

The influence of BMI on progression of DKD is controversial. While obesity and diabetes have a well-established association, role of higher BMI in DN progression needs further exploration [39]. Freidman et al. in their study on patients with advanced DN reported that short-term tight weight reduction significantly decreases serum creatinine by 12% [40]. Zoppini et al. also showed obesity to be independently associated with faster eGFR decline [21]. Contrary to this, some other studies have found no influence of higher BMI on rate of progression of DN [14, 41]. In this cohort, 70% patients were either overweight or obese suggesting a possible link between BMI and a rapid progression of DN in this population. This, however, calls for further studies in order to establish an association.

Histologically, around three-fourth of our patients showed nodular glomerulosclerosis and globally sclerosed glomeruli. More than half patients also had vascular hyaline changes. Our observation mirrored with histological findings

reported by Lim et al. in their case report [2]. A multicenter study involving 377 patients with biopsy proven DN indicated nodular glomerulosclerosis as an independent risk factor for rapidly progressive DKD [42]. Thus, it may be speculated that presence of nodular glomerulosclerosis serve as a pathological prognostic feature for rapid GFR decliners.

Presence of crescents in renal biopsy specimens of diabetics usually calls for a search for etiologies other than diabetes. It is interesting to note that almost one-quarter of the patients in our study (26.1%) were found to have crescents in the absence of any serologic or histologic evidence of immune-mediated glomerular disease. The presence of crescents in DN and possibility of an etiologic linkage between diabetic glomerular damage and formation of crescents has been described by some authors [43–45]. It is thought the information on morphology of histologic lesions in DM may have an intrinsic selection bias, and hence the presence of crescents within the glomeruli may often be overlooked particularly in those with advance renal damage. This is the first study reporting presence of crescents as an evidence of glomerular injury caused by T2DM and its association with a rapid decline of kidney function in the South Asian population.

Rapid decline in eGFR is associated with MACE as well as all-cause mortality [46–48]. Among our cohort, 53.3% experienced MACE, of which most common was CHF seen in 46.7%. It could be that MACE leads to cardio-renal syndrome causing a more rapid decline in kidney function. On the other hand, underlying endothelial dysfunction and vascular disease could result in MACE, and a more rapid decline in GFR. Later seems more intuitively plausible. Ragot and colleagues studied the trajectories of annual eGFR decline and serum creatinine increase over 6.3 years of follow-up in 1040 participants of discovery cohort [47]. They observed MACE was significantly associated with steeper trajectories, and rapid decliners had 4.11 times higher risk of MACE. Matsushita et al. also observed association of faster eGFR decline with cardiovascular outcome and all-cause mortality by assessing 3- and 9-year eGFR changes in 13,029 participants of the Atherosclerosis Risk in Communities Study [49].

Rapid decline of kidney function in a subgroup of diabetics emphasizes the need to identify novel biomarkers for early recognition of these rapid decliners. This may also open new horizons of therapeutic strategies to intervene and modify the disease course and outcomes. Researches have shown tumor necrosis factor family (TNFR1 and TNFR2) as strong predictors of accelerated eGFR decline in patients with T2DM [7, 50, 51]. Novel plasma biomarkers apoA4, CD5L, C1QB, and IBP3 have been reported as predictors of rapid decliners in a sub-study of FDS2 [9]. Whether it is causal or a mere association is yet to be established. Yamanouchi et al. have proposed a prognostic score using data from Joslin Kidney studies for early detection of fast decliners in DKD. The score has shown sensitivity and prognostic value of 72% and 81%, respectively. Once validated on larger cohorts, this may serve as a tool in future intervention trials for rapidly progressive DKD [52].

Retrospective study design and lack of a control group are the main limitations of our study. There is a possibility of missing important details that were not documented in the medical records. In addition, with this study design, we were unable to adjust treatment such as for glycemic and BP control and use of RAS blockers. Furthermore, estimated GFR was used instead of measured GFR, which is more accurate in assessing kidney function. Since this a small, single center data, results may not be generalized. Shorter follow-up period and fewer eGFR values did not allow determining eGFR trajectories that might show varying course and implications.

To the best of our knowledge, this is the first study from South Asia describing clinical and histological features of rapidly progressive variant of DKD. The prevalence of T2DM in both native and migrant South Asians is extremely high and continues to rise rapidly. This study may help identify modifiable risk factors and protective measures to slow the rate of renal function decline in this subgroup of DKD. It may help in devising community health strategies for not only timely identification but also prevention of rapid progression to ESKD, which will especially have profound impact on morbidity, mortality, and healthcare expenditure of low-middle income population. Our findings also shed light on areas of future research in terms of novel biomarkers, therapeutic targets, and prognostic tools.

Conclusion

Identifying the subset of diabetic patients at high risk for a rapid progression of their kidney disease is clinically and policy relevant especially in low-middle-income countries. This would help prioritizing resources targeting the highest risk patients to potentially avoid or significantly delay the need for kidney replacement therapy and potentially preventing cardiovascular and other adverse outcomes. Epidemiologic studies have identified various risk factors for a rapid kidney function decline in different populations. We, in our study, found younger age, higher BMI, coexisting hypertension, longer DM duration, overt proteinuria, and suboptimal glycemic and BP control to be more prevalent in rapid decliners of a diabetic South Asian cohort. We conclude that rapidly progressive diabetic kidney disease is a globally under-recognized entity, and this is the first experience shared from the South Asian region. We feel that the term "Rapidly Progressive Diabetic Kidney Disease" should be recognized in the sub-classification of DKD and used as

standard nomenclature. Identification of predictors of rapidly progressive DKD may help develop multifaceted preventive and interventional strategies to delay progression to ESKD and hence reduce burden on healthcare system.

Declarations

Research involving human participants and/or animals The study was approved by the Aga Khan University Hospital's Ethics Review Committee. This article is based upon review of medical records only and does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

Conflict of interest The authors declare no competing interest.

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ORIGINAL ARTICLE

The effect of using a reminder diabetic foot mirror on foot checking frequency and development of diabetic foot in people with diabetes

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Abstract

Purpose Diabetic foot is a serious and preventable complication. Foot self-inspection is one of the most important foot care behaviors to prevent diabetic foot. This experimental study aimed to assess the effect of using a reminder diabetic foot mirror on foot checking frequency and development of diabetic foot.

Methods This research was carried out between May 2019 and February 2020 at a university hospital in Istanbul. In this study, 133 people with diabetes were included and assigned to groups randomly. A reminder diabetic foot mirror was given to the experimental group 1, while the diabetic foot mirror was given to the experimental group 2. Diabetic foot examinations were performed at the beginning of the study and every 3 months. For 9 months, foot examination frequency data were recorded by interviewing the participants at the end of each month.

Results At the beginning of the study, the participants in all the groups had similar characteristics. However, at the end, it was found that the frequency of foot checking was higher in people with diabetes who used a reminder diabetic foot mirror when compared with those who did not, and this difference was statistically significant ($p < 0.05$). While none of the participants developed diabetic foot, it was found that the use of a reminder diabetic foot mirror led to positive changes in temperature, hydration, edema, and interdigital assessments in foot examination.

Conclusion Using a reminder diabetic foot mirror is an effective approach to increase the frequency of foot checking in people with diabetes.

Keywords Diabetes mellitus · Diabetic foot · Foot checking · Mirror · Self-care · Self-control · Type 2 diabetes

Introduction

Diabetes mellitus (DM) is one of the biggest global health problems of the twenty-first century. DM, defined as a group of metabolic diseases in which chronic hyperglycemia is the main finding, is caused by impaired insulin secretion, impaired insulin activity, or both [1]. According to the data of the International Diabetes Federation, the prevalence of DM in 2019 was 9.3% globally and 12% in Turkey [1].

Long-term uncontrolled glycemia leads to tissue and organ damage, dysfunction, and failure. One of the chronic complications of DM is diabetic foot, and its prevalence is increasing day by day [1, 2]. The global diabetic foot prevalence is 6.4% [1]. The lifetime risk of a person with DM to experience diabetic foot complications is 19–34% [3].

The main risk factors for diabetic foot include neuropathy, peripheral artery disease (PAD), and trauma. However, neuropathy and PAD lead to diabetic foot only when combined with trauma [4]. In the presence of neuropathy, when a minor trauma that disrupts tissue integrity occurs, the diabetic may not notice it due to loss of pain sensation. Continuing to step on the traumatized foot can easily trigger ulcer development [2, 4]. This reveals the importance of self-inspection. Active foot care, including foot checking, can prevent diabetic foot [5]. Foot checking permits early detection of problems and reduces the risk of developing diabetic foot [6].

Foot checking is an important behavior in preventing diabetic foot [7, 8]. Patients should check their feet every day for color, heat, hydration, edema, calluses, cracks, scratches,

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abrasions, blisters, swelling, fungus, scleronychia, ingrown nails, and deformity. Priority should be given to practices that diabetics can perform on their own, such as using a mirror to check their feet [2, 9]. Although checking the feet is a fairly easy preventive behavior, most people with diabetes do not have this habit [10].

Several studies have evaluated the effectiveness of education on foot care behaviors and have concluded that patient education and follow-up enable the diabetic to play an active role in self-care [11]. However, some studies have reported that diabetic foot care training alone is not sufficient [12, 13] since even diabetics with sufficient knowledge did not apply what they knew in their daily lives [13]. In addition, it has been stated that the positive effect of education does not last long [14].

It is difficult to change patients' behaviors and make them develop the habit of checking their foot daily [15]. Hence, more comprehensive interventions are required to instill positive behavioral changes that are permanent [16].

Impaired vision, restricted mobility, and inability to remember are some of the reasons for diabetics not checking their feet [9, 17, 18]. In this study, a special mirror was designed for diabetics, and its effectiveness was assessed.

This experimental randomized-controlled study aimed to evaluate the impact of using a reminder diabetic foot mirror on the frequency of foot checking and the development of diabetic foot.

Materials and methods

Participants

This is a randomized-controlled experimental study involving patients who presented to the Diabetes Outpatient Clinic of Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Hospital, between May 2019 and February 2020. We included patients who agreed to participate in the study, who were literate, who had type 2 DM for ≥ 10 years, who were aged ≥ 45 years, and who presented to the diabetes outpatient clinic. We excluded patients who had diabetic foot, psychiatric illness, difficulty in communicating, vision and/or hearing loss, type 1 DM, and gestational DM.

The sample size was 129 patients in total and 43 in each group, with 95% confidence, 80% power, $\alpha = 0.05$, and $\beta = 0.20$ according to the power analysis (G*Power 3.1.9.2).

Patients were assigned to groups randomly by drawing lots.

During data collection, we aimed to reach more patients than the sample number calculated considering that some might leave the study. Within the scope of the study, we assessed 196 patients for eligibility and used the data of 133 patients (Fig. 1).

Development of the diabetic foot mirror and reminder diabetic foot mirror

Diabetic foot mirror Created by the researcher, this tool is a 4-in. (10.16 cm) diameter, threefold magnifying circular mirror placed on a selfie stick with the handle adjustable between 30 and 80 cm. The magnifying feature of the mirror and the adjustable handle were designed to allow the patient to view their feet comfortably while sitting, lying, or standing (Fig. 2).

Reminder diabetic foot mirror A rectangular digital clock with alarm was fixed behind the diabetic foot mirror, thereby adding a feature to remind diabetics to check their feet daily and prevent negligence (Fig. 2).

Research design

Patients in the experimental group 1 were given a reminder diabetic foot mirror. One-to-one training was provided regarding the use of the mirror. The reminder (alarm) of the mirror was set at the most appropriate time. The experimental group 2 was given a diabetic foot mirror without a reminder, and the patients were familiarized with the mirror and its features. The control group was not given a diabetic foot mirror or a reminder diabetic foot mirror.

In the first interview, data were collected from all patients using face-to-face interview with the help of a patient identification form. The researcher performed a foot examination on all patients by using the Diabetes Nursing Association-Diabetic Foot Evaluation Form. Subsequently, all patients were educated by using the visuals in the diabetic foot self-monitoring criteria brochure, and this brochure was given to the patients. In this interview, the patients were given a diabetic foot check follow-up list that inquired about their daily foot checking status, and they were asked to mark the list on a daily basis. Afterwards, the data in the list were recorded by talking to the patient in the clinic and/or on the phone every month. At the end of every 3 months, the diabetic foot examination was repeated. At the end of the 9 months, the questions in the patient identification form about the diabetic foot were repeated to all three groups. The experimental groups were questioned about the diabetic foot mirror they used, and the groups were compared according to the data obtained.

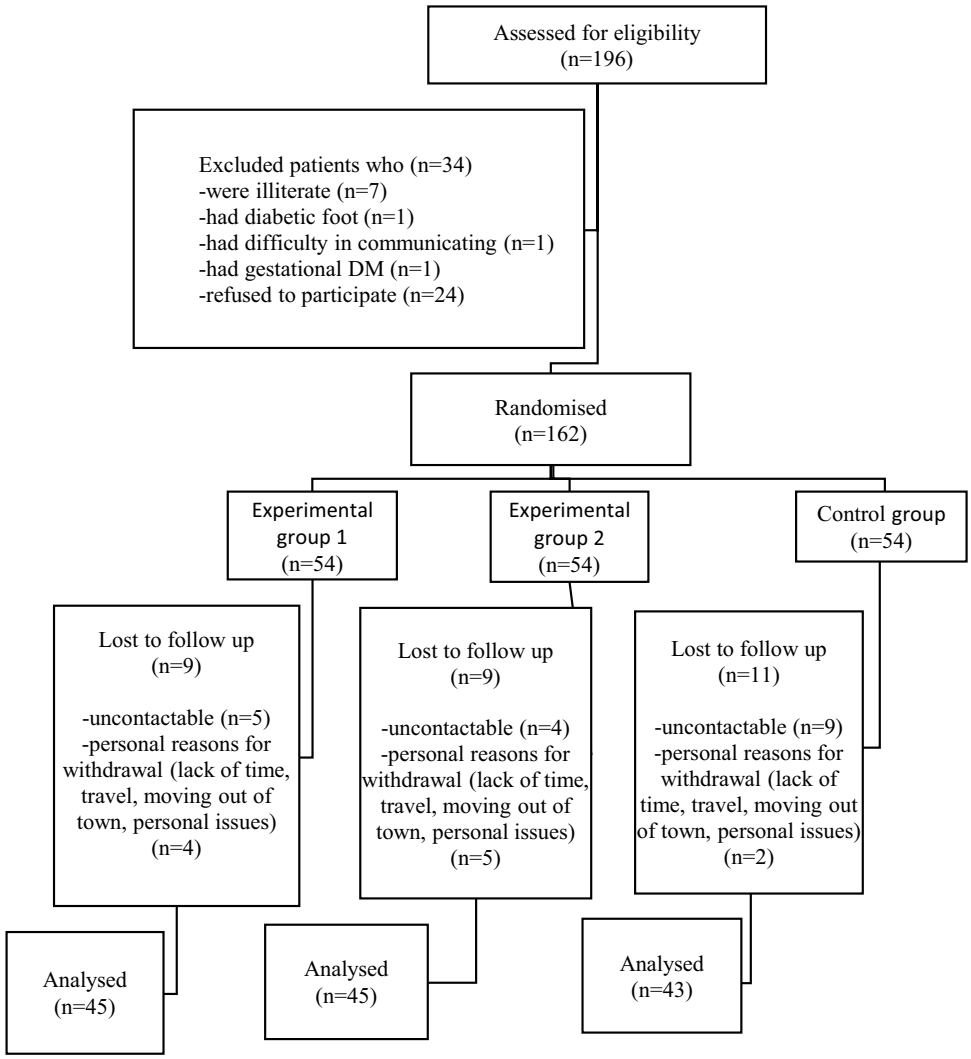

Fig. 1 The Consolidated Standards of Reporting Trials (CONSORT) flow diagram

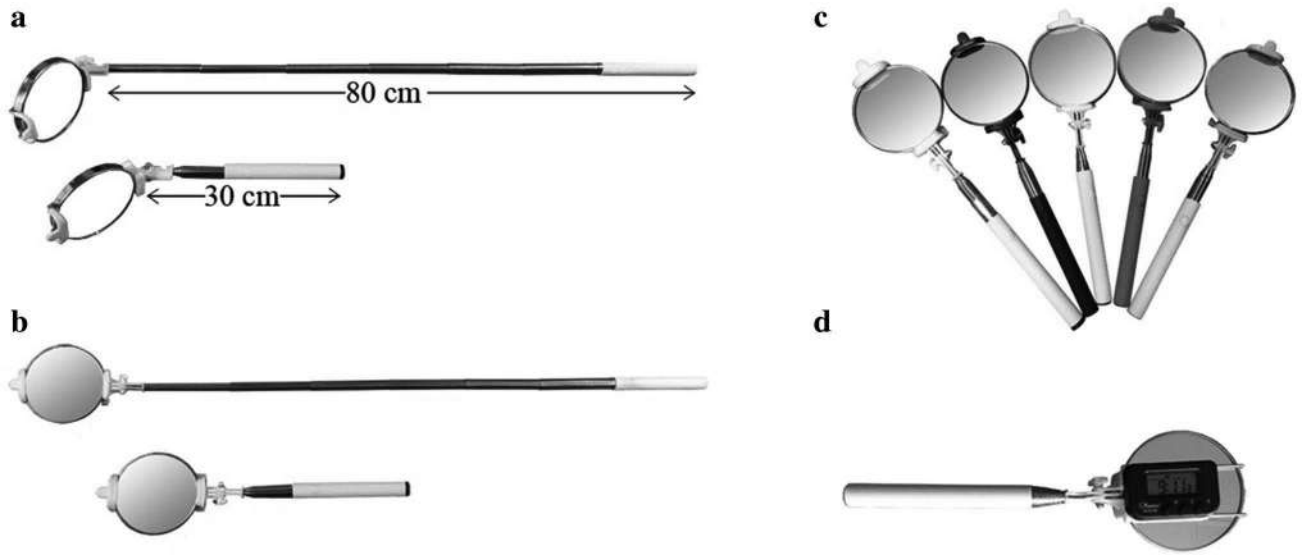

Fig. 2 Diabetic foot mirror and reminder diabetic foot mirror size (**a**, **c**) and digital clock at the back of the reminder diabetic foot mirror (**d**)

Data collection tools

Patient identification form

This form included questions about the patient's age, gender, marital status, metabolic findings, foot-related behaviors, and the use of mirrors.

Diabetic foot evaluation form

Created by the Diabetes Nursing Association, this form consisted of temperature, hydration, edema, color, pulses, nail trimming, interdigital evaluation, sensory test, muscle strength, deformities, and shoe evaluation to determine the risk category and devise an appropriate plan [19].

Diabetic foot-check follow-up list

The diabetic foot-check follow-up list was designed as a form containing the changes that the patient should check daily (discoloration, scratches-abrasions, dryness, swelling, blisters, calluses, warts, fungus, ulcers, thickening-stinging, and deformity of the nail) and the date.

Diabetic foot self-monitoring criteria brochure

This brochure was designed to allow the diabetic to recognize and standardize the foot checking criteria and consisted of visuals to help the patient recognize the changes in the foot.

Statistical analysis

The data were analyzed with IBM SPSS Statistics 25.0 program with 95% confidence level. The data were expressed as mean ± standard deviation (mean ± SD). The relationship of personal characteristics with the group was analyzed using the chi-square test, the difference in measurements according to the groups with one-way analysis of variance/Kruskal–Wallis, and the difference in measurements according to the time in the group separation with the Friedman test. In addition, the Wilcoxon test was used for numerical data and the McNemar test for categorical data to test the difference between the first and last measurements.

Results

Identifying characteristics

No significant difference was noted between the patients in the experimental group 1, experimental group 2, and the control group (Table 1).

Foot checking practice

Foot checking behavior was similar ($p > 0.05$) in all three groups at the beginning of the study and that it was high in all groups at the 9th month. This difference was statistically significant in all groups ($p < 0.05$). When the 9th month data were examined across the groups, the rate of foot checking was found to be higher in the experimental group 1 than in the other two groups, and this difference was statistically significant ($p < 0.05$) (Table 2).

Daily foot checking practice

In the 9th month, a statistically significant relationship was found between the daily foot checking behavior and the groups ($p < 0.05$). The rate of daily foot checking was higher in the experimental group 1 than in the other groups (Table 2).

The use of a mirror in foot checking and behaviors of practicing foot care are given in Table 2.

Monthly foot checking practice

In the 9th month, the difference in the monthly foot checking frequency among the groups was statistically significant ($p < 0.05$). The frequency of foot checking was higher in the experimental group 1 than in the other groups (Table 3) (Fig. 3).

During our study, none of the patients developed diabetic foot ulcer. At the beginning of the study, the patients' feet were evaluated in terms of temperature, hydration, edema, color, pulses, nail trimming, interdigital evaluation, sensory test, muscle strength, deformities, shoe evaluation, and risk category, and there was no significant difference among the three groups. In the foot examinations using the reminder diabetic mirror, a statistically significant difference was found in the 9th month with regard to temperature, hydration, edema, and interdigital assessment and in behaviors such as correct nail trimming technique and use of appropriate shoes ($p < 0.05$).

In our study, it was found that most of the patients (97.8%) who used a reminder diabetic foot mirror handled it without any problem, found the mirror useful, would like to continue using it, and would recommend it to others.

Discussion

Globally, there has been a gradual increase in the prevalence of type 2 DM and related foot complications [12]. Diabetic foot is a preventable complication with serious consequences, and foot checking is one of the critical behaviors that can prevent diabetic foot [8].

Table 1 Distribution of personal characteristics of the experimental and control groups

Characteristics	Total ($n=133$)		Experimental group 1 ($n=45$)		Experimental group 2 ($n=45$)		Control group ($n=43$)			
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		*F*	**p*
Age	59.01 ± 8.62		59.11 ± 9.19		59.26 ± 7.98		58.65 ± 8.86		0.59	0.942
Diagnosis duration	15.87 ± 6.07		16.46 ± 5.18		16.15 ± 6.40		14.95 ± 6.60		0.753	0.473
BMI	29.78 ± 5.50		31.17 ± 5.35		29.16 ± 5.54		29 ± 5.48		2.16	0.118
HbA1c	7.73 ± 1.70		7.99 ± 1.9		7.52 ± 1.48		7.7 ± 1.71		0.872	0.421
Gender	*n*	%	*n*	%	*n*	%	*n*	%	X^2	***p*
Female	83	62.4	29	64.4	30	66.7	24	55.8	1.224	0.542
Male	50	37.6	16	35.6	15	33.3	19	44.2		
Marital status										
Married	108	81.2	36	80	36	80	36	83.7	0.264	0.876
Single	25	18.8	9	20	9	20	7	16.3		
Education level										
Literate	13	9.8	1	2.2	5	11.1	7	16.3	10.832	0.094
Primary school	64	48.1	27	60	20	44.4	17	39.5		
Secondary/high school	32	24.1	7	15.6	11	24.4	14	32.6		
College/higher degrees	24	18.0	10	22.2	9	20	5	11.6		
Employment status										
Yes	22	16.5	8	17.8	5	11.1	8	18.6	1.611	0.447
No	111	83.5	37	82.2	40	88.9	35	81.4		
Income status										
Less than expenses	46	34.6	17	37.8	13	28.9	16	37.2	1.489	0.829
Equal to expenses	80	60.2	26	57.8	30	66.7	24	55.8		
More than expenses	7	5.3	2	4.4	2	4.4	3	7		
Smoking status										
Yes	24	18.0	5	11.1	9	20	10	23.3	2.369	0.306
No	109	82.0	40	88.9	36	80	33	76.7		
Alcohol status										
Yes	13	9.8	5	11.1	4	8.9	4	9.3	0.228	1.000
No	120	82.0	40	88.9	41	91.1	39	90.7		
Coexisting diseases										
Yes	98	73.7	32	71.1	34	75.6	33	76.7	0.411	0.814
No	35	26.3	13	28.9	11	24.4	10	23.3		
Type of treatment										
OAD	56	42.1	18	40	16	35.6	19	44.2	3.941	0.414
Insulin	29	21.8	7	15.6	13	28.9	9	20.9		
OAD + Insulin	48	36.1	20	44.4	15	33.3	13	30.2		

Table 1 (continued)

Characteristics	Total ($n=133$)		Experimental group 1 ($n=45$)		Experimental group 2 ($n=45$)		Control group ($n=43$)			
Had diabetic foot education										
Yes	30	22.6	10	22.2	10	22.2	10	23.3	0.18	0.991
No	103	77.4	35	77.8	35	77.8	33	76.7		
History of foot ulcer										
Yes	12	9.0	5	11.1	3	6.7	4	9.3	0.613	0.811
No	121	91.0	40	88.9	42	93.3	39	90.7		

OAD oral antidiabetic

* *p* values derived from the one-way analysis of variance

** *p* values derived from the chi-square test

Pre-study foot checking and daily foot checking data

According to foot checking findings of the pre-study period, less than half of the diabetics were checking their feet. Studies show that the rate of people with diabetes who checked their feet varied between 32.9 and 57.6% [7, 8, 10, 20]. Differences in the levels of development and education in countries might impact the patients' knowledge and behavior and may explain the different outcomes.

Dinesh et al. and Raithatha et al. reported that 0.5% and 9% of people with type 2 DM, respectively, checked their feet regularly [21, 22]. Other studies conducted in different countries found that between 12 and 41% of the participants exhibited daily foot checking behavior [15, 23–25]. Significant differences have been reported in daily foot checking practices among people belonging to diverse racial and ethnic groups [6], which can explain the differences in daily foot inspection rates in studies conducted in different societies.

Monthly foot checking frequency data obtained from the study for each group

In the control group, when the variation in the monthly foot checking frequency was examined, it was observed that the rate of monthly foot checking frequency peaked in the 1st month and declined from the 2nd month onward. According to these findings in the control group, the increased frequency in the first month might have been due to the short-term positive impact of the information given to patients at the beginning of the study. In a systematic review of the role of patient education in the prevention of diabetic foot, Dorresteijn and Valk stated that patients benefited from short-term education [14]. The subsequent decrease in the frequency of foot checking can be explained by the fact that diabetics do not apply what they know in their daily lives despite having a sufficient level of knowledge [15]. Notwithstanding this rise and fall, continued follow-up may be the reason why the frequency of foot checking among diabetics was lower than that among the other groups but higher than the baseline value and why it was found to be significant at the end of the study when compared with the baseline data.

In the experimental group 2, it was observed that the frequency of monthly foot check peaked in the 3rd month; besides, it was higher than that of the control group in the 9th month. It is thought that the use of the diabetic foot mirror by the experimental group 2 in addition to the explanations given to the control group might have affected the rate of increase in the frequency of foot checking. Studies have reported that complications such as loss of mobility and visual impairment prevent patients, especially the elderly people, from checking their feet [18, 26]. In a study

Table 2 Distribution of foot-related behaviors of the diabetics. Impact of using a reminder diabetic foot mirror on the foot checking and foot care behaviors of diabetics

			Experimental group 1 ($n=45$)		Experimental group 2 ($n=45$)		Control group ($n=43$)			
	Follow-up		n	%	n	%	n	%	X^2	*p
Do you check your feet?	Beginning	Yes	17	37.8	21	46.7	16	37.2	0.002	0.527
		No	28	62.2	24	53.3	27	62.8		
	9th month	Yes	44	97.8	37	82.2	27	62.8	17.475	0.001
		No	1	2.2	8	17.8	16	37.2		
		**p	0.001		0.001		0.001			
Do you check your feet daily?	Beginning	Yes	2	4.4	5	11.1	2	4.7	0.003	0.560
		No	43	95.6	40	88.9	41	95.3		
	9th month	Yes	35	77.8	10	22.2	5	11.6	41.138	0.001
		No	10	22.2	35	77.8	38	88.4		
		**p	0.001		0.125		0.250			
Do you use a mirror to check your feet?	Beginning	Yes	3	6.7	3	6.7	2	4.7	0.154	0.434
		No	42	93.3	42	93.3	41	95.3		
	9th month	Yes	42	93.3	31	68.9	13	30.2	0.154	0.001
		No	3	6.7	14	31.1	30	69.8		
		**p	0.001		0.001		0.001			
Dou you perform foot care?	Beginning	Yes	14	31.1	13	28.9	11	25.6	0.326	0.325
		No	31	68.9	32	71.1	32	74.4		
	9th month	Yes	37	82.2	26	57.8	16	37.2	18.362	0.001
		No	8	17.8	19	42.2	27	62.8		
		**p	0.001*		0.001*		0.063			
Dou you perform foot care daily?	Beginning	Yes	0	0	1	2.2	1	2.3	1.169	0.434
		No	45	100	44	97.8	42	97.7		
	9th month	Yes	14	31.1	6	13.3	3	7	8.954	0.001
		No	31	68.9	39	86.7	40	93		
		**p	0.001*		0.063		0.500			

*p values derived from the chi-square test

**p values derived from the McNemar test

Table 3 Impact of using a reminder diabetic foot mirror on the monthly foot checking frequency of diabetics

		Experimental group 1 ($n=45$)	Experimental group 2 ($n=45$)	Control group ($n=43$)		
		Mean ± SD	Mean ± SD	Mean ± SD	KW	*p
What is your foot checking frequency monthly?	Beginning	3.24 ± 6.99	5.98 ± 9.89	2.51 ± 6.53	2.523	0.283
	1st month	24.18 ± 10.51	10.38 ± 10.88	6.05 ± 9.22	47.885	0.001
	2nd month	23.82 ± 10.66	10.4 ± 10.86	5.84 ± 9.31	48.026	0.001
	3rd month	23.13 ± 10.94	10.84 ± 11.31	5.67 ± 9.34	44.164	0.001
	4th month	22.69 ± 11.49	10.78 ± 11.33	5.42 ± 9.44	43.344	0.001
	5th month	23.49 ± 11.11	10.78 ± 11.33	5.23 ± 9.34	46.425	0.001
	6th month	22.91 ± 11.19	10.62 ± 11.46	5.16 ± 9.33	45.841	0.001
	7th month	23.27 ± 11.08	10.58 ± 11.5	5.02 ± 9.39	45.385	0.001
	8th month	23.82 ± 10.5	9.73 ± 11.31	4.74 ± 9.48	51.275	0.001
	9th month	24.73 ± 10.13	10.4 ± 11.6	4.93 ± 9.42	52.534	0.001
	X^2	264.061	132.325	102.720		
	**p	0.001	0.001	0.001		
	Z	-5.673	−3.627	−3.216		
Beginning vs 9th month	***p	0.001	0.001	0.001		

*p values derived from the *Kruskal–Wallis test*

**p values derived from the *Friedman test*

***p values derived from the *Wilcoxon test*

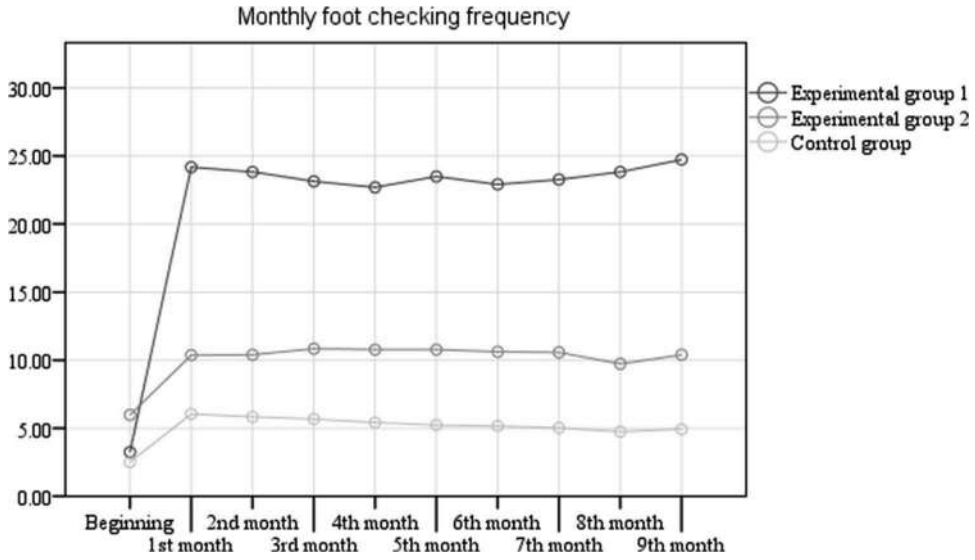

Fig. 3 Variation in the monthly foot checking frequency of diabetics according to observations

by Wallace et al., 7% of people with diabetes reported that they were not flexible and comfortable enough to bend and check their feet, while 72.2% reported wearing lenses that could affect their ability to see their feet clearly [17]. It has been stated that diabetics with visual impairments may have trouble checking their feet or noticing any changes in their feet, and it may be beneficial to advise such patients to use a magnifying mirror [8]. It is thought that using a diabetic foot mirror with threefold magnifying power for better observation and an adjustable handle (30–80 cm) for comfortably observing the feet while sitting, lying, or standing helps the patients to address these problems easily and increase the frequency of checks.

In the experimental group 1, it was found that the frequency of monthly foot checking reached the highest value in the 1st month, remained high until the end of the study, reaching the highest value in the 9th month, and was higher than the other groups. It is thought that the long-term increase in the frequency of foot checking in the experimental group 1 is due to the reminder alarm.

Studies have reported that some diabetics do not remember to look at their feet [9, 18]. Since there is no study in the literature evaluating the impact of a reminder diabetic foot mirror on the frequency of foot checking, the findings of the present study were compared with those assessing the use of mobile applications with a reminder feature on foot checks. Hovadick et al., in a systematic review of 23 studies, reported that individuals benefited from short message services that constantly reminded them of personal care practices. They also stated that improvement in foot care among the diabetics was noticeable as the short text messages motivated a proper management of the disease [27]. In a recent systematic review, it has been documented that daily reminders sent to people with diabetes in the form of text messages were helpful for them to check their feet regularly [28].

The statistically significant increase in daily foot checks observed in the experimental group 1 when compared with the other groups may be associated with the added feature of the reminder diabetic foot mirrors, which is similar to that of mobile applications.

Mirror usage in foot checking, foot care, and daily foot care data

Concerning the use of mirrors to check the feet, it was found that at the beginning of our study, very few diabetics in all the groups used mirrors to check their feet and this result was consistent with the literature [17]. Despite variations in identifying characteristics such as age, educational status, and socioeconomic status, studies clearly show that the frequency of using mirrors in foot checking is low among the diabetics. In preventing diabetic foot, it is important for the patients to check their feet regularly using a mirror, detect possible changes early enough, take precautions, and properly clean the small scratches-abrasions that are noticed.

While the vast majority of people with diabetes did not perform foot care at the beginning of the study, almost none practiced daily foot care. Ouyang et al. reported the rate of patients performing foot care to be 38% [29]. At the end of the study, it was found that the rate of increase in diabetics who performed foot care was significant in the experimental groups, while the rate of daily foot care increased significantly only in the experimental group 1. In a study involving the elderly, Miikkola et al. observed that the feet are neglected because they are not as visible as the other parts of the body. They reported that the elderly did not have the habit of inspecting their feet, and therefore, they often did not practice foot care [9]. Increased frequency of foot checking might have led to a corresponding increase in foot care because when patients see their feet, they may realize that their feet need more care and attention and this may avoid negligence.

Diabetic foot development

During our study, none of the people with diabetes developed diabetic foot ulcer. This result may be due to the fact that the data collection period was not long enough to monitor diabetic foot development. However, according to foot examinations of patients using a reminder diabetic mirror, it may be thought that statistically significant positive changes in temperature, hydration, edema, and interdigital assessment and in behaviors such as correct nail trimming technique and use of appropriate shoes may positively affect the risk of diabetic foot development.

To prevent diabetic foot, early recognition of changes that may occur in the feet before diabetic foot develops is important. It was found that the reminder diabetic foot mirror, designed as a non-pharmacological method that can be used by people with diabetes on a daily basis, significantly increased the frequency of foot checking. To this end, there are plans of getting this low-cost mirror patented, manufactured, and presented to diabetics to popularize the product. Our research asserted that the use of a reminder diabetic foot mirror increased the frequency of foot checking. There is a need to develop innovative solutions and evaluate their effectiveness in order to improve the existing systems for preventing diabetic foot and increase the frequency of foot checking among the diabetics.

Abbreviations DM: Diabetes mellitus; OAD: Oral antidiabetic

Acknowledgments The authors are grateful to all patients with type 2 diabetes who participated in this study.

Declarations

Ethics approval The study protocol followed the requirements of the Declaration of Helsinki. Prior to the study, we obtained written permission from the Ethics Committee of Istanbul University Cerrahpaşa Medical Faculty, dated April 04, 2019, No. 53375. We also obtained verbal and written consent from the study participants by explaining the researcher's identity, research subject, and purpose.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Dietary self-care and hospital readmission among individuals with diabetes mellitus

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Abstract

Background/Purpose Diabetes mellitus is one of the main public health problems worldwide. One important strategy for increasing the effectiveness for controlling diabetes mellitus and preventing complications is self-care supported by healthcare professionals. The aim of the study was to analyze dietary self-care strategies performed by individuals with diabetes mellitus (DM) after hospitalization and their possible influence on hospital readmissions.

Methods A cross-sectional study was conducted in the public health services qualified to hospitalize individuals with DM in a medium-sized municipality of Brazil. Adults were evaluated 24 months after discharge, assessing self-care strategies (dimensions: diet, physical activity, blood glucose monitoring, foot care, medication, and smoking); and clinical, anthropometric, and biochemical measurements.

Results A high death rate (33.7%) was verified after 24 months of hospitalization. Almost half (45.9%) of the 37 remaining patients were readmitted to hospital; however, during the 24 month period, only 42.0% received guidance about DM self-care from healthcare professionals. In the evaluation of dietary self-care, only 35.1% of the individuals reported taking care of their diet on 5 or more days a week. Individuals who adhered to self-care guided by health professionals presented twice the prevalence of not being readmitted to hospital (Prevalence ratio = 2.52; 1.27–5.01).

Conclusions After hospital discharge, individuals with DM presented poor adherence to self-care, especially regarding diet. However, when these individuals adhered to nutritional guidance, there were fewer hospital readmissions. These results reinforce the importance of interdisciplinary educational actions for DM management. However, due to the small sample size because of the high mortality, further studies for this purpose should be conducted.

Keywords Diabetes mellitus · Self-care · Hospitalization · Primary health care · Diet

Introduction

Diabetes mellitus (DM) is one of the main public health problems worldwide. It gives rise to complications with important repercussions for patient health, usually triggered by difficulties in controlling the disease. These difficulties result from poor performance of healthcare systems combined with the complexity of treatment [1].

DM is one of a number of ambulatory or primary care sensitive (PCS) conditions, for which it is strongly hypothesized that hospitalization would be reduced if effective preventive care was available [2].

One important strategy for increasing the effectiveness of primary care for controlling DM and preventing complications is the holistic approach of self-care supported by healthcare professionals. This approach involves analyzing the illness and the individuals with DM embedded in their context; providing healthcare that acknowledges and responds to the broad factors involved; promoting individual participation and involvement in the treatments, according to their realities; and empowering individuals

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to make decisions [3, 4]. Additionally, self-care relating to DM encompasses many requirements, such as a healthy diet, regular exercise, monitoring of blood glucose levels, and proper use of medications [5].

A healthy diet is particularly important due to its potential to contribute towards the improvement of clinical and metabolic parameters and also because of the difficulties that individuals have reported with regard to adhering to a diet. In addition to self-care, others factors can increase the risk of hospital readmission, including disease severity, metabolic control, CVD complications, previous hospitalization, age, and social conditions [6].

Although it is already recognized the importance of the diet in controlling DM and preventing complications, such as readmissions, the construction and maintenance of healthy eating practices remains a challenge, mainly because they are associated with social, economic, and cultural factors [7]. No studies were found that investigated the relationship of adherence to dietary care and its association with hospital admission. In view of this gap in literature, this study sought to identify dietary self-care strategies performed by individuals with DM, 24 months after they had been hospitalized, and the influence of these strategies on readmissions.

Methods

Selection and description of participants

This was a cross-sectional study conducted in the city of Divinópolis – Brazil, between August and October 2013. It included patients with DM, hospitalized for conditions sensitive to primary care in the Brazilian National Health System(SUS), who were invited to participate during the hospital admission. A previous study that aimed to estimate the prevalence of hospitalization due to PCS was conducted between July and October 2011 [8]. All hospitalizations were screened in the two public health services qualified to hospitalize individuals in the city. A total of 2775 hospitalizations were assessed and 95 were found that were due to DM (3.4%). At 24 months after the hospital discharge, these individuals were contacted by telephone and invited to participate in this study. The inclusion criteria for participants in this study were age ≥ 18 years and hospitalized due to DM. The interviews were conducted in a public health service, with home visits carried out for those individuals with difficulties in attending the service.

Divinópolis is a medium-sized city with an estimated population of 213,016 inhabitants [9]. Although Divinópolis is the reference center for healthcare for another 54 municipalities in this geographic region, at the time of this study, the city only presented 27.5% coverage by the Family Healthcare Strategy (the structural axis for Primary Care within the Brazilian National Health System. The scope of the ESF involves actions within the district covered, focused on families and the community) [8], unlike several other Brazilian municipalities, which present a mean coverage of 53.4% [10]. Considering this scenario, the study previously mentioned showed a high prevalence of PCS in the municipality, indicating an overburdening of urgent care services [11].

The face-to-face interview with the participants investigated self-care strategies, socioeconomic, health, and diet information. Clinical, anthropometric, and biochemical evaluations were also carried out. The socioeconomic data collected were age, sex, marital status, years of education, monthly family income, number of residents in the household, and occupation.

The clinical and healthcare information included the time that had elapsed since DM was first diagnosed, clinical complications and whether hospital readmission had occurred (for any reason) during the previous 24 months. In addition, the individuals were asked whether they participated in any kind of health care interventions, including guidance from healthcare professionals related to the control of DM, dietary and nutritional groups, and individual care with dietitians.

Food consumption was measured through a food frequency questionnaire (FFQ). This FFQ consisted of a list of 10 items covering foods relevant to DM, obtained from a FFQ calibrated for the population of a medium-sized municipality of Minas Gerais, reviewed based on a study conducted specifically with patients with DM within Primary Care [11, 12]. The frequencies investigated were daily, weekly, monthly, rarely, and never, which were then recategorized as either frequent or infrequent consumption, based on an adaptation of the criteria proposed by the American Diabetes Association (ADA) [4] and the Brazilian Society for Diabetes (*Sociedade Brasileira de Diabetes*, SBD) [13]. For example, sugar-rich food was considered frequent when consumed daily or weekly. Self-perception regarding changes in the consumption of foods that are important for controlling DM was also assessed.

The anthropometric parameters assessed were weight, height, waist circumference (WC), and hip circumference. The waist/hip ratio (WHR) and WC were used to identify risks of metabolic complications, according to the World Health Organization (WHO) recommendations [14]. Body mass index [BMI = weight(kg)/height(m)^2] was also calculated and the classification was assessed differently between the adult (<60 years, according to the WHO) (14) and older adult participants (≥ 60 years, according to Nutrition Screening Initiative) [15].

The biochemical evaluations were performed by an independent laboratory. The measurements investigated were fasting blood glucose (70–130 mg/dl), glycated hemoglobin (HbA1c) ($<7\%$), total (<200 mg/dl) and fractioned

cholesterol (high density lipoprotein, HDL > 45 mg/dl and low-density lipoprotein, LDL < 100 mg/dl), and triglycerides (< 150 mg/dl), observing adequate metabolic control according to the values recommended by the SBD [13].

Self-care strategies were assessed through the questionnaire for self-care activities with diabetes (QSAD) adapted and validated for the Brazilian context [16], which is a version of the Summary of Diabetes Self-Care Activities Measure (SDSCA) [5]. The QSAD is composed of the following dimensions: "general dietary habits," "specific dietary habits," "physical activity," "medication," "blood glucose monitoring," "foot care," and "smoking." The evaluation was conducted based on reports of care actions implemented according to the days of the week, on a scale from 0 to 7, corresponding to the past 7 days. For analysis purposes, "frequent self-care" was considered when the care action was implemented on 5 or more days a week [17].

The study was approved by the Research Ethics Committee of the Federal University of São João Del Rei (nº 258. 574) and all participants signed the written informed consent. The blood sample results were evaluated by medical staff and made available to the participants. Individuals with metabolic alterations were referred for treatment in the municipal healthcare services.

Data analysis

Variables with normal distribution were presented as mean values and standard deviations, while the others were presented as median values and interquartile ranges ($percentile_{25}$–$percentile_{75}$). The self-care dimensions were described as mean and standard deviation as proposed in the validation study [16] and in order to make the comparison with other studies that used the instrument [18–20].

Poisson regression with robust variance was used to investigate the association of the outcome variable "not readmitted to hospital" and the independent variables of frequent dietary care (5 or more days a week) with the items of the dimension "general dietary habits," namely, "following a healthy diet" and "following nutritional guidance from a healthcare professional" [21]. The analyses were adjusted for possible confounding variables: sex, age, time since diagnosis, educational level, and comorbidities, as well as number of complications and metabolic control (including glycemic and lipid control). Furthermore, a sensitivity analysis was performed regarding the items of the dimension "specific dietary habits." Prior to the sensitivity analysis, multicollinearity between these items was investigated and not identified. The results from the Poisson regression were presented using the prevalence ratio (PR) and respective 95% confidence interval (95% CI).

For all the analyses, a 5% significance level was considered.

Results

Of the 95 patients eligible to participate in the study, it was found that 32 had died (33.7%), 11 had moved to another municipality (11.6%) without leaving a contact address, and 15 refused to participate (15.8%). The remaining 37 patients (38.9%) were evaluated.

The study participants presented a mean age of 64.7 ± 13.4 years. The majority of them were older adults (62.2%), male (51.4%), and had low levels of education (Table 1).

The majority of the individuals had been diagnosed with DM at least 10 years earlier (64.8%) and presented two or more clinical complications (51.3%), of which retinopathy and renal disease were the most prevalent. Almost half of the participants (45.9%) reported having been readmitted to hospital during the previous 24 months, mainly due to uncontrolled glycemia or DM complications(including amputation, renal problems, and infections). During this period, only 42.0% received guidance from a healthcare professional regarding DM self-care, over 70.0% of the participants were not evaluated by a dietitian and only 2.7% were participating in educational groups related to nutrition (Table 1).

Approximately 80% of the individuals had less than 6 meals a day and 97.3%, 62.2%, and 56.8% reported infrequent consumption of whole meal foods, fruits, and vegetables, respectively (Fig. 1). After hospital admission, the majority reported not making changes to their diets (Fig. 2). However, 40.1%, 33.4%, and 33.3% reported positive changes regarding the consumption of fried food, sugary food, and fruits, respectively. Conversely, the consumption of processed meats, ready-made seasonings, and processed foods presented negative changes in 38.2%.

More than 60.0% of the participants were overweight, while approximately 70% presented increased fasting blood glucose, 40.5% increased glycated hemoglobin (HbA1c ≥ 7), and 30.0% an altered lipid profile (Table 1).

Regarding dietary self-care, "following a healthy diet" was the item for which the reports of self-care were the highest during the week (3.9 ± 3.2 days/week), while "consuming sweets" (1.2 ± 2.5 days/week) was the least frequent (Table 2). The items of the "physical activity" dimension presented the lowest means, along with care in examining the feet (24.3%). Regarding the dimension of "medication," approximately 80% of the individuals reported frequently taking care.

The items that composed the "general dietary habits" dimension were statistically associated with hospital readmission, after adjustment for sex, age, time since diagnosis, and educational level. Individuals with greater frequency of dietary self-care guided by a healthcare professional

Table 1 Sociodemographic, anthropometric, clinical, and biochemical characteristics of the participants, 24 months after hospital discharge. Divinópolis-MG, 2013

Variables	*n*	Descriptive measurement
Age (years)*	37	64.7 ± 13.4
Adults (< 60 years)	14	37.8
Elderly (≥ 60 years)	23	62.2
Females (%)	18	48.6
Marital status (%)		
Single/without partner	15	40.5
Married/civil union/with partner	22	59.5
Occupation (%)		
Paid work	9	24.3
Retired/pensioner/homemaker	28	75.7
Per capita family income (U$)*,†	34	219.23 ± 105.92
Years of school attendance		
0	6	16.7
1 to 7 years	27	75.0
≥ 8 years	3	8.3
Diabetes mellitus diagnosis time (years)		
Until 10	24	64.8
11 to 20	9	24.3
> 20	4	10.8
Hospital readmission over the past 24 months (%)	17	45.9
Orientations received at the healthcare services (%)		
Importance of dieting	19	51.4
Nutritional orientation	17	45.9
Importance of regular physical activity	14	37.8
Weight control or loss	12	32.4
Smoking cessation	11	29.7
Number of consultations with a dietitian		
None	27	73.0
1 to 3	5	13.5
> 3	5	13.5
Participation in educational groups on dietary habits and nutrition	1	2.7
Nutritional status (BMI)[§]		
Underweight	0	0
Eutrophic	23	37.8
Overweight	14	62.2
Waist circumference*,‖		98.2 ± 15.9
Normal	10	27.0
Increased risk	3	8.1
Substantially increased risk	24	64.9
Waist/hip ratio*,‖		0.98 ± 0.10
Normal	07	18.9
Substantially increased risk	30	81.1
Fasting blood glucose‡,¶		143.0 (109.0–211.5)
Normal (≤ 130 mg/dl)	12	32.4
Increased (> 130 mg/dl)	25	67.6
Glycated hemoglobin*,¶		7.02 ± 1.05
Normal (< 7%)	22	59.5
Increased (≥ 7%)	15	40.5
Total cholesterol*,¶		168.95 ± 43.26
Normal (< 200 mg/dl)	27	73.0

Table 1 (continued)

Variables	*n*	Descriptive measurement
Increased (≥ 200 mg/dl)	10	27.0
HDL‡,¶		54.0 (44.0–63.5)
Desirable (> 45 mg/dl)	26	70.3
Undesirable (≤ 45 mg/dl)	11	29.7
LDL‡,¶		73.0 (59.0–104.0)
Normal (< 100 mg/dl)	26	70.3
Increased (≥ 100 mg/dl)	11	29.7
Triglycerides‡,¶		116.0 (84.5–196.5)
Normal (< 150 mg/dl)	24	63.9
Increased (≥ 150 mg/dl)	13	36.1

BMI, body mass index; *HDL*, high density lipoprotein; *LDL*, low-density lipoprotein; *values described as mean ± standard deviation; †value of quotation in American dollars at the time of the study; ‡values described as median (P25–P75); other variables are described as percentage; §reference values proposed by WHO [21] and NSI[22]: underweight (< 18.5) < 60 years or (< 22.0) ≥ 60 years; eutrophic (≥ 18.5 *e* < 25.0) < 60 years or (22.0 *e* < 27.0) ≥ 60 years; overweight (≥ 25.0) < 60 years or (≥ 27.0) ≥ 60 years; ‖reference values proposed by WHO [17]: waist circumference—normal (≤ 80.0 cm) women or (≤ 88,0 cm) men; increased risk (80.0 < CC ≤ 88.0 cm) women or (94.0 < CC ≤ 102.0 cm) men; substantially increased risk (> 88.0 cm) women or (> 102.0 cm) men. Waist/hip ratio—normal (< 0.85) women or (< 0.90) men; substantially increased risk (≥ 0.85) women or (≥ 0.90) men. ¶Reference values proposed by ADA [4] and SBD [13]

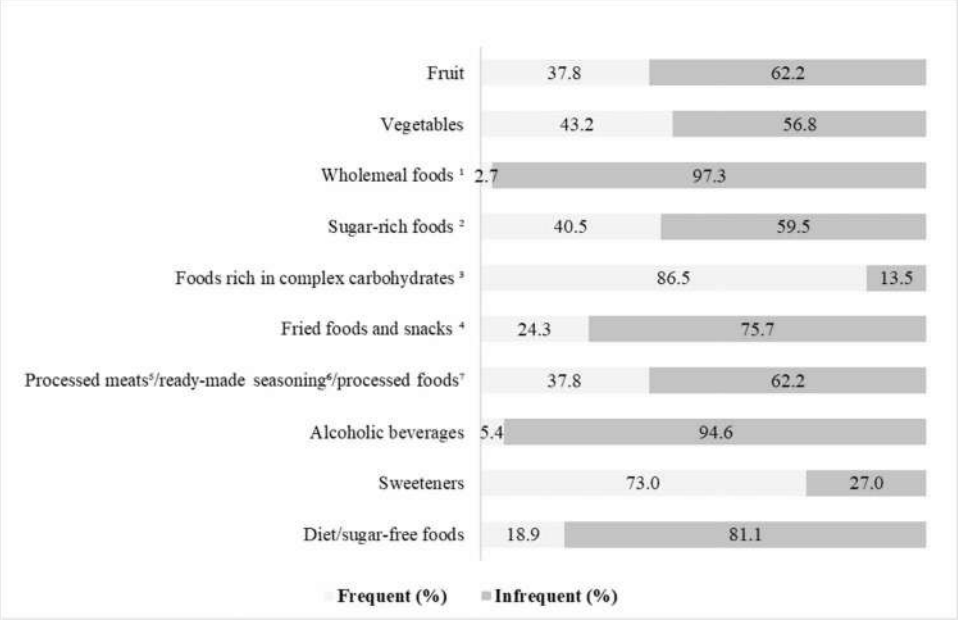

Fig. 1 Frequency of food consumption among the participants, 24 months after hospital discharge. Divinópolis-MG, 2013. [1]Rice, bread, cereals, etc.; [2]common carbonated drinks, powdered juice, sweets, candy, chewing gum, coffee with sugar, biscuits with filling, etc.; [3]rice, pasta, biscuit, flour, bread; [4]Fried snacks; [5]processed meats such as hot-dog sausages, salami, and ham; [6]manufactured seasoning such as beef and vegetable stock; [7]processed foods such as canned goods, soups, chips, sauces, and instant pasta

presented about twice the prevalence of not being readmitted to hospital(PR = 2.02; 95% CI: 1.15–3.54; $P = 0.015$). This association was even more notable after the sensitivity analysis relating to the items of the "specific dietary habits" dimension: frequent consumption of 5 or more portions of fruits and vegetables, and infrequent consumption of fatty food and sweets (PR = 2.09; 95% CI: 1.14–3.83; $P = 0.017$); and after the adjustment for number of complications and metabolic control (PR = 2.52; 95% CI: 1.27–5.01; $P = 0.008$) (Table 3).

Discussion

Low adherence to DM self-care strategies was observed, especially regarding diet and physical activity, in addition to low blood glucose monitoring and foot care among the individuals diagnosed with DM, 24 months after hospital discharge. Good adherence to self-care was only seen for medication use.

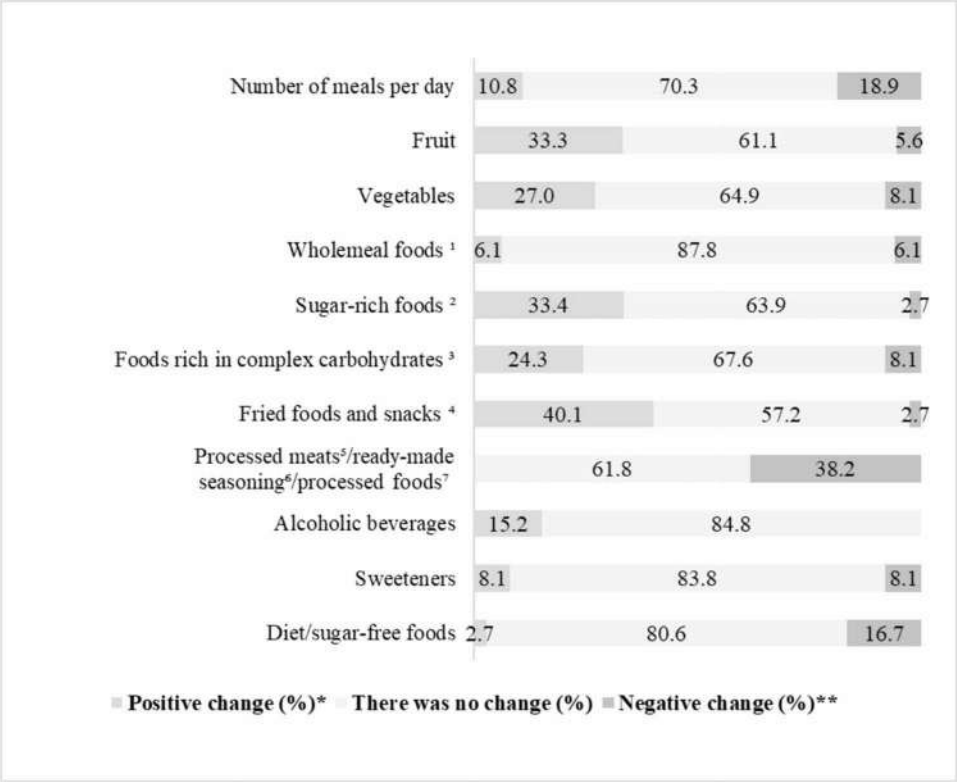

Fig. 2 Self-perceptions among the participants regarding changes in dietary habits, 24 months after hospital discharge. Divinópolis-MG, 2013. *Food consumption improved; **food consumption worsened; [1]rice, bread, cereals, etc.; [2]common carbonated drinks, powdered juice, sweets, candy, chewing gum, coffee with sugar, biscuits with filling, etc.; [3]rice, pasta, biscuit, flour, bread; [4]fried snacks; [5]processed meats such as hot-dog sausages, salami, and ham; [6]processed seasoning such as beef and vegetable stock; [7]processed foods such as canned goods, soups, chips, sauces, and instant pasta

When individuals presented good adherence to dietary care, as recommended by healthcare professionals, this was shown to be important for preventing readmission to hospital. This is because, the ability of diet to improve biomarkers of chronic diseases, such as DM, and is recognized as a first-line approach to optimal management of these diseases [22–24]. Dietary self-care can result in an improvement in the glycemic profile and consequent protection against hospital readmissions. This is because the consumption of fiber acts as a protective factor against weight gain and visceral fat deposition. In addition, the consumption of antioxidants is also seen as beneficial, as it is able to regulate the oxidative capacity and thus mitigate insulin sensitivity [25, 26].

However, we have to consider that most hospitalizations of diabetic patients occur due to DM complications such as diabetic nephropathy [27], yet it is important to take into consideration, that, glycemic control leads to an approximately 25% reduction in microvascular complications such as diabetic retinopathy, cataract, neuropathy, nephropathy, heart failure, and amputations [28].

Studies have demonstrated the benefits of lifestyle changes. There are multiple beneficial effects of progressive aerobic and resistance exercise training, on the improvement of blood lipid profile, blood pressure, and particularly on the mechanisms regulating glucose homeostasis (improve of the insulin sensitivity and decreased HbA1c) in patients with DM [29, 30]. The Chinese Da Qing Diabetes Prevention study assessed the lifestyle changes as food consumption and physical activity, and they found the beneficial effects of lifestyle modification can persist for 14 years after the active intervention [31].

Besides, the management of mental health is very important in patients with DM, since it is associated with an event called diabetes distress that comprises four interconnected domains, which include the emotional burden of living with diabetes, the distress associated with the diabetes self-care, the stress associated with social relationships, and the stress associated with the patient-provider relationship. This event is associated with elevated glycated hemoglobin [32]. Thus, psychosocial interventions should be integrated into diabetes self-care plans, including stress management strategies.

The participants also presented a high prevalence of excess weight and abdominal adiposity. Inadequate dietary habits and physical activity levels were also observed, with few positive changes in diet after hospital discharge. Despite this unfavorable scenario, more than half of the individuals assessed their dietary habits as adequate. This finding indicates that there was possibly a lack of knowledge or incorrect perceptions regarding appropriate dietary habits in relation to DM, which is supported by other studies in the literature [33]. This makes counselling even more challenging since the first step to be taken is to increase awareness of the need to make changes.

Table 2 Self-care in relation to diabetes mellitus reported by the participants, 24 months after hospital discharge. Divinópolis-MG, 2013

Items evaluated	Mean (no. of days/week) *	Infrequent (0–4 days)%	Frequent (5–7 days)%
General dietary habits			
Following a healthy diet	3.9 ± 3.2	43.2	56.8
Following nutritional guidance from healthcare professionals	2.9 ± 3.0	64.9	35.1
Specific dietary habits			
Consumption of 5 or more portions of fruit and/or vegetables	3.2 ± 3.0	59.5	40.5
Consumption of fat-rich foods (red meat, whole milk, and dairy products)†	4.3 ± 3.1	43.2	56.8
Consumption of sweets†	1.2 ± 2.5	83.8	16.2
Physical activity			
At least 30 min (total for continuous activities, including walking)	0.5 ± 1.6	91.9	8.1
Exercising (swimming, walking, cycling), except activities at home or at work	0.1 ± 0.5	100.0	0.0
Blood glucose monitoring			
Evaluation of blood glucose	3.1 ± 3.4	59.5	40.5
Evaluation of blood glucose as recommended	2.3 ± 1.2	70.3	29.7
Care with feet			
Examination of feet	2.0 ± 3.0	75.7	24.3
Examination of shoes before using them	3.7 ± 3.4	51.4	48.6
Drying of spaces between toes	4.4 ± 3.4	37.8	62.2
Medication			
Ingestion of medication/insulin, as recommended	4.4 ± 2.6	16.2	83.8
Ingestion of indicated number of pills/insulin	6.1 ± 2.4	13.5	86.5
Smoking			
Smoking over the past 7 days			
No	78.4	NA	NA
Yes	21.6		
Number of cigarettes per day*	5.6 ± 7.0	NA	NA
When the participant smoked his/her last cigarette			
Never	45.9	NA	NA
More than 2 years ago	29.7	NA	NA
Last month	8.1	NA	NA
Today	16.2	NA	NA

*Values described as mean ± standard deviation; *NA* non applicable

†For these items, infrequent self-care is desired

The sociodemographic characteristics of this sample (older adult individuals with low educational level and low income) may negatively influence self-care practices, and this needs to be taken into consideration by healthcare professionals, given the possible different levels of difficulties in assimilating information and care practices [7, 34].

These results are worrying given the reduced provision of self-care guidance, educational activities, and individual interdisciplinary care. The low coverage of ESF in the municipality may have limited the individuals participation in educational activities and individual care. A Brazilian research has shown that increasing population coverage in Primary Care was associated with reduced rates of hospitalization for PCS [35]. We also ponder that even if guidelines were carried out, they might not have been perceived or even sufficient or adequate for individuals' needs, given the food and metabolic profiles identified.

As a possible consequence of this context, a high percentage of individuals was readmitted to hospital during the 24-month period, suggesting low levels of problem solving by the primary healthcare service.

When the self-care strategies used by the interviewees were evaluated, low adherence to self-care related to "general dietary habits" and "specific dietary habits" was observed, similar to other studies [16, 17, 35–38]. Adherence to healthy dietary habits, compatible with DM, was only observed to be applied on half of the days of the week. This means adherence level became even lower when the participants were asked about nutritional guidance given by healthcare professionals. These results possibly indicate that

Table 3 Poisson's regression for the association between the outcome "not being readmitted to hospital" and the independent variables frequent self-care. Divinópolis-MG, 2013

Explanatory variables	Not being readmitted to hospital (outcome)											
	Crude model			Adjusted model1			Adjusted model2			Adjusted model3		
	PR	95% CI	*P* value	PR	95% CI	*P* value	PR	95% CI	*P* value	PR	95% CI	*P* value
Following a healthy diet												
No	1.0											
Yes	1.41	0.73–2.73	0.30									
Following nutritional guidance from a healthcare professional												
No	1.0											
Yes	1.85	1.05–3.25	0.034	2.02	1.15–3.54	0.015	2.09	1.14–3.83	0.017	2.52	1.27–5.01	0.008
Evaluation of the adjustment‡	0.99			0.98			0.98			0.91		

PR, results as prevalence ratio; *CI*, confidence interval

1, Adjusted for age, sex, time since diagnosis, educational level, and comorbidities; 2, adjusted for age, sex, time since diagnosis, educational level, comorbidities, and variables from the sensitivity analysis (frequent consumption of 5 or more portions of fruits and vegetables; infrequent consumption of fat-rich foods; infrequent consumption of sweets); 3, adjusted for number of complications, glycemic control, and lipid control;‡evaluation of the adjustment (Pearson goodness-of-fit, Hosmer and Lemeshow test)

the perception of these individuals of what constitutes an adequate diet did not necessarily correspond to the guidance of the healthcare professionals. Another possible explanation for these results could be that these individuals had an optimistic dietary perception regarding their own food intake that hindered their self-evaluation. As an example, Plotnikoff et al. [18] found that individuals may make 1 or 2 changes to their diet and have the incorrect perception of then having a healthy diet, while ignoring other necessary changes. Illustrating, an individual starts to avoid fried foods and understands that he began to have a healthy diet in a global way, without considering the other aspects involved.

All the different self-care strategies investigated were applied over less than half of the days of the week, with the exception of medication use. Although there is strong evidence for the benefits of modifying behavior to achieve metabolic control and prevent complications relating to DM [4, 13, 19, 38, 39], changes in diet and physical activity (lowest mean) presented lower adherence. The adherence to non-drug treatment is more complex since it involves incorporating new ways of living, and changes involve ingrained cultural values related to traditions and social life. Also, evidence suggests that individuals with limited knowledge about the subject are less likely to practice diabetes self-care [20]. In addition, it is important to consider that DM complications may limit the practice of physical activity, and the majority of individuals in this sample presented 2 or more clinical complications, and 25% foot ulcer.

Adequate care regarding dietary habits can have a positive reflection on the health of individuals with DM. This was demonstrated in this study through the identification of the association between adherence to the dietary care recommended by the healthcare professionals and the higher prevalence of not being readmitted to hospital. Furthermore, infrequent self-care regarding dietary habits predominated among individuals (82.4%) who had been readmitted to hospital during the preceding 24 months. These results corroborate the importance of conducting nutritional counselling for individuals with DM at all levels of healthcare, including during hospitalization [40]. Individuals need to be empowered so that they can become co-participants, with the healthcare team, in developing treatment goals and overcoming self-care barriers, thus favoring appropriate everyday dietary choices [3].

The limitations of this study include the small sample size and the inability to generalize the results. Unfortunately, the high death rate verified after 24 months of hospitalization being an uncontrollable aspect of the study design. However, it is possible to infer internal validity, since all individuals hospitalized for DM in the municipality were investigated, with the high death rate verified after 24 months of hospitalization being an uncontrollable aspect of the study design. These results might have been even more statistically relevant if the losses due to mortality in this study had not been so high. In addition, other studies with larger sample sizes have presented similar results [16, 17, 33–35], which reinforce the internal validity of these results.

A second limitation is the cross-sectional design, not being possible to imply a causal relationship. However, the results provide evidence for the need to conduct longitudinal studies. Thirdly, a single evaluation of adherence to self-care may not represent the variation of behaviors

throughout the lives of the interviewees. Besides, we did not evaluate the reasons for hospital readmission that may not be related with the individuals diet care. It is, however, important to note that a validated questionnaire was used, with positive results regarding its applicability. In addition, further studies are recommended, in which an analysis of before and after dietary education interventions could be performed.

Despite these limitations, the present study presents important implications. It represents an initiative for deepening knowledge regarding self-care strategies implemented by individuals with DM after hospital discharge, especially in relation to diet and its influence on hospital readmission. Thus, the study provides contributions by revealing the importance of investing in interdisciplinary actions relating to diet and nutrition. It also shows the need to more assertively address the barriers that limit the construction of healthy dietary habits, thereby contributing towards adequate management of DM. Furthermore, the results indicate the need for change in clinical practices, in order to better meet the needs of individuals with DM, especially those in a more critical condition who live with recurrent complications and hospitalizations, with significant repercussions on their health and quality of life.

After hospital discharge, individuals with DM presented poor adherence to self-care, especially regarding diet. However, when these individuals adhered to nutritional guidance, there were fewer hospital readmissions. These results reinforce the importance of interdisciplinary educational actions for DM management. It is important to clarify that this study focused on dietary self-care strategies, but others self-care management also plays a role in DM controlling and should not be ignored in the same group who is benefitted. Also, due to the small sample size due to high mortality, further studies for this purpose should be conducted.

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Author contribution Mariana Carvalho de Menezes—analysis and interpretation of data; drafting and critical review; and final approval. Márcia Machado Cunha Ribeiro—conception and study design; analysis and interpretation of data; drafting and critical review; and final approval. Hillary Nascimento Coletro—critical review and final approval. Cláudia Di Lorenzo Oliveira—conception and study design; data acquisition; critical review; and final approval. Clareci Silva Cardoso—conception and study design; data acquisition; critical review; and final approval. Aline Cristine Souza Lopes—conception and study design; interpretation of data; critical review; and final approval.

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Data availability All materials used in this study are available.

Declarations

Ethics approval The study was approved by the Research Ethics Committee of the Federal University of São João Del Rei (nº 258. 574) and all participants signed the written informed consent.

Consent to participate All participants consented to participate as research volunteers, by signing the Informed Consent Form.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

To study the awareness of gestational diabetes mellitus in antenatal women, and medical and paramedical trainees in teaching hospital in North India

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Abstract

Introduction Gestational diabetes mellitus (GDM) increases pregnancy complications and future development of diabetes. Success of initiatives to increase GDM awareness depends upon the knowledge level of healthcare providers and antenatal women.

Materials and method It is a descriptive, cross-sectional study done in a teaching hospital in North India. Two pre-tested, semi-structured questionnaires assessing the basic and advanced knowledge on GDM were used. Participants included antenatal women, undergraduate trainees who filled the level 1 questionnaire, and postgraduate residents, MBBS 3rd professional students, who filled the level 2 questionnaire. The level of awareness was compared across groups using chi-square. Kruskal–Wallis H test was applied to check if the distribution of scores varied significantly across the three groups for both levels.

Results A total of 1402 study subjects filled the level 1 questionnaire (nursing and paramedic students 680/1402, 48.5%; MBBS 1st and 2nd professional students 422/1402, 30.1%; antenatal women 300/1402, 21.4%) and 500 study subjects (postgraduate residents: obstetrics 50/500, 10%; physiology 25/500, 5%; medicine 25/500, 5%; MBBS 3rd professional students 400/500, 80%) were asked level 2 questionnaires. Antenatal women had significantly better basic knowledge than medical and paramedic trainees ($p < 0.001$). Among level 2 respondents, obstetrics residents had the highest score followed by MBBS 3rd year professionals ($p < 0.001$).

Conclusion Antenatal women had better knowledge than medical and paramedical trainees due to intensive education and counselling at every point of contact. Significant learning gaps exist in medical and paramedic trainees which need attention. For the advanced level of awareness, regular continued medical education programs are required.

Keywords GDM awareness · Medical trainees · Paramedical trainees · Patient education

Introduction

As per the International Diabetes Federation (IDF), there are 223 million women (20–79 years) living with diabetes (2019), and this number is projected to increase to 343 million by 2045 [1, 2]. With a current estimate of 50.8 million diabetic people, India has the dubious distinction of being the diabetes capital of the world. This is reflected in the high prevalence of gestational diabetes mellitus (GDM) at 13.9% in India [3]. In addition, diabetes during pregnancy increases the risk of pregnancy complications like miscarriage, macrosomia, polyhydramnios, and increased operative delivery, and poses the risk of development of diabetes in the mother and child in the future [4].

Preventive strategies can check the rising trends and the associated adverse events. The primary strategy until recently

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has been the detection and treatment of GDM. Due to advances in technology, improved means of communication allow faster dissemination of information. However, paradoxically, the same system can facilitate the spread of false information, which can be deleterious to health. Quinary prevention overlaps and includes all other levels of prevention. It is a method to stop the propagation of health-related misinformation by improving awareness among all. Quarternary prevention is the set of health activities to avoid unnecessary intervention by preventing overdiagnosis and overtreatment of medical conditions by health professionals. Both these preventive measures can be implemented by educating women and involved health personnel [5, 6].

International and Indian associations such as the Diabetes in Pregnancy Study Group in India (DIPSI) and Research Society for the Study of Diabetes in India have marked National GDM Awareness Day on March 10 and aim at raising awareness about the link between maternal health and diabetes nationwide. However, the real success of these initiatives depends upon the knowledge and commitment level of healthcare providers and the general population at risk, i.e., pregnant women. They directly help the cause and act as disseminators of information for the public as a whole. Therefore, it is imperative to know their existing knowledge regarding the subject so that gaps are identified, and rational planning and resource allocation can be done.

This study was planned to evaluate the awareness about GDM among antenatal women and healthcare trainees, mainly medical students and paramedical students of a teaching institute of India. Though many studies have been conducted in the past to understand GDM awareness in antenatal women, there is none reported in medical and paramedical trainees. Undergraduate trainees and residents are the future community healthcare workers. Efforts to understand the gaps in their knowledge and strategies to overcome them can have far-reaching implications in management of GDM.

Materials and method

This study was carried out in a tertiary care center and teaching hospital in North India, over a period of 6 months from March 2019 to August 2019, in the Department of Obstetrics and Gynecology (OBG) in collaboration with the Department of Nursing and Paramedical Sciences. Ethical clearance was obtained from the Institute's Ethics Committee.

Sample size calculation for the study was done using the following formula for proportions:

$$n = \frac{Z^2(1-p)}{e^2}$$

The above formula is for estimating a population proportion (single proportion) with specified absolute precision. This formula was used for estimating the prevalence (or proportion) of a binary outcome in the population [7].

Studies advocating 20–30% awareness of GDM in the Indian population and expecting at least 25% awareness (prevalence) of GDM with 5.0% margin of error (type I error) and 80.0% power (type II error), the minimum sample size required was 288 ~ 300 [8].

Antenatal women from the outpatient department, undergraduate trainees in medical and paramedical courses, and postgraduate resident doctors of the Department of Obstetrics (OBG), Department of Medicine, and Physiology were included in this study. Only those respondents who were willing and gave written informed consent were recruited. There were no exclusion criteria.

There were two levels of the questionnaire—basic (level 1) and advanced (level 2) based on the complexity of the questions. Three groups, including the antenatal women attending OPD, MBBS students of 1st and 2nd professional (not exposed to GDM module), nursing, and other paramedical trainees (enrolled in physiotherapy, optometry courses), were given the level 1 questionnaire. Since this group was not exposed to any training modules, the awareness was solely through social media, print media, and knowledge acquired through relatives and friends.

Level 2 questionnaire was given to 3 groups, including MBBS students of 3rd professional and postgraduate resident doctors from the Department of Obstetrics (OBG), who are the cornerstone in the management of women with GDM; and postgraduate residents of the Department of Medicine, who deal with the bulk of diabetic patients in the community; and physiology, who are taught the intricacies of development of GDM. These respondents had received training through modules in the academic curriculum.

It is a descriptive, cross-sectional study which involved the use of two pre-tested, semi-structured questionnaires.

Questionnaire validation

The questionnaires were designed in the English language. The level 1 questionnaire regarding essential awareness of GDM comprised eight questions. These questions (Q1–6) were based on knowledge of harmful effects of GDM on mother and child, and need and timing of screening method. This questionnaire also looked at the potential risks of GDM to mothers and children born to them. In addition, there were two questions on the source of knowledge about GDM and if they were acquainted with a GDM patient. For questions 1 to 6, multiple options were provided with one correct response. For question no. 7, familiar sources of information were given, whereas for question no. 8, the response was recorded as yes/no.

The level 2 questionnaire regarding advanced awareness of GDM comprised nine questions, dealing with screening

population, cutoff screening criteria for GDM, and management-based questions including target blood sugar levels and standard of care. Questions were also related to the risk of development of diabetes post-pregnancy in a woman with GDM. The number of correct responses was recorded as mean and compared. Both the questionnaires are included as supplementary material.

A pre-test of the questionnaires was carried out on 15 students (MBBS and paramedical), and corrections were made accordingly. All information obtained from this study have been kept confidential and not linked to the participants in any way.

Data analysis

The level of awareness was compared across groups and chi-square test of association was performed to check if there was a significant association between levels of awareness among various groups. The score calculated was compared across the categories for both levels. Since it followed a non-normal distribution, the Kruskal–Wallis H test was applied to check if scores varied significantly across the three groups for both levels. Furthermore, if the difference was found to be significant, Mann–Whitney U test was performed to check for inter-group differences. p value < 0.05 was considered to be statistically significant. All the statistical analyses were done using SPSS v20.

Results

The study was conducted in a tertiary care center to understand the awareness of GDM among the selected population. The total number of respondents was 1902 (level 1 = 1402; level 2 = 500).

A total of 1402 study subjects, i.e., nursing and paramedical students (n = 680; 48.5%), MBBS 1st and 2nd professional students (n = 422; 30.1%), and antenatal women (n = 300; 21.4%), were interviewed on level 1 questionnaire. Table 1 draws a comparison between the level of awareness among nursing and other paramedical students, MBBS 1st and 2nd professional students, and antenatal women. Around 90% of nursing and paramedical students and MBBS 1st and 2nd professional students were aware of the harmful effect of GDM on mothers. The percentage was slightly lower for antenatal women (around 84%). A maximum number of MBBS 1st and 2nd professional students (88.9%) knew about the harmful effect of GDM on a child. The percentage was slightly lower for antenatal women (84.0%) and nursing and other paramedical students (79.3%). Among antenatal women enrolled in the survey, around 90% were aware that all of them should undergo screening for GDM; however, only 71% of nursing and other paramedic students were

Table 1 Comparison of question-wise correct responses among antenatal women, paramedics, nursing students, and MBBS 1st and MBBS 2nd professional students

Item no	Description	Total (N = 1402)		Paramedics and nursing students (n = 680)		MBBS 1st and 2nd Prof. (n = 422)		Antenatal women (n = 300)		Significance of difference	
		No	%	No	%	No	%	No	%	X^2	p value
1	Harmful effect of GDM on mother	1239	88.4	616	90.6	372	88.2	251	83.7	65.89	< 0.001
2	Harmful effect of GDM on child	1166	83.2	539	79.3	375	88.9	252	84.0	67.83	< 0.001
3	Category of pregnant women eligible for GDM screening	1098	78.3	483	71.0	350	82.9	265	88.3	104.89	< 0.001
4	Appropriate period of gestation for GDM screening	696	49.6	336	49.4	129	30.6	231	77.0	231.78	< 0.001
5	Risk of diabetes in GDM mothers	872	62.2	383	56.3	264	62.6	225	75.0	143.26	< 0.001
6	Risk of diabetes in children born to GDM mothers	893	63.7	432	63.5	238	56.4	223	74.3	125.60	< 0.001
7	Mean number of correct responses ± SD (out of maximum of 6)	4.25 ± 1.41		4.10 ± 1.29		4.09 ± 1.29		4.82 ± 1.68		X^2	

Kruskal–Wallis H test

Mann–Whitney U test (paramedics and antenatal women sig.—antenatal higher; paramedics and MBBS—non sig.; MBBS and antenatal women sig.—antenatal higher)

aware of the correct answer. Surprisingly, more than three-fourths of pregnant women enrolled in the survey knew that the appropriate period for GDM screening was 1–3 months; however, the level of awareness was deficient among nursing and other paramedic students, and MBBS 1st and 2nd professional students. Around 50% of nursing and other paramedical students were aware of the appropriate gestation period, while only 31% of MBBS students knew about the correct period.

Furthermore, following the same trend, around three-fourths of pregnant women were aware that diabetes in pregnancy would increase the chance of diabetes in mothers within 10 years. The level of awareness was slightly lower among MBBS 1st and 2nd professional students (62.6%) and nursing and other paramedic students (56.3%). Around 75% of pregnant women enrolled in the survey knew that GDM could increase the chances of diabetes in offspring later in life. The majority of nursing and other paramedic staff and MBBS 1st and 2nd year professional students came to know about GDM from doctors, while the remaining came to know from relatives, and about 15–20% of them had still not heard about GDM. The difference was also statistically significant (p value < 0.01). A large proportion of nursing and paramedical students (62.3%) were acquainted with GDM patients followed by antenatal women (19.5%) and MBBS 1st and 2nd professional students (18.3%) (p value < 0.01).

A total of 500 study subjects were interviewed based on the level 2 questionnaire. Among these, 100 (20%) were residents (OBG, 10%; physiology, 5%; medicine, 5%) and 400 (80%) were MBBS 3rd professional students.

Table 2 shows the level of awareness among residents of OBG, residents of the Physiology and Medicine department, and MBBS 3rd professional students. Almost all the medical professionals (98.4%) knew that all pregnant women should be tested for diabetes. The proportion has gone down for residents of the Physiology and Medicine department (94%), accounting for a significant difference across the groups. Eighty-eight percent of OBG residents were aware of the cutoff for diagnosing GDM; however, only 70% of MBBS students knew about it. Almost all OBG residents knew that pregnant women have to be first tested for diabetes during the first trimester, while only half of the physiology and medicine residents were aware of it. There was a significant difference in the awareness about the correct number of times a pregnant woman be tested during pregnancy. The highest percentage (65%) was for MBBS students, followed by physiology and medicine residents (58%) and lastly OBG residents (40%). Almost 50% of OBG residents knew about the preferred test for diagnosing GDM, while less than 20% of physiology and medicine residents and MBBS 3rd professional students knew about the one-step test. However, the distribution was different for recommended tests for diagnosing GDM. Again, there was a significant difference

Table 2 Question-wise correct responses of level 2 respondents (N = 500)

Item no	Total (N = 500)		Obs. and Gynae. residents (n = 50)		Physiology and medicine residents (n = 50)		MBBS 3rd and 4th professional (n = 400)		Significance of difference	
	No	%	No	%	No	%	No	%	X^2	p value
Should all pregnant women be tested for diabetes?	492	98.4	50	100.0	47	94.0	395	98.8	7.27	0.026
The cutoff for diagnosing gestational diabetes	359	71.8	44	88.0	36	72.0	279	69.8	7.31	0.026
Period of gestation for 1st test for GDM	405	81.0	48	96.0	23	46.0	334	83.5	48.73	< 0.001
Number of times the test for GDM should be done	310	62.0	20	40.0	29	58.0	261	65.2	12.40	0.002
Preferred test for diagnosing GDM	105	21.0	23	46.0	8	16.0	74	18.5	21.10	< 0.001
Recommended test according to DIPSI standard	367	73.4	32	64.0	23	46.0	312	78.0	25.82	< 0.001
The target blood sugar for a pregnant women	181	36.2	30	60.0	0	0	151	37.8	41.05	< 0.001
Risk of diabetes in GDM mothers	482	96.4	46	92.0	48	96.0	388	97.0	3.23	0.199
The standard of care for gestational diabetes management	168	33.6	31	62.0	15	30	122	30.5	20.09	< 0.001
Mean ± SD	5.74 ± 1.61		6.48 ± 1.79		4.58 ± 0.95		5.79 ± 1.59		$X^2 = 40.21$; $p < 0.001$	

Kruskal–Wallis H test

Mann–Whitney U test (OBG and Physio sig.—OBG higher; OBG and MBBS sig.—OBG higher; MBBS Physio sig.—MBBS higher)

across all three groups. Almost 80% of MBBS 3rd year professional students knew about the DIPSI test, while 64% of OBG residents and 46% physiology and medicine residents were aware. None of the physiology and medicine residents knew about the target blood sugar level for a pregnant woman, while more than half of the OBG residents were aware of the target blood sugar levels for pregnant women. Almost all the medical professionals knew that GDM does not always become maternal diabetes; hence, there was no significant difference across this item. More than 60% of OBG residents knew the standard care for GDM management, while approximately one-third of other residents and MBBS students knew that medical nutrition therapy and exercise were the standard care for GDM.

All the correct responses were added up, and the scores were compared across the three categories. The Kruskal–Wallis H test results indicated a significant difference across the scores for the three categories of medical professionals. To further identify the inter-group differences, the Mann–Whitney U test was applied, which indicated a significant difference in each pair of groups of medical professional scores in level 2 respondents. However, OBG residents had the highest score, followed by MBBS 3rd year professional students and physiology and medicine residents.

Discussion

GDM has emerged as a common medical disorder of pregnancy affecting 17.8% in urban, 13.8% in semi-urban, and 9.9% in rural areas in a field study in Tamil Nadu under "Diabetes in Pregnancy"- awareness and prevention project in the year 2008 [9]. In the last decade, health professionals have taken numerous steps to spread awareness of diabetes and its complications. This study was undertaken to understand its awareness and knowledge gaps in antenatal women and medical and paramedical trainees. Two different levels of the questionnaire were devised as antenatal women and medical and paramedical professionals have different levels of understanding.

Through the level 1 questionnaire, it was evident that antenatal women who underwent testing for GDM had better knowledge than paramedical and early professional MBBS students. This finding highlights the impact of (IEC) information, education, and counseling, reinforcing the existing knowledge at every antenatal visit. It was seen that around 75% of antenatal women knew the right time for GDM screening, and also the risk of developing future diabetes. It is particularly encouraging as it reflects the women's receptivity to attempts made at educating them. A large survey on 3182 women by Murugesan et al. in Chennai in 2007 showed that women had lesser awareness about diabetes than men. Moreover, higher education and professional jobs were significantly associated with better awareness [10]. Another large multi-centric study conducted by ICMR in four zones of India in 2014 to assess knowledge and awareness of diabetes in the general population showed that only 50% of the population had ever heard about diabetes. Even though the understanding among the self-reported diabetic population was better than the rest, overall knowledge was not satisfactory [11]. These surveys showed ignorance in the considerable size of the population regarding diabetes and its consequences.

Among pregnant women, Hussain et al. in 2015 in Malaysia evaluated the knowledge regarding GDM. They found that a majority of women had good knowledge about the anticipated complications of GDM for the mother and baby [12]. It is similar to this study where three-fourths of antenatal women and half of the others were aware of the future risk of development of diabetes in the mother and the child.

On the contrary, Bhavadharni et al., in their study on awareness of GDM among pregnant women in south Tamil Nadu in 2017, found that almost half of the urban women knew GDM would lead to diabetes in the future, and the rest either did not know or felt that GDM does not lead to diabetes. Among rural women, only 24.4% believed that GDM would lead to diabetes, and 19.5% reported that GDM was only a temporary problem during pregnancy. The vast majority (56.1%) did not know anything about the progression to diabetes [13]. Shriraam et al. found only a small proportion of rural antenatal women had good knowledge about GDM [14]. Dhyani et al. also found poor knowledge (36%) regarding diet and lifestyle modification among pregnant women with GDM. They further suggested using mass media to educate women and increase knowledge among healthcare workers to improve awareness. Better awareness among antenatal women in the present study could be attributed to heightened counseling in antenatal clinics in a tertiary center [15].

Level 2 questionnaire was devised at understanding the logistics of testing, guidelines, and management strategies. OBG residents at the forefront of managing pregnant women with GDM showed better knowledge than their counterparts in other departments. Almost all of the level 2 respondents were aware of the universal testing for GDM in India and most correctly identified the cutoff value of blood sugar for diagnosing GDM and the apt period of gestation for GDM testing. In contrast, Mahalakshmi et al. (2016) reported that more than half of the diabetologists/endocrinologists and obstetricians all over India do not consistently follow any recommended guideline for the proper diagnosis [16]. Concerning the DIPSI criteria, the level 2 participants in this study had varying levels of knowledge, with medicine residents being grossly unaware of the criteria. Mahalakshmi et al. also concluded that although 36.7% of OB/GYNs and 29.4% of physicians/

diabetologists/endocrinologists said they used the DIPSI criteria, in reality, only 12.7% and 3.8%, respectively, used the DIPSI criteria correctly [16]. The knowledge levels were deficient regarding target blood sugar levels in pregnancy, and standard care for women with GDM among all the participants in this study.

The strengths of this study are its large sample size and inclusion of antenatal women with undergraduate trainees in the medical, paramedical field, and postgraduate residents of different clinical departments. The diverse sample population helped us know the awareness regarding a very commonly encountered complication in pregnancy among heath staff who deal with patients as point of first contact.

It is noteworthy that no such study has been done previously to assess the lacunae in understanding GDM among various healthcare professionals including undergraduate medical and paramedical trainees. It is realized that the undergraduate curriculum should be more focused on practical aspects of disease management, especially diabetes to address the community level. It is well known that most medical and paramedical graduates do not opt for specialty training and directly serve the masses at the community level. Hence, the paramedics, community health workers, and nursing staff, who serve as the backbone of the medical fraternity should be well informed about GDM, and its diagnosis and management. Nurses and midwives must be adequately trained on pregnancy-specific lifestyle modifications, treatment, and screening for complications in GDM. The paramedical staff should receive quality training so the scope of common diseases like GDM is widened beyond specialty training.

It is heartening to note that antenatal women had a better understanding than the rest, contributed by information education and counseling practiced at each point of contact. However, this activity should be broadened to cover other aspects of antenatal care besides GDM in each woman.

The present study highlights the importance of providing training to residents of medicine and physiology departments, who deal with diabetes regularly to understand special nuances in pregnancy. Initiation of workshops, continued medical education programs, and seminars focusing on GDM, its prevention, diagnosis, and management should be conducted regularly. These initiatives will also serve as portals for new updates and changing guidelines regarding the subject.

India has earned the distinction of being the diabetes capital in the world. GDM is a preventable health issue and can be effectively dealt with by increasing awareness among antenatal women and medical and paramedical staff. Regular training sessions of healthcare professionals will ensure accurate dissemination of knowledge and bring a visible change in the management of GDM at the community level. Better GDM outcomes will pave the way for a healthier community by reducing a load of diabetes in the future generation.

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Declarations

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Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Resveratrol exerts antiproliferative effects on high-glucose-cultured vascular smooth muscle cells via inhibition of STAT3 and upregulation of mitochondrial gene GRIM-19 which is responsible for STAT3 activation

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Abstract

Background and aim The current study aimed to investigate the antiproliferative effect of Resv on the growth of VSMCs and to determine the association between Resv and STAT3 and the mitochondrial biogenesis signaling pathway under high-glucose conditions.

Materials and methods Male Wistar rats weighing between 180 and 200 g were killed by cervical vertebra dislocation. Primary VSMCs were obtained by outgrowth methods. Cells from different treatment groups were then collected, and changes in cell proliferation and signaling pathway activity were analyzed by PCR and Western blotting. And the MTT assay was used to investigate the effect of Resv on VSMC proliferation.

Results Resv inhibited proliferation of VSMCs under high-glucose conditions. Resv suppressed the transcriptional activity of STAT3, but the mitochondrial gene GRIM-19, which is responsible for STAT3 activation, was simultaneously upregulated. The mitochondrial biogenesis signaling pathway components NRF1, NRF2, TFB1m, and TFB2m were also upregulated. In addition, the mitochondrial genes NDUFA1, NDUFA2, and NDUFA3 were involved in the function of Resv.

Conclusion The results of the study suggest that Resv has an antiproliferative effect in high-glucose-cultured VSMCs partly through inhibiting STAT3 function and upregulating mitochondrial biogenesis.

Keywords Resveratrol · Proliferation · Signal transducer and activator of transcription 3 · Vascular smooth muscle cells · Mitochondria

Introduction

Hypertension in patients with diabetes is an important public health issue worldwide that is associated with an increased risk of cardiovascular disease [1]. The proliferation of vascular smooth muscle cells (VSMCs) is important in hypertension-induced vascular changes, including vascular remodeling. Despite the development of antihypertensive medication, these vascular changes remain a major clinical challenge. Therefore, signal transducer and activator of transcription 3 (STAT3) and mitochondrial biogenesis signaling pathways have been identified as potential therapeutic targets for blocking VSMC proliferation. Additionally, the activation of STAT3 signaling pathways in VSMCs contributes to their proliferation [2].

STAT3, one of the seven STAT transcription factors, may be regulated by various cytokines, growth factors, and hormones, including interleukin-6 (IL-6) and epidermal growth

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factor [3, 4]. In response to extracellular stimuli, STAT3 may be activated by non-receptor tyrosine kinases, such as Janus kinase 2 (JAK2) and Src, and inhibited by sirtuin 1(SIRT1) and mitochondrial proteins, including gene associated with retinoid-IFN-induced mortality 19 (GRIM-19) and suppressor of cytokine signaling 3 (SOCS3) [5–7]. Activated STAT3 forms dimers, which are then translocated into the nucleus and regulate the transcription of target genes. STAT3 mediates physiological responses and pathological changes, including vascular stenosis and arterial wall thickening. STAT3 activation by external stimuli influences the proliferation and viability of VSMCs. Previous studies have demonstrated STAT3-mediated proliferation in VSMCs and identified that mitochondria are also involved in this process [8, 9]. STAT3 may be activated by pulmonary hypertension, and thrombin and interferon-γ may also induce the proliferation of VSMCs, partly via the STAT3 signaling pathway, and thus may regulate various genes involved in cell proliferation (cyclin D1, cyclin B1, and vascular endothelial growth factor) and survival (B-cell CLL/lymphoma 2 (Bcl-2)) [10, 11]. Therefore, STAT3 is considered to be a potential molecular target for antiproliferative research in VSMCs.

Resveratrol (Resv), a polyphenolic stilbene derivative found in grape skin and other food products, has been proposed as an inhibitor of cell proliferation and moderates inflammation. The antiproliferative effect of Resv on cancer cells is partly mediated by AKT and STAT3 inhibition [12, 13]. Furthermore, Resv exerts antiproliferative, hypertrophy, and differentiation effects on VSMCs [14, 15]. However, the potential antiproliferative activity of Resv and its molecular target STAT3, and the role of mitochondria have not been extensively investigated in VSMCs. The present study evaluated the antiproliferative activity of Resv under high-glucose culture conditions in VSMCs, and investigated the underlying molecular mechanism associated with STAT3 and the mitochondrial biogenesis signaling pathways.

Materials and methods

Animal treatment conditions

Male Wistar rats weighing between 180 and 200 g, and about 6–8 weeks old, were obtained from the Experimental Animal Center, Fudan University, Shanghai, People's Republic of China. The rats used in the experiment were killed by cervical vertebra dislocation.

Cell lines and treatment conditions

VSMCs were maintained in Dulbecco's Modified Eagle's Medium (DMEM)/F12 containing high glucose, L-glutamine, and sodium pyruvate (GIBCO; Thermo Fisher Scientific, Inc., Walham, MA, USA) to which 10% fetal bovine serum (FBS) and 100 U/ml penicillin–streptomycin (all Gibco; Thermo Fisher Scientific, Inc.) were added. Primary VSMCs were obtained by outgrowth methods. The aortas of Wistar rats were obtained under sterile conditions, and then, the vessels were washed. Following the removal of adherent fat and connective tissue, the aortas were washed with phosphate-buffered saline (PBS) and cut longitudinally. The vessel was then transferred to the dish containing culture medium, and two ophthalmic forceps were used to remove the outer membrane. Endothelial cells were then removed by gentle scraping with fine forceps. The aortas were then minced into small pieces and allowed to attach to the bottom of the culture vessel. DMEM/F12 containing 20% FBS was carefully added, and explants were incubated for 72 h. Culture medium was replaced at this time point and thereafter at 48-h intervals. The resulting primary cells were seeded at a density of $3–5 \times 10^5/cm^2$ and cultured at 37 °C in 5% CO_2. Cells were used at passages 5–8 for all experiments. The phenotype of the cultured VSMCs was determined by α-SM actin (Santa Cruz Biotechnology Inc., Dallas, TX, USA) staining in > 95% of the cells.

The effects of high glucose (25.5 mM) and Resv on the function of cultured VSMCs were evaluated. The primary cells were seeded at a density of $3.0–5.0 \times 10^5/cm^2$. And after 48 h, different concentrations of Resv (1, 10, 50, 100, and 200 μM) were added to VSMCs and incubated for 24 h. Cells from different treatment groups were then collected, and changes in cell proliferation and signaling pathway activity were analyzed by reverse-transcription polymerase chain reaction (RT-qPCR), RT-PCR, and Western blotting.

Reagents

Resv and 3-(4,5-dimetrylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against SIRT1, protein kinase AMP-activated catalytic subunit α 1 (AMPK), phosphorylated (p)-AMPK Threonine-172 (p-AMPK Thr172), STAT3, and p-STAT3 (tyrosine-705) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against GAPDH were purchased from Santa Cruz Biotechnology (CA, USA). Ethidium bromide stain and AG490 were obtained from Beyotime Institute of Biotechnology (Jiangsu, People's Republic of China), and secondary antibodies were purchased from Biosynthesis Biotechnology (Beijing, China). Penicillin, streptomycin, DMEM/F12, and FBS were obtained from GIBCO (Grand Island, NY, USA).

Western blotting analysis

Western blotting was performed as previously described [15], with minor modifications. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Novex; Thermo Fisher Scientific, Inc.). Membranes were blocked in 5% (w/v) non-fat milk for 1 h at room temperature and then incubated with primary antibodies against SIRT1(1:2000), GRIM-19(1:2000), STAT3(1:3000), p-STAT3 Tyr705(1:1500), AMPK(1:2000), p-AMPK(1:1000), and GAPDH(1:5000). Subsequent to washing, membranes were incubated with the appropriate secondary antibodies and most of the secondary antibodies were from rabbit and the goat which depended on the first antibody. Protein bands were detected using the enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.) and band intensities were quantified using Quantity One software (BioRad Laboratories, Inc., Hercules, CA, USA).

Reverse-transcription PCR and RT-PCR analysis

Total RNA was isolated from VSMCs 24 h after treatment with 1 μM or 100 μM Resv under high-glucose conditions. Total RNA was purified using TRIzol® Reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol and quantified by spectrophotometric absorbance at 260 nm. RNA purity was confirmed by the absorbance (260/280) ratio and RNA integrity evaluated by ethidium bromide staining following electrophoresis on a denaturing agarose gel. cDNA was synthesized, and PCR was performed as previously described [16]. PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Each sample was analyzed in three biological replicates, and at least three reactions were used to calculate RNA expression levels. The primer sequences used are presented in Table 1, and β-actin served as the internal control. Relative gene expression was quantified densitometrically using the Gel Image System version 3.74 (Tianon, Shanghai, China). CyclinB1 and Bcl-2 primers were also used by qPCR using SYBR green PCR master mix (Thermo Fisher Scientific, Inc.) to observe the mRNA expression levels of cyclinB1 and Bcl-2. Fluorescence was monitored and analyzed using a GeneAmp 7000 detection system instrument (Thermo Fisher Scientific, Inc.). Also, the SIRT1-related NAMPT was detected; β-actin was used to normalize cDNA input levels. The relative quantification $2^{-\Delta\Delta Cq}$ method was used for comparisons between groups.

Table 1 Oligonucleotide primer sequences used in this study

Name	Sequence(5′–3′)	Product size (bp)
cyclinB1_F	TAGGTGTGGGCAGCCAGAGGT	158
cyclinB1_R	ACTGCCACAGGCACACGCTT	
SOCS3_F	TCTTTACCACCGACGGAACC	191
SOCS3_R	TGACCGTTGACAGTCTTCCG	
Bcl-2_F	AGAGAGGCCGCCCTCGATCTG	105
Bcl-2_R	GGCCGGGATCATGCGACCTG	
SIRT1_F	CCAGATCCTCAAGCCATGT	201
SIRT1_R	TTGGATTCCTGCAACCTG	
NAMPT_F	TGCTACTGGCTCACCAACTG	198
NAMPT_R	TATGCCGGCAGTCTCTTGTG	
GRIM-19_F	CTACTGGAGAATAATGAGGTGGAAC	175
GRIM-19_R	CCAGTTGGGCACATCTTTCA	
NDUFA1_F	TGCTGCCGGAAGAGCGGTGA	189
NDUFA1_R	TCCTTGCCCCCGTTGGTGAACT	
NDUFA2_F	ACTGAGGACTGAACAAGCCCACCA	223
NDUFA2_R	GCGACATCCCAGCGGGTAGC	
NDUFS3_F	CGTGCCCTTGAGGCTCCGTG	152
NDUFS3_R	ACAGCACTGAGGGTCGCCCA	
NRF1_F	ACACAGCATAGCCCATCTCG	226
NRF1_R	GGTCATTTCACCGCCCTGTA	
NRF2_F	AGCAAGACTTGGGCCACTTA	112
NRF2_R	TCTGGCTTCTTGCTCTTGGG	
TFB1m_F	CCGTTACCCACCATTCGAGA	216
TFB1m_R	TTCAACCACCAGAAGCTCGG	
TFB2m_F	TGTTTGATCTGTACTCCTGCGA	186
TFB2m_R	GCTGAGAGCAAACCATGTGC	
Actin_F	GCGTCCACCCGCGAGTACAA	118
Actin_R	ACATGCCGGAGCCGTTGTCG	

F, forward; *R*, reverse

Cell proliferation assay

Cell viability was determined using MTT assays according to the manufacturer's protocols. VSMCs were seeded into 96-well culture plates at a density of 1×10^4 cells/well. At 70–80% confluence, the cells were treated with Resv (1, 10, 50, 100, and 200 μM) for 24 h and then PBS containing MTT to obtain a final concentration of 0.5 mg/ml was added to each, which were then incubated at 37 °C for 4 h. The medium was gently removed, and 150 μl DMSO was added to each well. Culture plates were shaken for 10 min to dissolve the formazan product. A control well containing DMSO but no cells served as a blank control, and the optical density at 490 nm was determined using a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The number of VSMCs was counted after 24 h following the addition of different concentrations of Resv and the experiment was

repeated for three times and three wells were used in each group. And later, in order to detect the function of AG490, AG490 is added 30 min before the addition of Resv.

Statistical analysis

Data are expressed as means ± standard deviation for three or more independent experiments. Statistical significance was estimated using t-test for pairwise comparison. $p < 0.05$ suggested that the difference was statistically significant. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Resveratrol inhibits proliferation in high-glucose-exposed VSMCs

The MTT assay was used to investigate the effect of Resv on VSMC proliferation. Different concentrations of Resv (1, 10, 50, 100, and 200 μM) were added to the cell culture medium and incubated for 24 h prior to the MTT analysis. Resv-treated cells (Resv100 and Resv200) demonstrated significantly lower proliferation compared with the control group ($p < 0.05$; Fig. 1).

Resveratrol inhibits the STAT3 signaling pathway

To investigate the effect of different concentrations of Resv on the STAT3 signaling pathway, cells were treated with varying concentrations of Resv for 24 h prior to Western blot analysis. The protein expression levels of STAT3 and p-STAT3-Tyr705 were determined. Resv significantly suppressed p-STAT3 expression compared with the control group ($p < 0.05$; Fig. 2B). The expression levels of STAT3-dependent genes that regulate cell proliferation and viability were also determined. The express levels of cyclinB1 and Bcl-2 were significantly downregulated following a 24-h Resv treatment compared with the control group ($p < 0.05$; Fig. 3A). Additionally, another p-STAT3 target gene, SOCS3, which exerts a

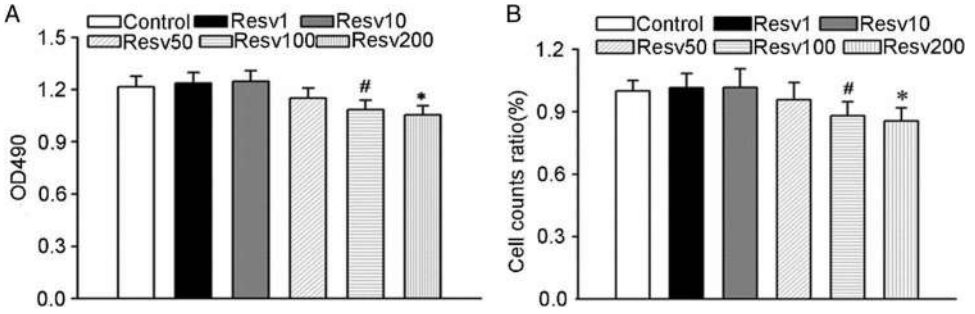

Fig. 1 **A** Resveratrol inhibited proliferation in high-glucose-exposed VSMCs. VSMCs were treated with different concentrations of Resv (1, 10, 50, 100, and 200 μM) or 0.1% dimethyl sulfoxide (control) for 24 h prior to 3-(4,5-dimetrylthiazol-2-yl)-2,5-diphenyltetrazolium bromide analysis. **B** Count ratio of VSMCs. Data are presented as the mean ± standard deviation of three independent determinations. *$p < 0.05$ Resv200 group vs. control group; #$p < 0.05$ Resv100 vs. control group

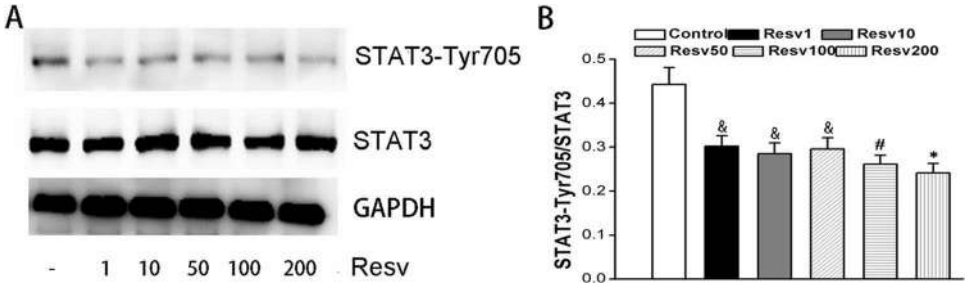

Fig. 2 Resveratrol inhibits the STAT3 signaling pathway. Vascular smooth muscle cells were treated with different concentrations of Resv (1, 10, 50, 100, and 200 μM) or control for 24 h. Western blot analysis demonstrating the expression of **A** STAT3-Tyr705 and STAT3 expression. **B** The abundance of STAT3-Tyr705 relative to STAT3. Data are presented as the mean ± standard deviation of three independent experiments. *$p < 0.05$ Resv200 group vs. control group; #$p < 0.05$ Resv100 vs. control group; &$p < 0.05$ Resv1.10.50 groups vs. control group

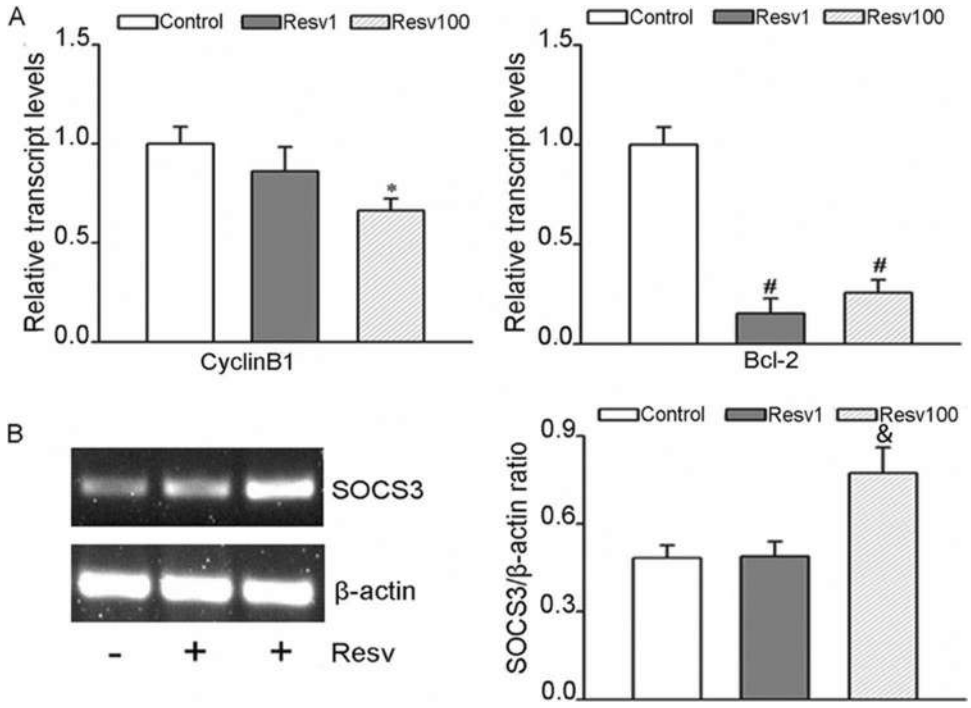

Fig. 3 Resveratrol induced the downregulation of cyclinB1, Bcl-2, and upregulation SOCS3. Vascular smooth muscle cells were incubated without or with 1 μM Resv (Resv1) or 100 μM Resv (Resv100) for 24 h. **A** Expression of cyclinB1 and Bcl-2 mRNA by RT-PCR. **B** Expression levels of SOCS3 mRNA by reverse-transcription PCR. Data are presented as the mean ± standard deviation of three independent determinations. $^{*}p < 0.05$ cyclinB1 Resv groups vs. control group; $^{\#}p < 0.05$ Bcl-2 Resv groups vs. control group; and $^{\&}p < 0.05$ SOCS3 Resv100 group vs. control group

negative feedback effect on p-STAT3 expression levels, was significantly upregulated following Resv100 treatment under high-glucose conditions ($p < 0.05$; Fig. 3B). The effect of AG490, a JAK2 inhibitor, was also investigated. It was determined that AG490 significantly suppressed the proliferation of VSMCs under high-glucose conditions when compared with the control ($p < 0.05$; Supplementary Fig. 1).

Resveratrol promotes the upregulation of p-AMPK and SIRT1

SIRT1 was previously demonstrated to affect p-STAT3 signaling, and p-AMPK and SIRT1 expression are associated with mitochondrial biogenesis [7, 16, 17]. To assess the effect of Resv on high-glucose-induced p-AMPK and SIRT1 expression levels, VSMCs were cultured under high-glucose conditions with increasing concentrations of Resv for 24 h. As indicated by Fig. 4A, treatment with Resv significantly upregulated p-AMPK expression compared with the control group ($p < 0.05$). Concurrently, SIRT1 expression was significantly greater in cells treated with Resv compared with the control group ($p < 0.05$; Fig. 4B). To analyze the mechanism that is responsible for the changes in SIRT1 expression, the expression of nicotinamide phosphoribosyltransferase (NAMPT), an enzyme that controls SIRT1 production, was also determined. As indicated by Fig. 4C, NAMPT transcription levels ware significantly upregulated along with SIRT1 in VSMCs following Resv treatment when compared with their respective control group ($p < 0.05$). Therefore, these findings demonstrate that Resv increases p-AMPK expression and SIRT1 signaling in VSMCs under high-glucose conditions compared with controls.

Resveratrol affects mitochondria biogenesis

In order to determine the effect of Resv on mitochondrial biogenesis, expression levels of the mitochondrial gene, GRIM-19, which is for the suppression of p-STAT3, were detected [16]. The expression of other mitochondrial genes (NADH:ubiquinone oxidoreductase subunit A1(NDUFA1), NDUFA2, and NDUFS3) and mitochondrial biogenesis-associated transcription factors were analyzed by RT-qPCR. It was observed that Resv significantly upregulated the expression levels of GRIM-19, NDUFA1, NDUFA2, and NDUFS3 ($p < 0.05$; Supplementary Fig. 2A and C). The transcription factors nuclear respiratory factor 1 (NRF1), NRF2, transcription factor B1, mitochondrial (TFB1m), and TFB2m were also significantly upregulated by Resv treatment under high-glucose conditions ($p < 0.05$; Supplementary Fig. 2B and D). These findings clearly demonstrate that Resv induced upregulation of mitochondrial gene transcription factors associated with p-STAT3 regulation and biogenesis in VSMCs.

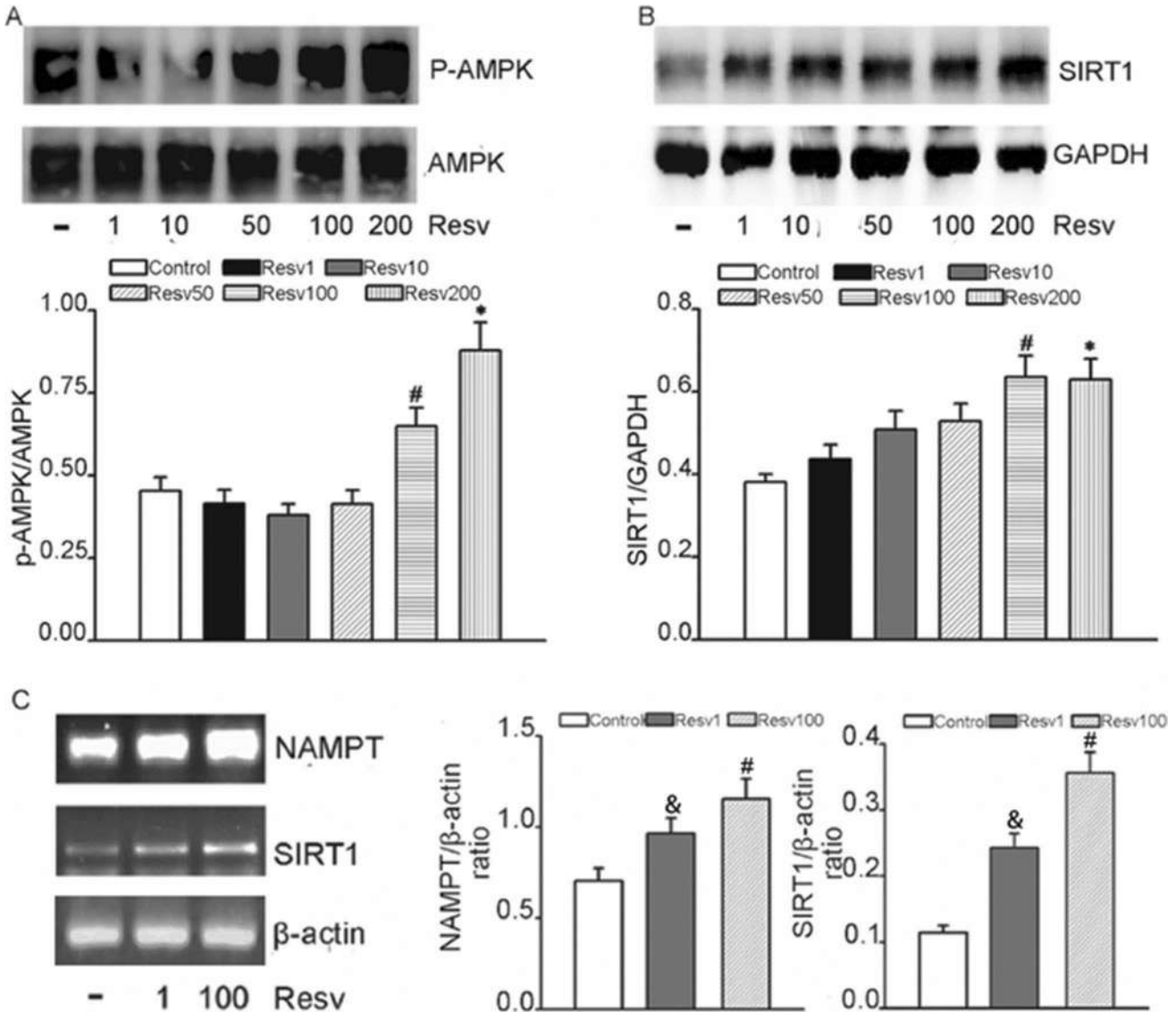

Fig. 4 Resv promoted the p-AMPK and SIRT1 upregulation in VSMCs. VSMCs were cultured in high-glucose medium and then incubated with different concentrations (1, 10, 50, 100, 200 μM) of Resv for 24 h. Normal medium was used as a control. Western blotting analysis indicated the expression levels of **A** p-AMPK and AMPK, the abundance of p-AMPK relative to AMPK; **B** SIRT1 and GAPDH, and the abundance of SIRT1 relative to GAPDH; and **C** NAMPT and SIRT1 expression relative to β-actin. Data presented as the mean ± standard deviation of three independent determinations. $^{*}p < 0.05$ Resv200 group vs. control group; $^{\#}p < 0.05$ Resv100 group vs. control group; $^{\&}p < 0.05$ SOCS3 Resv1 group vs. control group

Discussion

The antiproliferative effects of Resv on VSMCs have been demonstrated in vitro in the present study along with the molecular mechanism involved in this process. Resv simultaneously inhibited VSMC proliferation, STAT3 transcriptional activity, and the expression of STAT3 target genes. However, the importance of STAT3 in cell proliferation requires further investigation. The upregulation of the expression levels of GRIM-19 and SOCS3, which regulate p-STAT3 signaling, indicated that STAT3 may be a molecular target of Resv. Additionally, increased SIRT1 expression following Resv treatment was detected, which may be associated with the antiproliferative function of Resv in VMSCs. Mitochondria are important for the regulation of cell proliferation. The current study determined that Resv may induce the expression of p-AMPK, mitochondrial biogenesis genes, and transcription factors including SIRT1. Therefore, the present findings suggest that Resv acts partly through the inhibition of activated STAT3 and by stimulating mitochondrial biogenesis.

Resv is a stilbenoid, a natural phenol and phytoalexin produced by a variety of plants such as the skin of grapes, blueberries, raspberries, mulberries. Resv exerts multiple beneficial effects, including cancer prevention, cardioprotection, and antidiabetic effects [18–22]. Its antiproliferative function is mediated by NF-κB inhibition, endoplasmic reticulum modulation, and connexin 43 (Cx43) phosphorylation [23–25]. However, the antiproliferative properties of Resv cannot be completely explained by the regulation of these transcription factors and the mechanism remains to be fully elucidated. In the present study, Resv inhibited VSMC proliferation in vitro. Additionally, STAT3 was identified as a possible novel molecular target of Resv in VSMCs. Consistent with these findings, Resv may inhibit the proliferation of medulloblastoma, human multiple myeloma, and prostate cancer cell lines, which contain constitutively active STAT3 [18]. Therefore, the present study indicates that Resv may represent an effective antiproliferative treatment for hypertension in diabetes under conditions in which the STAT3 signaling pathway is activated in VSMCs.

Although external factors can have an effect, STAT3 activation in VSMCs is primarily due to overexpression or deregulation of upstream signaling molecules, including Src and JAK2 [26, 27]. For example, high levels of IL-6, thrombin, and heparin-binding EGF like growth factor and angiotensin II may result in STAT3 activation and thus induce proliferation in VSMCs [9, 26, 28]. The proliferative effects of these signaling pathways are mediated by STAT3-dependent gene expression. STAT3 has been shown to be an antiproliferative therapeutic target [28]. The present study determined that Resv may inhibit STAT3 phosphorylation, possibly by controlling SIRT1, GRIM-19, and SOCS3 expression. Overexpression of GRIM-19 and of the SIRT1 suppressor EX527 reduces STAT3 target gene expression associated with cell viability and proliferation, as we previously reported [16]. Collins et al. reported that SOCS3 expression may affect p-STAT3 activity. A previous study determined that GRIM-19 is important for mitochondrial function. In addition, mitochondria are vital for VSMC proliferation and viability [8], and metabolism and oxidative stress, which also contribute to cell proliferation. Notably, the present study demonstrated that Resv may induce the upregulation of p-AMPK, mitochondrial genes, and transcription factors such as NRF1, NRF2, TFB1m, and TFB2m. Therefore, Resv may suppress STAT3 phosphorylation and STAT3 target gene expression in VSMCs via the regulation of SIRT1, GRIM-19, and SOCS3 expression and mitochondrial biogenesis. The mechanism linking Resv to STAT3 activity, which is demonstrated in VSMCs in the current study, may be important for future treatment of cardiovascular disease. However, further studies are required to conclusively demonstrate that the effects of Resv on STAT3 are mediated by SIRT1, GRIM-19, and SOCS3 signaling and changes in mitochondrial biogenesis.

Clinicians and researchers are more frequently investigating the potential utility, and mechanism of action, of plant-based therapeutic agents in treating diseases. Various herbal medicines, including curcumin, vitamin E, and Resv, have been shown to exert antiproliferative and anti-inflammatory activities [29, 30]. The use of natural compounds represents an attractive strategy for inhibition of cell growth and inflammation. It is increasingly apparent that Resv may be an important therapeutic modality for VSMCs. A previous study indicated that Resv inhibits cell proliferation through regulating Cx43 phosphorylation and estrogen receptor-dependent nitric oxide production, and inhibits cell migration by regulating Rac1 [24, 31]. Inhibiting constitutive STAT3 often leads to inhibition of VSMC proliferation [32]. However, the effects of Resv on STAT3 signaling in VSMCs are not fully understood. In the present study, Resv treatment altered p-STAT3 expression and upregulated signaling pathways involving SIRT1, GRIM-19, and SOCS3. Furthermore, Resv induced mitochondrial biogenesis and suppressed VSMC proliferation under high-glucose conditions. In addition, the delineation of the mechanism involving p-STAT3 and the biogenesis of mitochondria in the current study indicates that this natural compound may be a promising antiproliferative agent. The concentration of Resv (100 μM and 200 μM) required to inhibit cell proliferation is rather high. Therefore, further research is required in order to develop a practical use for this antiproliferative compound in VSMCs during hypertension.

Conclusion

The present study demonstrated that Resv has an effect on the proliferation of VSMCs under high-glucose conditions in vitro. The action of Resv may partly depend on the regulation of STAT3 and mitochondrial biogenesis. The effect of Resv on the signaling pathway upstream of STAT3 is complex; therefore, further research is required to fully elucidate the underlying mechanism of the antiproliferative activity of Resv on VSMCs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-00963-3.

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Declarations

Ethics approval The animal research study protocol was in compliance with the policy of the Institutional Animal Care and Use Committee (IACUC) of Shanghai Jiao Tong University affiliated with Shanghai Sixth People's Hospital. Animal testing was performed in accordance with the international guiding principles for biomedical research.

Conflict of interest The authors declared no competing interests.

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ORIGINAL ARTICLE

Liraglutide may affect visceral fat accumulation in diabetic rats via changes in FTO, AMPK, and AKT expression

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Abstract

Purpose The aim of this study is to explore the effects of liraglutide (LRG) on the expression of FTO, AMPK, and AKT in the visceral adipose tissues of obese and diabetic rats and the underlying mechanisms thereof.

Methods Thirty SPF-grade, male SD rats were randomly divided into the healthy control, diabetic model (DM), and DM + LRG groups. The DM and DM + LRG groups were administered normal saline and LRG (0.6 mg/kg/d), respectively. After 12 weeks, the body weight of the rats was measured, and their visceral adipose tissues were collected and weighed; the levels of serum biochemical indicators and FTO, AMPK, and AKT in these tissues were then measured using qRT-PCR and western blotting.

Results Compared to the control group, the body weight and visceral fat accumulation and blood glucose, TG, TC, and LDL-C levels increased significantly, while the HDL-C levels decreased significantly, in the DM group ($p < 0.05$). After LRG treatment, the HDL-C levels increased significantly, but the levels of the other indicators decreased significantly ($p < 0.05$). Compared to the control group, the visceral adipose tissue levels of FTO and AKT increased significantly, while the AMPK levels decreased significantly in the DM group ($p < 0.05$). After LRG treatment, the FTO and AKT levels decreased significantly, and the AMPK levels increased significantly ($p < 0.05$).

Conclusion LRG may activate and inhibit the AMPK and AKT pathways, respectively, and decrease FTO expression, thereby alleviating abdominal obesity in type 2 diabetes.

Keywords Liraglutide · Diabetes · Obesity · FTO · AMPK · AKT

Introduction

Diabetes mellitus (DM) is a common chronic disease. The prevalence of type 2 diabetes (T2DM) and obesity is rapidly increasing worldwide because of lifestyle changes and accelerated aging, posing a global public health issue [1]. Being overweight and abdominal obesity are the greatest risk factors for T2DM, which aggravate insulin resistance and adversely affect blood glucose control [2]. The fat mass and obesity-associated (FTO) gene, discovered by Frayling et al. is abundantly expressed in adipose tissues [3, 4]. FTO is a transcription co-factor that may affect the process of obesity by modulating the growth, development, and adipogenesis of adipocytes [5]. Accumulating evidence suggests that FTO polymorphisms are closely related to obesity and T2DM [6]. AMP-activated protein kinase (AMPK), a key factor in lipid metabolism, reduces the accumulation of lipids by inhibiting the expression of FTO in skeletal muscle cells [7]. The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is a classic pathway affected by the development of T2DM. The activation of PI3K/AKT signaling plays an important role in lipid metabolism and insulin resistance. In endometrial cancer, the expression of FTO is induced by the estrogen-activated pPI3K/AKT pathways in adipocytes [8].

Liraglutide (LRG) is a glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA). Similar to GLP-1, LRG

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promotes insulin secretion, inhibits glucagon release, and maintains blood glucose stability in a glucose concentration-dependent manner [9]. LRG may also regulate glucose metabolism by improving the constitution of intestinal flora, promoting the enrichment of short-chain fatty acid-producing bacteria (probiotic bacteria, e.g., bifidobacteria). In such a manner, it could alleviate systemic inflammation and elicit a beneficial effect on diabetes [10]. In addition, GLP-1RAs reduce energy intake by delaying gastric emptying, increasing satiety, and suppressing appetite, posing a direct impact on energy balance and body weight control [11]. Long-term combined application of LRG for the treatment of T2DM effectively regulates the level of glycated hemoglobin, markedly reduces the body weight of overweight and abdominally obese T2DM patients, alleviates hyperlipidemia, and reduces the risk of cardiovascular disease [12]. In db/db mice, LRG administration reduces body weight and visceral fat production by activating AMPK and inhibiting AKT [13].

Herein, a streptozotocin (STZ)-induced obese rat model of T2DM was used to observe LRG-induced changes in the visceral adipose tissue expression of FTO, AMPK, and AKT, and how these changes may affect metabolism and visceral fat accumulation. Our study provides an experimental basis for examining the effects of LRG on FTO, AMPK, and AKT expression and suggests a mechanism whereby LRG alleviates abdominal obesity in diabetic rats.

Materials and methods

Animals

SPF-grade male Sprague Dawley (SD) rats, weighing 180 – 220 g, were provided by the Laboratory Animal Center of Xinjiang Medical University (Xinjiang, China). A high-fat and high-sugar diet was provided by Beijing Botai Hongda Biotechnology Co., Ltd (China).

Reagents and instruments

STZ and LRG were purchased from Sigma and Novo Nordisk, Denmark, respectively. Primers for the FTO, AMPK, and AKT genes were obtained from the Beijing Genomics Institute. RNA extraction kits, cDNA reverse transcription kits, and real-time PCR kits were purchased from QIAGEN, Germany. Antibodies against FTO, AMPK, and AKT were purchased from Abcam, USA. A portable blood glucose monitor and test papers were purchased from ACCU-Chek, Germany, and the enzyme-labeled analyzer was obtained from Thermo, USA. The real-time PCR machine, CFX96, and the gel imaging and electrophoresis system were obtained from BIO-RAD, USA.

Establishment of the T2DM model

After adaptive feeding for 1 week, searching the related researches, considering the ethics and modeling rationality of animal experiments, thirty SPF-grade male SD rats were randomly divided into healthy control (n = 10) and diabetic model (DM) (n = 20) groups. Rats in the control group were fed an ordinary diet, while those in the DM group were fed with a high-fat, high-sugar diet for 8 weeks. When the average body weight of DM rats reached 400 – 450 g, streptozotocin was intraperitoneally injected at a dose of 30 mg/kg, and the high-fat, high-sugar diet was continued. Blood was collected from the tail vein of all DM rats after 3 days. Blood glucose ≥ 16.7 mmol/L indicated successful induction of diabetes. Then, the twenty diabetic rats were randomly divided into two subgroups (10 per group). One group received LRG (0.6 mg/kg/d) subcutaneously twice a day, while the other was injected with saline as a control. Meanwhile, the high-fat, high-sugar diet was continued. Accordingly, a total of three groups were created, a healthy control group, a DM + LRG group, and a DM control group. All rats were anesthetized and sacrificed after 12 weeks of LRG or standard saline treatment.

Sample collection

After 12 weeks of treatment, rats were fasted overnight for 12 h and anesthetized by intraperitoneal injection of 3% sodium pentobarbital (40 mg/kg). Blood was collected from the abdominal aorta. The visceral adipose tissues were harvested, weighed, and stored at – 80 °C.

Detection of serum biochemical indicators

Whole blood samples were centrifuged, and the supernatants were collected and stored on ice until analysis. For the detection of the levels of serum glucose, triglycerides, total cholesterol, and high- and low-density lipoproteins, ELISA was performed using specific kits according to the manufacturer's protocols.

Real-time PCR

Total RNA was extracted from visceral adipose tissue and reverse transcribed into cDNA. The FTO, AMPK, and AKT primer sequences used for real-time RT-PCR are shown in Table 1. The mRNA expression levels of FTO, AMPK, and AKT were determined using qPCR in a total volume of 20 µl with the following parameters: denaturation at 95 °C for

Table. 1 Primers for each gene

Gene	Forward primer	Reverse primer
FTO	5'-GACCGTCCTGCGATGATGAAGTG-3'	5'-CCTGTCCACCAAGTTCTCGTCATG-3'
AMPK	5'-ATGATGAGGTGGTGGAGCAGAGG-3'	5'-GTTCTCGGCTGTGCTGGAATCG-3'
AKT	5'-CTGCCTTCTGCCAAGCCACAC-3'	5'-ACTCCTCTGAGACCTGCCAAGATG-3'
β-actin	5'-CAACCTTCTTGCAGCTCCTC-3'	5'-CGGTGTCCCTTCTGAGTGTT-3'

10 min, annealing at 60 °C for 1 min, extension at 95 °C for 15 s, for a total of 40 cycles. β-actin was used as an internal control.

Western blotting

Liquid-frozen visceral adipose tissue was homogenized in lysis buffer containing phosphatase inhibitor and protease, sonicated, and centrifuged at 4 °C for 15 min. The supernatants were collected, and protein concentrations were determined using a BCA protein detection kit. Denatured proteins (20 μg/well) were subjected to 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was suspended in 5% BSA at ambient temperature for 2 h, left to react with the primary antibodies (FTO, 1:1000; AMPK, 1:2000; AKT, 1:1000; β-actin, 1:2000) and incubated overnight at 4 °C. After washing with TBST thrice, the membrane was incubated with an HRP-labeled secondary antibody (1:10,000) at ambient temperature for 2 h. After washing with TBST thrice, signals were detected by high-sensitivity chemiluminescence (ECL) and quantified using a gel analysis system. β-actin was used as the internal control.

Statistical analysis

Experimental data were analyzed using SPASS 25.0 software. Data were presented as $\overline{x} \pm$ sd. Differences among the three groups were analyzed using ANOVA, followed by least significant difference *t*-test(LSD). Results with p values < 0.05 were considered statistically significant.

Results

Body weight and visceral fat accumulation

Compared with healthy control rats, the body weight of the DM + LRG and DM rats was significantly increased ($p < 0.05$) (Table 2, Fig. 1A). However, the body weight of the DM + LRG rats was significantly lower than that of the DM rats ($p < 0.05$) (Table 2, Fig. 1A). In addition, the weight of visceral fat in DM + LRG and DM rats was significantly higher than that in control rats ($p < 0.05$) (Table 2, Fig. 1B); however, the visceral fat weight in DM + LRG rats was significantly lower than that in the DM rats ($p < 0.05$) (Table 2, Fig. 1B).

Glucose and biochemical indicators

Blood glucose (Table 3, Fig. 2A) and the serum triglyceride and total cholesterol (Table 3, Fig. 2B) levels were significantly higher in the DM + LRG and DM rats than in control rats ($p < 0.05$); however, these levels were significantly lower in DM + LRG rats than in DM rats ($p < 0.05$) (Table 3, Fig. 2). Moreover, serum high-density lipoprotein cholesterol (HDL-C) levels were significantly lower, while those of low-density lipoprotein cholesterol (LDL-C) were significantly higher in the DM + LRG and DM rats, than in the control rats ($p < 0.05$) (Table 3, Fig. 2C). However, the DM + LRG rats had significantly higher HDL-C levels, but significantly lower LDL-C levels, than DM rats ($p < 0.05$) (Table 3, Fig. 2C).

Expression of FTO, AMPK, and AKT in visceral adipose tissues

Compared with control rats, visceral adipocyte FTO and AKT mRNA levels were significantly higher ($p < 0.05$) (Table 4, Fig. $3A_{(1)}$ and $3C_{(1)}$), while those of AMPK were significantly lower ($p < 0.05$) (Table 4, Fig. $3B_{(1)}$) in DM and DM + LRG rats. However, DM + LRG rats showed significantly lower FTO (Table 4, Fig. $3A_{(1)}$) and AKT (Table 4, Fig. $3C_{(1)}$) mRNA levels ($p < 0.05$), and significantly higher AMPK levels ($p < 0.05$) (Table 4, Fig. $3B_{(1)}$) than DM rats.

Similarly, the visceral fat of DM and DM + LRG rats showed significantly higher FTO (Table 5, Fig. $3A_{(2)}$, Fig. 4) and AKT (Table 5, Fig. $3C_{(2)}$ and Fig. 4) protein levels, and significantly lower AMPK (Table 5, Fig. $3B_{(2)}$ and Fig. 4)

Table 2 Body weight andvisceral adipose levels

Group	Number	Body Weight (g)	Adipose Weight (g)
Control	10	222.51±22.24	6.90±1.75
DM+LRG	10	475.86±67.21*	10.58±1.76*
DM	10	596.67±36.46*#	18.14±1.94*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *P<0.05 vs Control; #P<0.05 vs DM+LRG

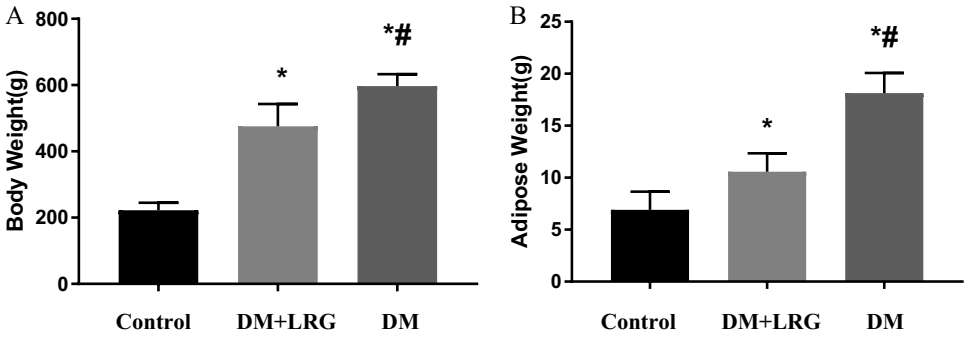

Fig. 1 Body weight and visceral adipose levels

Table 3 Blood glucose andbiochemical levels

Group	Number	Glucose (mmol/L)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	10	5.64±0.97	1.07±0.17	2.40±0.40	1.74±0.21	1.56±0.37
DM+LRG	10	8.56±0.95*	1.68±0.17*	3.44±0.44*	1.06±0.20*	2.52±0.22*
DM	10	11.16±1.29*#	2.62±0.29*#	5.25±0.89*#	0.63±0.15*#	3.90±0.59*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *P<0.05 vs Control; #P<0.05 vs DM+LRG

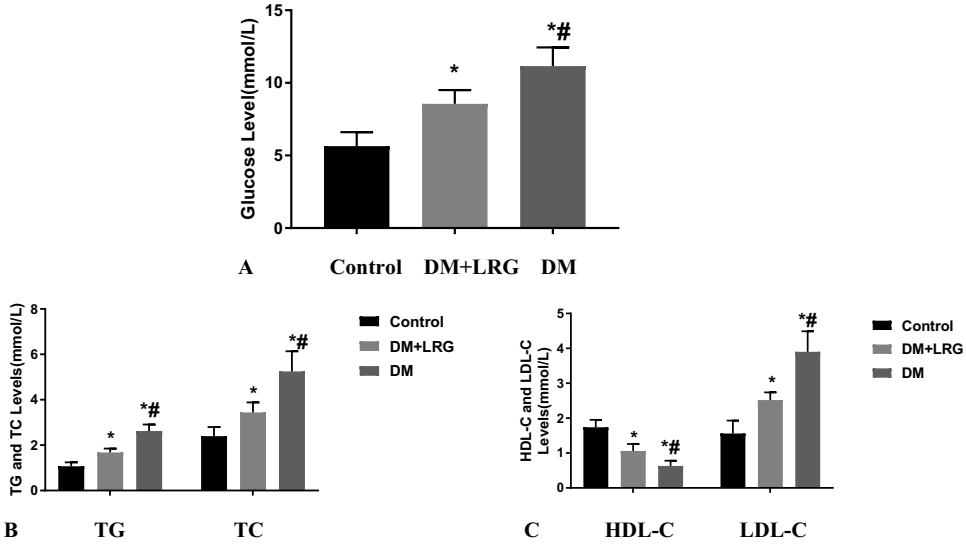

Fig. 2 Blood glucose and biochemical levels

Table 4 mRNA expression of FTO, AMPK and AKT

Group	Number	mRNA FTO	mRNA AMPK	mRNA AKT
Control	10	0.64±0.22	2.42±0.29	0.37±0.14
DM+LRG	10	1.44±0.56*	1.88±0.26*	0.70±0.11*
DM	10	2.74±0.68*#	0.86±0.28*#	1.16±0.18*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *P<0.05 vs Control; #P<0.05 vs DM+LRG

protein levels. However, DM + LRG rats showed significantly lower FTO (Table 5, Fig. $3A_{(2)}$ and Fig. 4) and AKT (Table 5, Fig. $3C_{(2)}$ and Fig. 4) protein levels, but significantly higher AMPK protein levels, than DM rats ($p < 0.05$) (Table 5, Fig. $3B_{(2)}$ and Fig. 4).

Discussion

In this study, we used a STZ-induced diabetic obese rat model to observe LRG-induced changes in metabolism and visceral adipose tissue expression of FTO, AMPK, and AKT. Our study provides an experimental basis to study the effects of LRG on FTO, AMPK, and AKT expression and

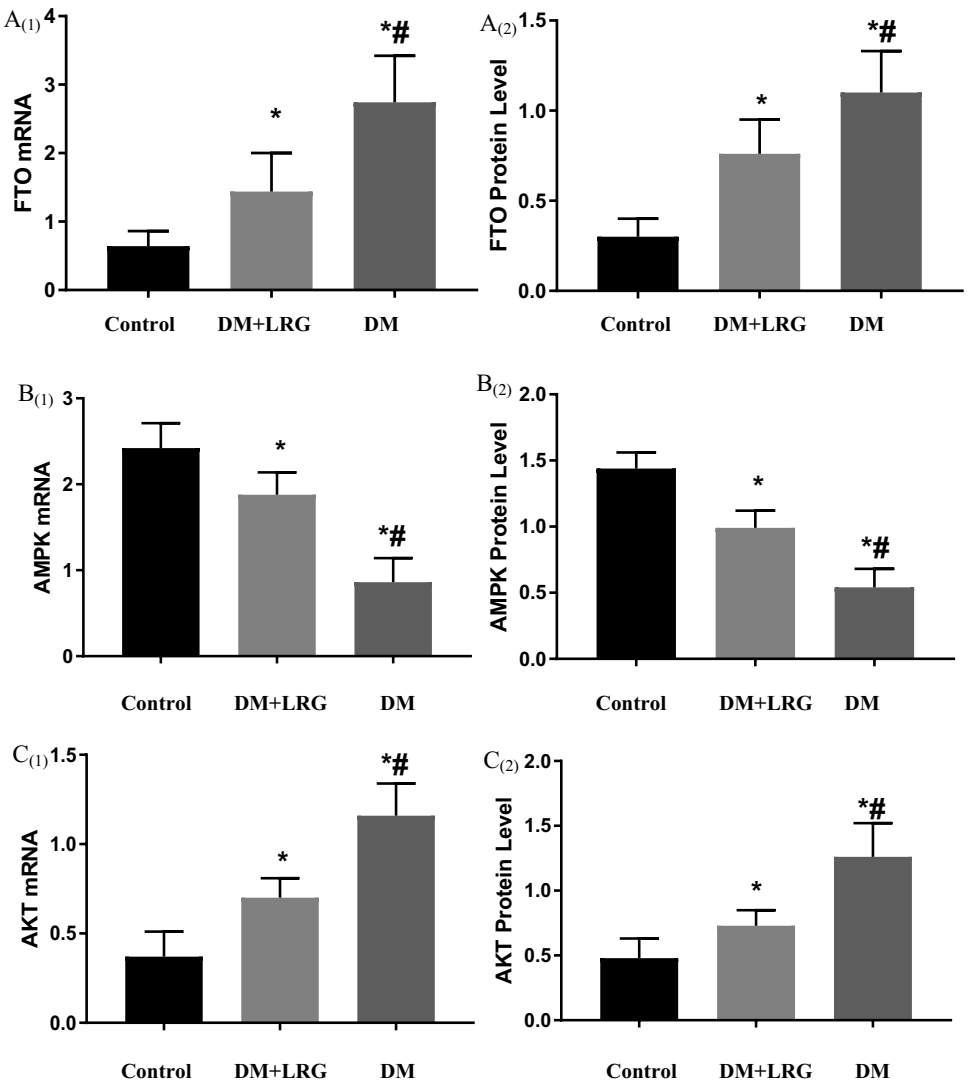

Fig. 3 mRNA and protein expression of FTO, AMPK, and AKT

Table 5 Protein expression ofFTO, AMPK and AKT

Group	Number	Protein FTO	Protein AMPK	Protein AKT
Control	10	0.30±0.10	1.44±0.12	0.48±0.15
DM+LRG	10	0.76±0.19*	0.99±0.13*	0.73±0.12*
DM	10	1.10±0.23*#	0.54±0.14*#	1.26±0.26*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *P<0.05 vs Control; #P<0.05 vs DM+LRG

suggests a mechanism whereby LRG may exert its effects in diabetic and obese rats. Many studies have shown that an allele at FTO, rs8050136, is closely related to insulin resistance, inflammatory factors, and obesity markers such as BMI and waist and hip circumference [14–16]. Similarly, a polymorphism at FTO rs9939609 is significantly associated with hyperlipidemia, obesity, and insulin metabolism, and this association is mediated by target tissue receptors. Moreover, activation of the SIRT-AMPK signaling pathway reduces the expression of fatty acid synthases and associated transcription factors, increases the oxidation rate of fatty acids, and regulates lipid and energy metabolism [17–19]. In a high-fat-induced obesity model in C57BL/6 mice, the activation of AMPK significantly reduced liver fat accumulation and prevented diabetes by inhibiting gluconeogenesis. Insulin-sensitive PI3K/AKT signaling affects glucose and lipid homeostasis in the body [20]. The PI3K/AKT pathway is also involved in the defocused low-energy shock wave

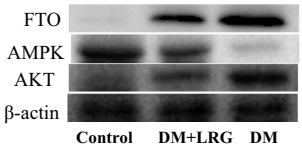

Fig. 4 Western blotting shows the expression of FTO, AMPK and AKT protein

in activated adipose tissue-derived stem cells [21]. Inhibition of the PI3K/AKT signaling pathway during liver lipid accumulation promotes hepatocyte autophagy and reduces liver steatosis in db/db mice [22]. In addition, an elevated expression of FTO has been detected in cancer cells, which may regulate cell metabolism and growth via the PI3K/AKT pathway, whereas FTO may be suppressed by the activation of AMPK [23].

Based on the abovementioned findings, the present study explored the expression of FTO, AMPK, and AKT in visceral fat tissue of diabetic obese rats. We found that body weight and visceral fat accumulation were significantly increased in diabetic rats compared to control rats ($p < 0.05$). In addition, blood glucose, triglyceride, total cholesterol, and LDL-C levels were significantly increased, while those of HDL-C were notably decreased ($p < 0.05$), in diabetic rats ($p < 0.05$). The FTO and AKT mRNA and protein levels were significantly elevated, while those of AMPK were decreased, in visceral fat of diabetic rats compared to that of control rats ($p < 0.05$). These findings suggest that increased FTO and AKT expression, and decreased AMPK levels in visceral fat, may affect fat accumulation in diabetic obese rats. LRG decreases blood sugar levels and may potentially lower apolipoprotein III and triglyceride levels, reducing the levels of lipoprotein particles in atherosclerotic lesions in patients with hyperglycemia [24]. In obese patients, LRG reduces the risk of cardiovascular events by restoring endothelial function [25, 26]. In addition, LRG could alleviate the metabolic status and vascular dysfunction of high-fat-induced obese mice by activating the PKA-AMPK pathway, improving their antioxidative capacity and effecting a protective role within the cardiovascular system [27]. Moreover, inhibition of AKT activity can decrease lipid accumulation [13]. AMPK can promote the dephosphorylation of AKT/PKB via PP2A and thereby affect the activity of AKT in MDA-MB-231 cells [28]. Treatment of diabetic and obese mice with LRG was shown to activate AMPK and inhibit AKT in visceral adipose tissue (composed of perinephric, epididymal, and omental fat), decrease visceral fat accumulation, and reduce body weight [13]. In the present study, we first found that increases in FTO and AKT expression, and reductions in AMPK levels may be involved in the visceral fat accumulation of diabetic and obese rats. Furthermore, we explored the effect of LRG treatment on metabolism and visceral fat tissue expression of FTO, AMPK, and AKT in diabetic and obese rats. We found that treatment with LRG significantly reduced body weight and visceral fat accumulation ($p < 0.05$); decreased blood glucose, triglyceride, total cholesterol, and LDL-C levels; and increased HDL-C levels in diabetic and obese rats ($p < 0.05$). Compared with diabetic control rats, the mRNA and protein levels of FTO and AKT were lower, and those of AMPK were higher, in LRG-treated diabetic rats ($p < 0.05$). These results suggest that LRG may reduce abdominal obesity in type 2 diabetic patients by activating AMPK and suppressing AKT and FTO expression in visceral fat tissue. However, the mechanisms whereby LRG alleviates visceral fat accumulation in type 2 diabetes via the FTO, AMPK, and AKT pathways require further investigation.

In summary, changes in the expression levels of FTO, AMPK, and AKT may affect visceral fat accumulation in diabetic conditions. Mechanically, LRG activates the AMPK pathway, while inhibiting the AKT pathway and decreasing FTO expression, thereby alleviating abdominal obesity in diabetic rats. These findings provide a theoretical basis for the prevention and treatment of type 2 diabetes and abdominal obesity.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Xinjiang Medical University (Xinjiang, China), and the procedure was strictly performed according to the relevant regulations.

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ORIGINAL ARTICLE

Protective effect and mechanism of *Schistosoma japonicum* soluble egg antigen against type 1 diabetes in NOD mice

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Abstract

Objective To study the effect and mechanism of *Schistosoma japonicum* soluble egg antigen (SEA) on protecting against type 1 diabetes, 4-week-old female BALB/c and NOD mice were divided randomly into four groups: BALB/c control, BALB/c treated with SEA, NOD control, and NOD treated with SEA.

Methods Treated mice were injected intraperitoneally with 50 μg of SEA twice a week for 6 weeks, and control mice received the same volume of phosphate-buffered saline. Blood glucose in all mice was determined weekly from 8 weeks of age. Flow cytometry was used to detect the percentages of regulatory T cells of splenocytes in each group. Enzyme-linked immunosorbent assays were used to detect the levels of interferon-γ, interleukin (IL)-2, IL-4, and IL-5 in splenic cell culture supernatants.

Results Compared with those of the NOD group, the blood glucose level and percentage incidence of diabetes in NOD mice treated with SEA decreased significantly. This indicated that SEA treatment prevented spontaneous type 1 diabetes. After SEA administration, the frequency of splenic regulatory T cells increased significantly, and the secretion of IL-4 and IL-5 by splenic cells increased.

Conclusions These results demonstrated that SEA can prevent type 1 diabetes by enhancing regulatory T cells and the T helper 2 cell immune response in NOD mice.

Keywords Soluble egg antigen · Type 1 diabetes · Regulatory T cells · Cytokines · NOD mice

Introduction

Diabetes is one of the biggest healthcare challenges of the twenty-first century. The number of diabetes is expected to reach 930 million by 2045, which increases the risk of microvascular and macrovascular diseases [1]. Diabetes can be categorized into type 1 diabetes (T1D), type 2 diabetes, gestational diabetes, and others. T1D is an autoimmune disease in which cells of the immune system destroy pancreatic β cells that secrete insulin. In recent decades, the prevalence of T1D has increased significantly. The first nationwide study in Serbia on the incidence of T1D showed that, in adolescents under 19 years of age, the incidence was 11.82/100,000 and that in the 0–14-year-old group, the incidence was 14.28/100,000, with an average annual increase in incidence of 5.9% [2]. A reduced exposure to parasitic worms and their strong influence on host immunity may be the cause of the increased incidence of T1D [3]. There is no cure for T1D, and the current treatment is to control blood sugar levels by daily or continuous subcutaneous insulin injections. There is an urgent need to find new treatments.

In recent decades, several parasitic worms and their by-products, including *Filaria*, *Heligmosomoides polygyrus*, *Fasciola hepatica*, and *Schistosoma*, have been reported to prevent or suppress T1D. Recombinant *Wuchereria bancrofti* L2 and *Brugia malayi* abundant larval transcript 2 proteins

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have been investigated individually, and in combination, for their therapeutic potential in streptozotocin-induced T1D [4]. In the streptozotocin-induced T1D mouse model, *H. polygyrus* infection has a protective effect on reducing islet size and hyperglycemia. It plays these roles through the independent actions of interleukin (IL)-10 and STAT6 [5]. At 30 weeks of age, 84% of NOD mice maintain normal blood glucose and are insulitis-free after the intraperitoneal injection of excretory/secretory products from *F. hepatica* [6]. In addition, the regulation of the immune response in *S. mansoni*-infected mice with *S. mansoni* egg deposition can inhibit autoimmune T1D induced by multiple low doses of streptozotocin [7]. *S. mansoni* also partially prevents the degradation of pancreatic islets and hyperglycemia in mice treated with multiple low doses of streptozotocin [8]. Soluble egg antigen (SEA) of *S. japonicum* is an antigen secreted by the eggs that can activate the immune system, sensitizing T cells through this secretion. Studies on the mechanisms of SEA-induced immune regulation may help to develop new therapies for inflammatory diseases. Our previous study demonstrated that *S. japonicum* SEA protects against type 2 diabetes in $Lepr^{db/db}$ mice by enhancing regulatory T cells and Th2 cytokines [9]. In the current study, we used NOD mice to study the effect and mechanism of *S. japonicum* SEA on the development of T1D.

Methods

Animals and SEA preparation

Four-week-old female BALB/c and NOD mice were obtained from the Model Animal Research Centre of Nanjing University and placed in a specific pathogen-free environment. All animals experiment protocols were approved by the Committee on Animal Research of Wuchang Hospital (No. 2018–0022). The preparation of SEA was based on a previous publication [10]. Briefly, for SEA, eggs were obtained from the livers of infected mice that were homogenized and washed with phosphate-buffered saline (PBS) on ice. Polymyxin B agarose beads (Sigma-Aldrich, St. Louis, MO, USA) were used for sterile filtration and endotoxin removal to < 1 EU/mg.

Immunization schedule and blood glucose detection

BALB/c and NOD mice were injected intraperitoneally with 50 μg of SEA twice a week for 6 weeks, and the same volume of PBS was used as control. Glucose was monitored weekly in caudal venous blood with an automatic glucose monitor (Roche), and the incidence of T1D in each group of mice was calculated. After 15 weeks of SEA administration, all mice were sacrificed to detect blood glucose.

Flow cytometry analysis of Tregs in spleen cells

To detect the percentage of Tregs, a single cell suspension of spleen cells was prepared. Cells were stained with a mouse Treg staining kit (eBioscience, San Diego, CA, USA) and analyzed by CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA). Cell suspensions were stained with the following antibodies: 1 μl of fluorescein isothiocyanate-labeled anti-mouse CD4, 1 μl of an allophycocyanin-labeled anti-CD25 monoclonal antibody, and 2 μl of phycoerythrin-labeled anti-mouse forkhead box p3 (Foxp3). The three target cell populations were then sorted by flow cytometry using a FACSCalibur (BD Biosciences). The appropriate isotype antibody came from the specific antibody of the same manufacturer.

Cytokine detection

Fifteen weeks after the last treatment, the spleen was removed from each mouse, and 5×10^6 cells/well were cultured in RPMI-1640 plus with 10% FCS and 1% penicillin and streptomycin (all from Sigma, St. Louis, MO, USA) for 72 h at 37 °C under 5% CO_2. SEA (5 μg/mL) was used to stimulate the growth and proliferation of spleen cells and the secretion of cytokines. Then, the supernatant was collected, and interferon (IFN)-γ, IL-2, IL-4, and IL-5 were measured according to the manufacturer's instructions of the enzyme-linked immunosorbent assay kits (eBioscience).

Statistical analysis

Data are expressed as means ± SD. SPSS 17.0 (IBM, Chicago, IL, USA) was used for statistical analyses. Variance analysis was used in each group. A value of $p < 0.05$ was considered statistically significant.

Results

The effect of SEA on T1D in NOD mice

Zaccone et al. [11] reported that SEA of *S. mansoni* had a complete preventive effect on T1D in NOD mice when injected at the age of 4 weeks. In the current study, 4-week-old female NOD mice were injected once a week for 6 weeks with 50 μg of SEA or an equal volume of PBS as control. SEA prevented T1D in NOD mice after six injections (Fig. 1). None of the mice injected with SEA had diabetes, while 70% of the control group had diabetes at 25 weeks of age in NOD mice ($p < 0.05$).

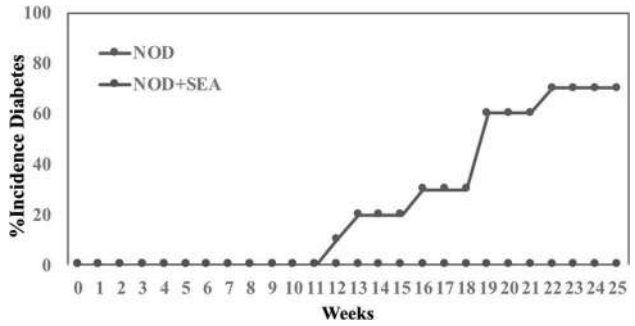

Fig. 1 Treatment of NOD mice with SEA of *S. japonicum* inhibits spontaneous type 1 diabetes. Four-week-old female NOD mice were injected with 50 μg *S. japonicum* SEA once a week for 6 weeks or an equal volume of PBS as control (n = 10 per group)

Effect of SEA on the frequency of Tregs in spleen cells

Because Foxp3 is a specific marker of Tregs, we used $CD4^+CD25^+Foxp3^+$ T cells to define Tregs. As shown in Figs. 2 and 3, compared with BALB/c mice, the frequency of Tregs decreased significantly in NOD mice. It was restored significantly even higher in NOD mice after SEA administration ($p < 0.05$).

Cytokine production by splenocytes after SEA administration

To determine the effect of SEA on cytokines, we used enzyme-linked immunosorbent assays to detect the levels of IFN-γ, IL-2, IL-4, and IL-5 in spleen cell supernatants. As shown in Fig. 4, the levels of the Th2-type cytokines, IL-4, and IL-5, in NOD mice treated with SEA, were significantly higher than those in the control NOD group ($p < 0.05$). However, there was no significant difference in the levels of the Th1-type cytokines, IFN-γ, and IL-2, among groups.

Discussion

T1D is an autoimmune disease caused by the immune destruction of islet cells, and NOD mice are an excellent animal model of T1D because about 80% of females and 20% of males develop hyperglycemia at 30 weeks of age. Several immunological aspects of human T1D are mimicked in the NOD mouse model, such as islet infiltration and destruction autoimmune insulitis (as early as 4 weeks) and spontaneous progression to overt diabetes in adults [12]. Schistosoma has been reported to be used for the treatment of T1D. Cooke et al. [13] published the first report showing that *S. mansoni*

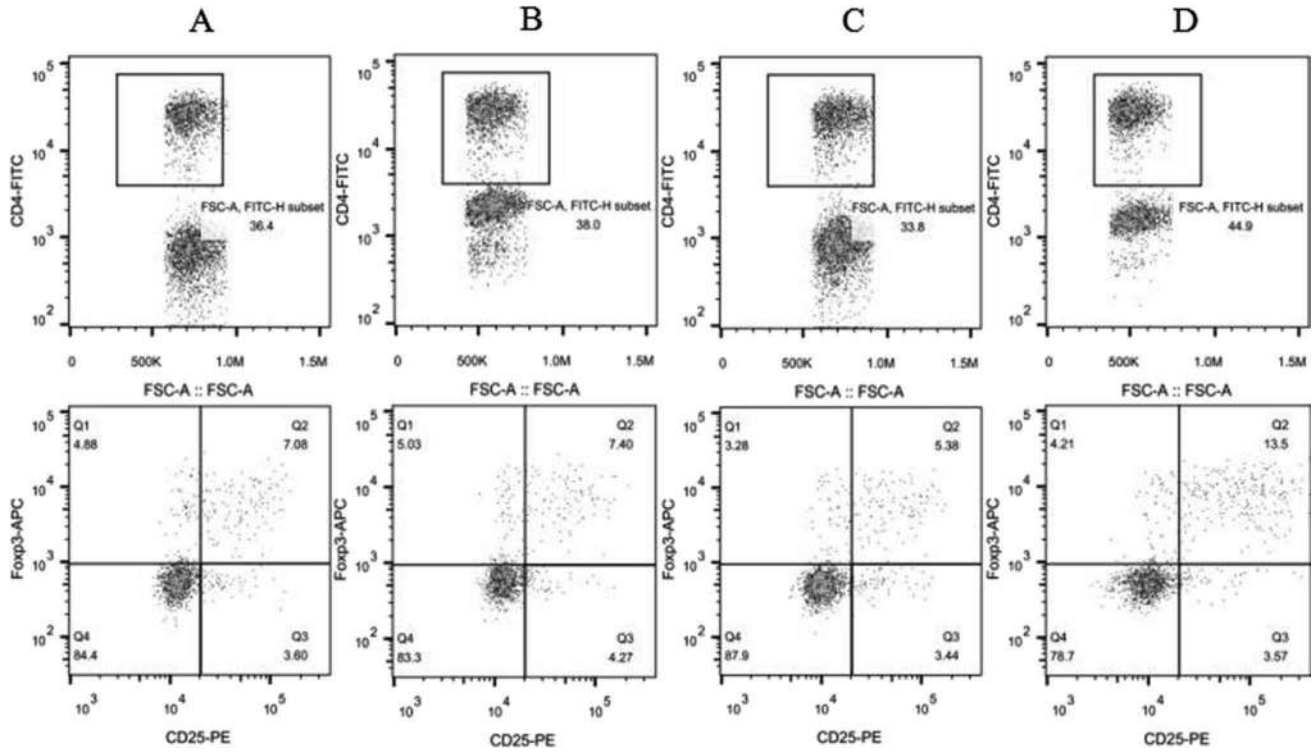

Fig. 2 Representative flow cytometry results of Tregs from one experiment. **A** BALB/c control; **B** BALB/c + SEA; **C** NOD control; **D** NOD + SEA. The upper panels show the frequency of $CD4^+$ T cells in spleen cells. The right upper quadrant in the lower panels shows the frequency of $CD25^+Foxp3^+$ T cells from $CD4^+$ lymphocytes

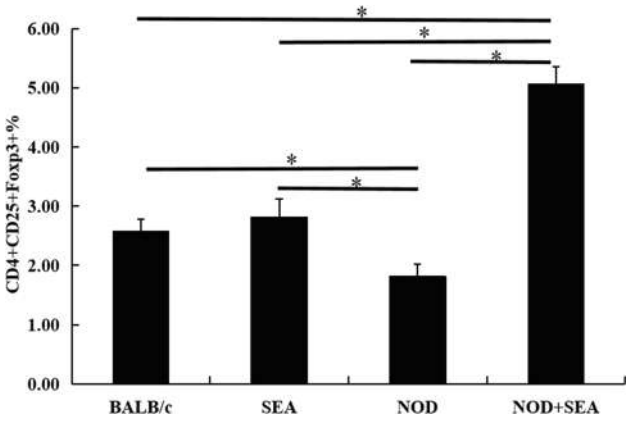

Fig. 3 The effect of SEA treatment (50 μg twice a week for 6 weeks) on the frequency of Tregs within total splenocytes. Data are shown as means ± SD. The experiment was done three times with 10 mice per group. $^*p < 0.05$

infection significantly reduced the spontaneous incidence of T1D in NOD mice and injecting parasite eggs only also prevented T1D. Worm-derived immunomodulatory products mimicking the beneficial immune regulation stimulated by worm infection open up potential new avenues for identifying novel T1D treatment strategies [14]. Soluble extracts of *S. mansoni* worms or eggs mediate potent protection in the NOD mouse but only if the injection is performed in 4-week-old animals [15]. In this study, we used *S. japonicum* SEA to treat 4-week-old NOD mice and demonstrated that, compared with the control NOD group, the percentage incidence of diabetes decreased significantly with SEA treatment. This suggests that *S. japonicum* SEA can prevent T1D in NOD mice.

In the NOD mouse, Treg deletion accelerates the progression of T1D, while Treg transfer prevents disease progression [16]. Kukreja et al. [17] showed that there was a lower frequency of Tregs during the onset of T1D or in individuals with established T1D than in healthy controls. A high dose of cyclophosphamide causes diabetes in the NOD mouse, which is related to a reduced number of Tregs [18]. To further study the mechanism by which SEA prevented T1D, we used flow cytometry to detect the percentages of Tregs in splenic lymphocytes from all groups and demonstrated that SEA induced the expansion of Tregs. El-Ahwany et al. [19]

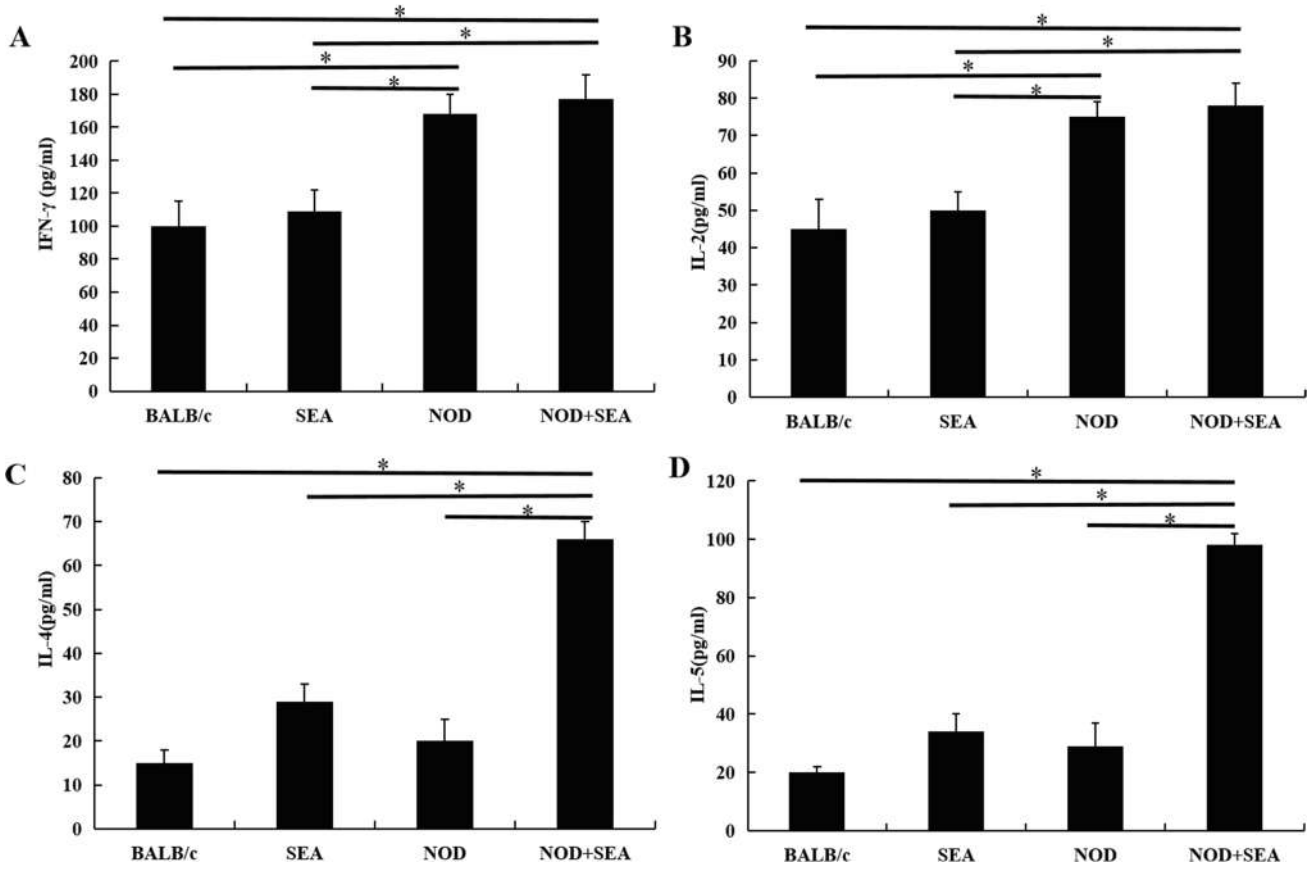

Fig. 4 Levels of interferon (IFN)-γ (**A**), interleukin (IL)-2 (**B**), IL-4 (**C**), and IL-5 (**D**) in spleen cell supernatants were determined by enzyme-linked immunosorbent assays. SEA was administered twice a week for 6 weeks. Data are presented as means ± SD from triplicate experiment. $^*p < 0.05$

reported that, after injection with multiple doses of SEA for 7 days before infection, the percentage of Tregs increased significantly in the immunized compared to the control group (infected but not SEA-treated) at 8 and 16 weeks post-infection. The *S. japonicum* stress protein, HSP60, derived from SEA, significantly induces Tregs in vivo and in vitro [20]. In addition, previous reports show that SEA induces Tregs in vitro and in vivo in murine models of asthma [21] and inflammatory bowel disease [22]. Based on the above findings, it is speculated that the therapeutic effect of SEA on NOD mice may be mediated by Tregs.

Because T1D is a Th1-mediated disease, it is presumed that an immune response tilted toward Th2, and the regulatory axis may inhibit the diabetogenic Th1 response. Kikodze et al. [23] reported that the production of Th1-type cytokines (IL-2 and IFN-γ) was associated with T1D, while Th2-type cytokines (IL-4 and IL-10) were associated with protection. A transgenic NOD mouse expressing IL-4 in the pancreatic islets was protected from the development of diabetes [24]. In this article, we reported that SEA induced significant increases in Th2-type cytokines (IL-4 and IL-5). Therefore, SEA may improve T1D by inducing Th2 immune responses. A previous study reported that SEA injected into mice induced a Th2 immune response [25]. In addition, omega-1 (a glycoprotein from *S. mansoni* eggs) induced the rRNAs and mRNAs that tilt the immune response toward a Th2 distribution [26]. Maron et al. [27] evaluated the cytokine profiles and proliferative responses of lymphocytes in the NOD mouse after oral administration of insulin β-chains with or without *S. mansoni* SEA. The authors demonstrated that Th2 responses to oral insulin could be enhanced synergistically by providing both SEA and the insulin β-chain. In addition, SEA treatment increases the bioactivity of transforming growth factor-β, which is important not only for Treg expansion, but also for the successful Th2 response to SEA [28]. Overall, our results suggest that SEA of *S. japonicum* can be used to treat T1D by enhancing Tregs and Th2-type immune responses.

The use of helminthic antigens as treatment for diabetes by immunoregulation is still at a very nascent hypothetical stage, but more work may eventually shed more light on this theory. Jackson-Thompson et al. [29] demonstrated that *Axenic Caenorhabditis elegans* antigen protects against the development of type-1 diabetes in NOD mice increases in total IgE and total IgG1, consistent with induction of a type 2 immune response similar to that typically seen in parasitic worm infection. The administration of recombinant *Schistosoma japonicum* cystatin or fructose-1, 6-bisphosphate aldolase, significantly reduced the diabetes incidence and ameliorated the severity of T1D by increasing Tregs [30]. This work is definitely a step in strengthening the hypothesis in that direction, and the mechanism was needed to be further elucidated.

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Declarations

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

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SHORT ARTICLE

What drives glycemic control in a person living with diabetes?

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Abstract

Background Glycemic control has remained an enigma despite a large number of anti-diabetic drugs being available. Clinical practice and guidelines have focussed largely on optimal and rational use of anti-diabetes drugs to achieve care goals. This study aims to delineate factors impacting glycemic control from real-world data.

Methods Retrospective, cross-sectional data comprising 15,689 prescriptions from 4647 people living with diabetes and attending an endocrine clinic over a 5-year period was extracted from EMR (electronic medical records) of the clinic. Data pertaining to drugs prescribed, glycemic control attained and patient behavioral factors like diet, drug and exercise adherence was analysed to delineate the contribution of patient or care team-dependent factors towards glycemic control.

Results Factors related to patient behavior affected glycemic control linearly with statistically lower HbA1c in people with better adherence to diet, medications and exercise. People who did any self-monitoring of blood glucose at home had significantly better glycemic control. On the other hand, a number of medications were negatively associated with glycemic control. Hypoglycemia had no impact on glycemic control. A number of visits to treating physician were positively associated with glycemic control but impact plateaued after 6 visits.

Conclusion Patient behavior and activation-related factors are predominant drivers of glycemic control.

Keywords Diabetes mellitus type 2 · Glycemic control · Behavioral determinants · Patient factors

Introduction

In developing countries, including India, chronic disease care is an out of pocket expense for patients [1]. Diabetes care in these countries is delivered largely by primary care physicians and a smaller number of specialists. Clinical care is typically confined to medication prescription with minimal emphasis on patient education and improving health-related behavior. Understanding factors influencing glycemic control in people living with diabetes can pave the way for devising strategies for better diabetes care. This is especially important in India as, in our population, diabetes happens at an earlier age and has already reached epidemic proportions. This manuscript represents a retrospective analysis of data on factors responsible for driving glycemic control. The aim of this study was to delineate factors in a patient's domain or physician's domain impacting glycemic control in real-world situations.

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Material and methods

Retrospective, cross-sectional data from electronic medical records (EMR) was extracted from an endocrine practice in a highly urbanised area of national capital territory. Practice employs an EMR software "Healthvriksh EMR, version 1 (Kalpavriksh Healthcare, Delhi, India)" for recording patient visits and generating prescriptions. Data of patients visiting from May 2015 to Feb 2020 was extracted and analysed. Additional feature to record behavioral parameters of patients was introduced in March 2018

and subsequent patient visits had an option of recording behavioral factors displayed by the patient in the period since the last patient visit. These behavioral questions are asked by treating endocrinologist directly from patients and recorded. Recording of these behavioral parameters is organised in an objective way on a scale of 4–5.

Factors influencing the attainment of glycemic control can largely be divided into three broad categories viz. (1) factors driven by the care team, i.e. number of medications and incidence of hypoglycemia; (2) factors driven by patient, i.e. dietary adherence, physical activity, self-monitoring of blood glucose, compliance to diabetes medications and follow-up with treating physician and (3) factors influenced by disease processes like duration of diabetes.

Disease characteristics impact glycemic control indirectly via the actions of the care team and patient. For example, the number of medications prescribed increases with the duration of diabetes. This observation is reported from a smaller subset of current data and published previously [2]. In the current report, we analyse the relative importance of actions taken by both stakeholders. Data on age, sex, body mass index, HbA1c, nature and number of medications used, incidence of hypoglycemia at each patient visit and bahavioural factors (diet adherence, exercise adherence, compliance to medications and self-monitoring of blood glucose levels and monitoring habits) was accessed and analysed. HbA1c is used as a measure of glycemic control. As glycemic control varies even in an individual patient over time, the decision was made to analyse data at the prescription level except for the effect of the number of visits on the last HbA1c level.

Data processing and descriptive analysis were done using Google analysis tools. For trends, ordinal logistic regression was done using open-source software "Jamovi" (Version 0.9).

Results

We analysed diabetes prescriptions from 4647 patients under the care of a private endocrine practice. The mean age of these patients was 52.58 ± 12.55 years with 2715 men (mean age 51.51 ± 13 years) and 1930 women (mean age 54.08 ± 11.72 years). A total of 4647 patients followed with a variable number of visits with an endocrinologist (mean number of visits 3.14 ± 3.74, median 2 (range 1–39; 25th percentile 1; 75th percentile 4) and this resulted in 15,689 prescriptions being generated over a span of 5 years. Out of these 15,689 prescriptions, 8557 prescriptions had information on HbA1c. In a previous study by authors, the duration of diabetes correlated with the number of medications being prescribed to a patient [2].

We analysed various factors that can have bearing on glycemic control achieved in people living with diabetes viz. number of medications and visits to the doctor.

Furthermore, 3861 diabetes prescriptions also had information regarding patients' behavioral factors in the preceding 3 months viz. compliance to diet, compliance to physical activity, compliance to drugs and occurrence of hypoglycemia. The impact of these factors on glycemic control was then analysed. Out of these 3861 prescriptions, 2434 prescriptions had HbA1c reading recorded.

Results are depicted in Table 1.

Discussion

Glycemic control for all people with diabetes is still a distant dream in clinical practice with ~ 50% of people achieving the HbA1c goal in the USA and mean HbA1c in India being in the range of 8–9% [3–5]. In this manuscript, we analysed retrospective data from a clinic's electronic medical records to assess factors impacting glycemic control.

Care team factors

More number of prescription medications were associated with worse HbA1c. This indicates that poor glycemic control is being chased by medication additions without much success. Similarly, previous studies in the literature have shown no improvement in glycemic control on therapy escalation [6, 7]. Hypoglycemia is considered a great barrier in achieving glycemic control [8]. However, in the current analysis, no degree of hypoglycemia was associated with any improvement or worsening of glycemic control. In fact, 90% of people reported to have no hypoglycemia symptomatically or on self-monitoring of blood glucose. This may represent the very conservative nature of practice vis-a-vis medication prescription.

Patient factors

Dietary and exercise adherence has been shown to be consistently associated with good glycemic control [9]. In the current analysis, 52.5% of diabetes prescriptions had good adherence to dietary recommendations. In this population, diet recommendations provided to them are very non-prescriptive and just do's-and-don'ts. This is in contrast to dieticians who provided diet plans that have poor compliance rates [10]. Exercise compliance was found to be good in 41.9% of the diabetes prescriptions. This compliance rate is also higher than 19.5% compliance reported in urban communities from southern India [11]. All the grades of compliance were associated with progressive improvement in glycemic control.

Table 1 Results of analysis of various factors affecting glycemic control

Care team domains	Prescription medicines	Number of medications	0–1	> 1–2	> 2–3	> 3	-	For trend: $p < 0.001$ Odds ratio 1.18. (95% CI; 1.16–1.21)
		HbA1c ± SD	7.59 ± 1.84	7.91 ± 1.67	8.30 ± 1.74	8.43 ± 1.50	-	
		n = 8556 prescriptions with HbA1c	3236	3190	1871	259	-	
	Hypoglycemia incidence	Hypoglycemia incidence (BG values < 70 mg/dl)	Daily	Once a week or more	Once a month or more	Rarely	Never	For trend: *p = 0.931*
		HbA1c ± SD	7.36 ± 1.36	7.35 ± 1.42	7.25 ± 1.41	7.29 ± 1.29	7.76 ± 1.71	
		n = 2271 diabetes prescriptions	5	49	106	61	2050	
Dual domain factors	Follow-up visits	Number of appointments	Number of appointments < 4	Number of appointments 4–6	Number of appointments > 6	Number of appointments > 9	-	For trend (results for < 4, 4–6, > 6 visits): $p < 0.001$ Odds ratio 0.72. (95% CI; 0.69–0.75)
		HbA1c ± SD	8.63 ± 2.06	7.62 ± 1.63	7.48 ± 1.38	7.46 ± 1.33	-	
		n = 3142 patients	1879	639	624	325	-	
Patient domains	Diet adherence	Diet compliance	Taking sugars daily	Sugars on and off and sweets > once a week	No sugars but on and off sweets < once a week	Sweets < once a month	Full compliance	For trend: $p < 0.001$ Odds ratio 0.65. (95% CI; 0.62–0.68)
		HbA1c ± SD	9.18 ± 2.04	8.64 ± 1.95	7.85 ± 1.55	7.29 ± 1.40	6.50 ± 0.80	
		n = 2433 diabetes prescriptions	243	243	662	1277	8	
	Exercise adherence	Exercise compliance	No exercise	No dedicated exercise but moderate NEPA	< 150 min/week exercise or good NEPA	150–200 min/week spread over at least 5 days	Exercise Compliance > 200 min/week	For trend: $p < 0.001$ Odds ratio 0.68. (95% CI; 0.65–0.71)
		HbA1c ± SD	8.78 ± 1.95	7.91 ± 1.69	7.41 ± 1.40	7.31 ± 1.42	7.26 ± 1.21	
		n = 2433 diabetes prescriptions	571	380	462	837	183	
	Medication compliance	Medication compliance	Misses regularly	Misses occasionally	Never misses	-	-	For trend: $p < 0.001$ Odds ratio 0.67. (95% CI; 0.63–0.71)
		HbA1c ± SD	8.96 ± 1.86	7.93 ± 1.71	7.46 ± 1.47	-	-	
		n = 2318 diabetes prescriptions	282	147	1889	-	-	
	Self-monitoring of blood glucose (SMBG)	SMBG	No	Yes	-	-	-	$p < 0.001$
		HbA1c ± SD	8.39 ± 1.87	7.47 ± 1.52	-	-	-	
		n = 2420 diabetes prescriptions	775	1645	-	-	-	

Compliance with medications is reported to be much better in the Indian population as compared to western studies [12–14]. Compliance to medications in the current study population was 78.15% and compliance improved glycemic control linearly.

Self-monitoring of blood glucose has been shown to result in lower HbA1c in previous studies and the same fact is reasserted in the current study with ~ 0.9% lower HbA1c in people who practice any SMBG as compared to those who do it at all.

Factor in dual domain

Number of follow-ups is at crossroads of domains of care team and patient. The decision to visit is taken by the patient but it allows the care team to make an impact. The number of appointments with endocrinologists positively correlated with glycemic control most of the effect coming in the first 6 appointments and plateauing after that. In a study from the Kaiser Permanente Northern California Diabetes Registry, pharmacologically treated patients missing more than 30% of scheduled visits had a HbA1c value 0.7 points higher relative to those with perfect attendance [15]. In developed countries, doctor visits and medicine refilled are generally by either national health systems or insurance. Even in these countries, loss to follow-up is a significant issue with 4 to 68% of people missing their scheduled appointments [16, 17]. In India, doctor visits and medicine refills are out of pocket expenses. Though clinic EMR has a reminder feature to alert patients 5 days before the date of appointment, the rate of follow-up remains dismal as 70% of patients in this population followed for less than 4 visits. There is > 1% difference in HbA1c between people following for less than 4 appointments and those following for > 6 appointments.

The limitation of this study included the recording of behavioral factors based on history provided by patients and there had been no verification based on any monitoring device or medication/glucometer strip fulfillment. The strength of this study lies in recording behavioral factors by treating the physician himself at the time of appointment and therefore minimal chance of any misinterpretation.

Conclusion

This analysis of large retrospective real-time data firmly establishes the patient-related factors as firm drivers of glycemic control. The number of medications increases with the duration of diabetes and increased HbA1c but their contribution to glycemic control seems secondary to patient attitude and activation. An activated patient in partnership with their physicians can achieve better glycemic control even with lesser medications. Thus, in clinical practice, equal emphasis needs to be placed on patient education and empowerment as is placed on medication prescription. So, the answer to "What drives glycemic control in a person living with diabetes?" is patient attitude and activation.

Author contributions Rajiv Singla (R.S.), Geetu Gupta (G.G.) performed the research. R.S. and Yashdeep Gupta (Y.G.) designed the research study. R.S. analyzed the data. R.S., G.G., and Y.G. wrote the paper. All authors critically edited and endorsed the manuscript.

Data availability (data transparency) Available with authors on request.

Declarations

Ethics approval Retrospective study, ethics approval not needed.

Consent to participate Retrospective study.

Consent for publication Retrospective study.

Conflict of interest The authors declare no competing interests.

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https://doi.org/10.1007/s13410-021-01043-2

CORRECTION

Correction to: Genetic association of vascular endothelial growth factor (VEGF) gene variants with the risk for diabetic retinopathy: a meta-analysis

Sanober Kafeel[1] · Khan Muhammad Nangrejo[2] · Roberto Gonzalez-Salinas[3]

Published online: 3 January 2022

Correction to: International Journal of Diabetes in Developing Countries (2021) 41:180–188
https://doi.org/10.1007/s13410-020-00874-9

Out of them, n=189 studies contained duplicated data.

Flowchart represents the assessment criteria of inclusion and exclusion.

The original article can be found online at https://doi.org/10.1007/s13410-020-00874-9.

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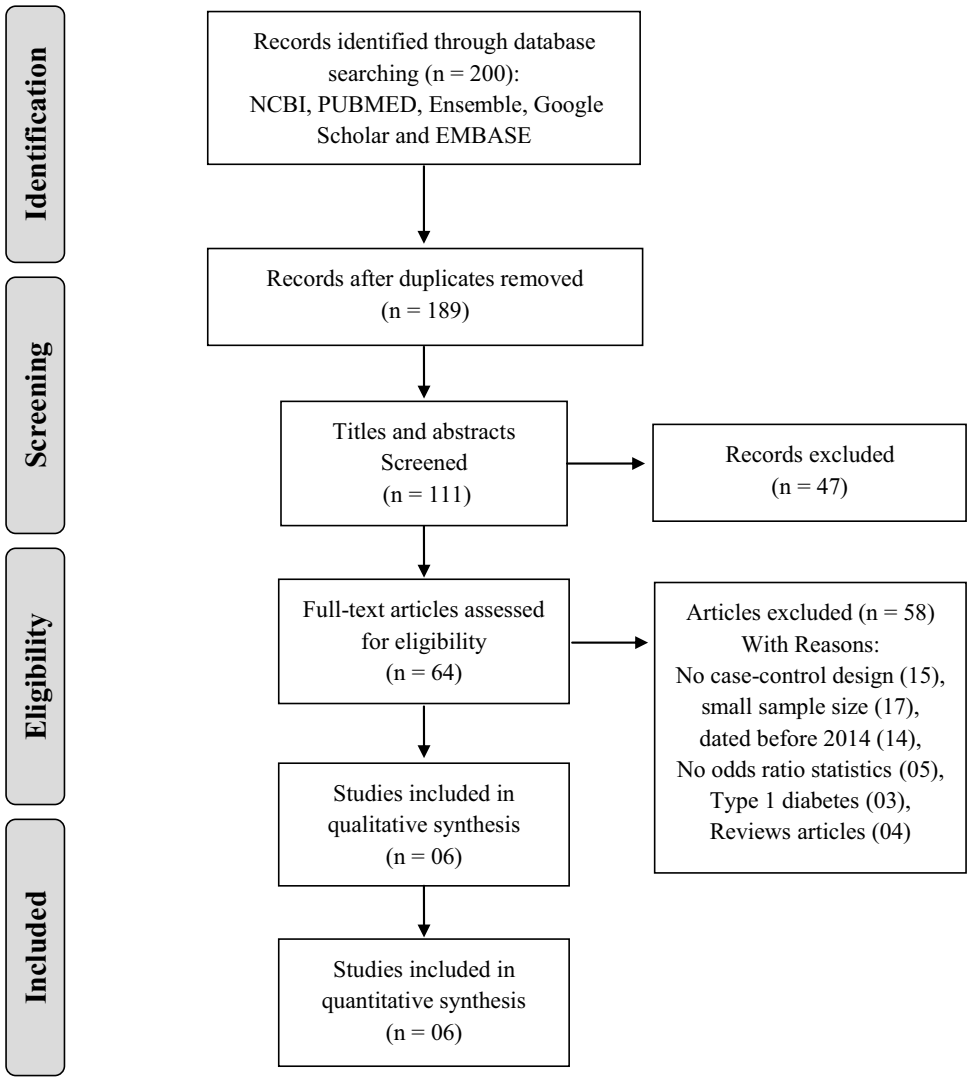

VISION STATEMENT

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

NEW EXECUTIVE COMMITTEE AND OFFICE BEARERS 2021-2022

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TRAINEE GRANTS (Up to 10 grants)

Research Grants upto INR 200000 to support outstanding thesis/ research work by first year MD/DNB/ PHD students/Research fellows from India.

Eligibility Criteria
All Postgraduates in First year MD, DM /DNB from any of the institutions in the country are eligible to apply

How to apply?
Send in your Research proposals by email to the RSSDI Secy/ Chairman research committee by email/ apply directly on web site.

Research proposal should have following proofs-

1. A supporting letter from your guide/ head of department stating that this is a bonafide project for your thesis and also mentioning the dates of you joining the program and expected date of graduation. The guide must also state that he/she will stand guarantee for the work done
2. A detailed budget
3. Thesis proposal approved by the department/appropriate institutional authority
4. Approval by the ethics committee

Selection Process
Proposals will be reviewed by the research committee of the RSSDI.

Disbursement of Grant
A minimum of 50% of the grant amount will be disbursed initially. Further disbursement will be done annually based on submission of progress reports on the work done and utilisation of sanctioned amount. These reports must be filed to the secretary of the RSSDI

Responsibility:
All grant awardees are expected to present their work at RSSDI Annual Conference during research presentation's session. Failure to file progress reports annually and when requested by the RSSDI and failure to present progress at RSSDI Annual conf may result in the forfeiture of the grant.

All awardees are expected to follow the tenets of responsible and ethical conduct of research. Unethical or fraudulent use of RSSDI research funds will warrant adverse action from the society including

forfeiture of grant, black listing in the society's databases and other legal recourses that are available to the society.

Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

All awardees are encouraged to submit their work to the RSDDI Journal IJDDC

CALL for RESEARCH PROPOSALS for GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI

The proposals may of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization, of funding sought from RSSDI

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
All applications should be addressed to:
1. The Secretary , RSSDI
2. Soft copy of the research proposal should be sent to Secretary, RSSDI

When to apply
Proposals will be accepted Twice a year. Once between 1st Jan - 31st April & then July 1st to 30th Nov.

All research proposals will be reviewed by Research committee over a period of 4-6 weeks & approved proposals will be provided Research Grant after fulfilling all documentation by 30th June & then 31st December of each year.

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health-care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmacahri Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh
22.	Galaxy Speciality Centre	Sodala, Jaipur

carefully looked into all aspects of this course & has accredited & recognized 22 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

COURSE DETAILS

Name of the Course: Advanced Certificate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given !

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

Session: Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

ANNOUNCEMENTS

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

RSSDI 50th Golden Jubilee Year Celebrations
(look out for more details on our website)

RSSDI JNU certificate course in Diabetes:

Last date of submission of Application Form - 30th June 2022
Screening Interview - 7th July 2022
Declaration of Exam Result - 10th July 2022
Last date of payment of course fee - 15th July 2022
Commencement of course - 16th July 2022
Prospectus release date 15th May 2022

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