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EDITORIAL

Teneligliptin: A potential therapeutic approach for diabetic cardiomyopathy

Ashraf Al Madhoun

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Abstract

In this editorial, we comment on the article by Zhang *et al*. Diabetes mellitus is a chronic disorder associated with several complications like cardiomyopathy, neuropathy, and retinopathy. Diabetes prevalence is increasing worldwide. Multiple diabetes medications are prescribed based on individual patients' needs. However, the exact mechanisms by which many of these drugs exert their protective effects remain unclear. Zhang *et al* elucidates molecular mechanisms undelaying cardioprotective effect of the dipeptidyl peptidase-IV inhibitor, teneligliptin. Briefly, teneligliptin alleviates the activation of NOD-like receptor protein 3 inflammasome, a multiprotein complex that plays a pivotal role in regulating the innate immune system and inflammatory signaling. Suppression of NOD-like receptor protein 3 inflammasome activity reduces the expression of cytokines, oxygen radicals and inflammation. These findings highlight teneligliptin as an anti-diabetic cardioprotective reagent.

Key Words: Teneligliptin; Diabetes mellitus; NOD-like receptor protein 3 inflammasome; Inflammation; Cardiomyopathy

Core Tip: Zhang *et al* provided evidence that teneligliptin mitigated diabetic cardiomyopathy. The authors also clarified the undelaying molecular mechanisms, showing that teneligliptin inhibits NADPH oxidase 4, NOD-like receptor protein 3 inflammasome and activates activated protein kinase to maintain myocyte homeostasis. Researchers are encouraged to implement similar studies on humans to delineate the precise mechanism by which teneligliptin influences activated protein kinase and NOD-like receptor protein 3 signaling.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia. DM increases the risk of microvascular and macrovascular complications including heart disease, retinopathy, neuropathy, and nephropathy. DM is a global health crisis affecting an estimated 425 million individuals worldwide[1]. Diabetic cardiomyopathy (DCM) is a serious complication of DM that affects approximately 12% of patients with DM and leads to heart failure and death[2]. DCM is a chronic progressive myopathy characterized by ventricular chamber dilation and myocyte hypertrophy, leading to impaired systolic function and heart failure[3]. The exact mechanisms underlying DCM are not fully understood; however, chronic hyperglycemia is believed to play a role. One critical pathway implicated in DCM development is the NOD-like receptor protein 3 (NLRP3) inflammasome. The NLRP3 inflammasome is a multiprotein complex that activates inflammatory responses. Recent studies have demonstrated that NLRP3 inflammasome activation contributes to DCM by promoting inflammation and cell death in the heart[4,5].

In the context of glucose homeostasis, dipeptidyl peptidase-4 (DDP-4) plays a key role in the regulation of incretin hormones, including glucagon, glucagon-like peptide (GLP)-1, GLP-2, and gastric inhibitory peptide[6]. These incretins are secreted into the bloodstream and stimulate postprandial insulin secretion and peripheral glucose uptake. The inactivation of these incretins *via* dipeptidyl peptidase-4 (DPP-4) digestion is essential for maintaining glucose homeostasis[7]. In DM characterized by impaired incretin function, DPP-4 inhibition is a therapeutic strategy to enhance incretin activity and improve glycemic control.

DPP-4 inhibitors or gliptins are a class of oral antihyperglycemic drugs that suppress DDP-4 activity, leading to elevated and sustained incretin levels[8]. Several gliptins, including sitagliptin, saxagliptin, linagliptin, alogliptin, and vildagliptin, have been approved for clinical use in Western countries to manage type 2 DM management[9]. Teneligliptin, anagliptin, and trelagliptin are gliptins approved in some Asian markets, but are not yet widely available globally[10]. Omarigliptin was initially used in Japan and is currently undergoing further evaluation in clinical trials[11,12].

Although extensive research has established the efficacy of DPP-4 inhibitors in the management of DM, a comprehensive comparison of all the available gliptins is beyond the scope of this editorial. Here, we focus specifically on the article by Zhang *et al*[13] published in the recent issue of the *World Journal of Diabetes* (PMID: 38680706, DOI: 10.4239/wjd.v15.i4.724) elucidating the potential therapeutic role of teneligliptin in DCM and its unique characteristics compared to other established gliptins.

TENELIGLIPTIN: POTENTIAL THERAPEUTIC ADVANTAGES

Teneligliptin is an orally administered medication belonging to the class of DPP-4 inhibitors. Compared to other DPP4 inhibitors, teneligliptin is relatively cost-effective and economical for patients[14]. Although it shares similar efficacy and safety profiles with other gliptins, teneligliptin exerts unique pharmacokinetic and pharmacodynamic properties owing to its distinct chemical structure. Classified as a class III DPP-4 inhibitor, teneligliptin exhibits a stronger binding affinity to the enzyme than other class II gliptins[15]. This strong binding is attributed to the formation of an additional subsidized bond with DPP-4, potentially leading to more extensive and longer-lasting DPP-4 inhibition[16,17]. However, large-scale clinical trials are needed to investigate whether this translates into significant advantages in glycemic control compared to other gliptins. While a recent meta-analysis suggested favorable efficacy and acceptable safety of teneligliptin compared to other DDP-4 inhibitors, further well-designed clinical trials are warranted to definitively confirm these findings[18]. Interestingly, recent research has suggested that teneligliptin may have additional benefits beyond glycemic control. Previous studies have reported that teneligliptin exerts anti-inflammatory and protective effects on myocardial and neuronal cells[19-22]. These findings suggest that teneligliptin is a potential therapeutic agent for the management of DCM.

INVESTIGATING TENELIGLIPTIN'S EFFECTS ON DCM

To explore the potential therapeutic effects of teneligliptin on DCM and delineate the associated molecular mechanisms, Zhang *et al*[13] conducted a study on streptozotocin-induced diabetes in mice. In this study, one group of diabetic mice was treated with teneligliptin (30 mg/kg) and the other group served as a control. Notably, teneligliptin treatment alleviated the myocardial hypertrophy phenotype observed in streptozotocin-induced diabetic mice, improved heart function parameters, and reduced the cardiomyocyte damage markers (creatine kinase-MB, aspartate transaminase, and lactate dehydrogenase). Mechanistically, the study revealed that teneligliptin inhibited NADPH oxidase 4 and NLRP3 inflammasome activation and the subsequent release of reactive oxygen species and interleukin 1β – key inflammatory

molecules - in diabetic mice.

IN-VITRO CONFIRMATION: TENELIGLIPTIN PROTECTS CARDIOMYOCYTES

To strengthen these findings, Zhang *et al*[13] conducted additional experiments using isolated primary mouse cardiomyocytes. These cells were exposed to high glucose (HG) conditions mimicking the diabetic environment, with or without teneligliptin treatment. Notably, HG exposure triggered NLRP3 inflammasome activation in cardiomyocytes. Teneligliptin treatment effectively suppresses NLRP3 inflammasome activation in these cells. Moreover, teneligliptin significantly reduced the levels of creatine kinase-MB, aspartate transaminase, and lactate dehydrogenase in HG-treated cardiomyocytes, suggesting that it protected against cell damage.

Interestingly, this study also revealed that the beneficial effects of teneligliptin on cardiomyocytes were mediated by the activation of activated protein kinase (AMPK), a cellular energy sensor that plays a crucial role in maintaining metabolic homeostasis. Researchers have found that teneligliptin increases the levels of phosphorylated AMPK, an activated form, in cardiomyocytes exposed to HG levels. Furthermore, blocking AMPK signaling using compound C, a specific inhibitor, abolished the protective effects of teneligliptin[13]. These findings suggested that AMPK activation is a key mechanism underlying the cardioprotective effects of teneligliptin.

PROMISING POTENTIAL OF TENELIGLIPTIN FOR DCM

This preclinical study provides compelling insights into the potential use of teneligliptin in DCM. This study suggests that teneligliptin may exert its effects through the inhibition of NADPH oxidase 4 and the NLRP3 inflammasome, and the activation of AMPK, all of which are key players in reactive oxygen species formation and inflammation[13]. To translate these findings into clinical applications, further research is needed to validate these mechanisms in human patients with DCM, and to elucidate the precise pathways through which teneligliptin influences AMPK and NLRP3 signaling for a complete understanding of its therapeutic potential.

BROADENING THE SCOPE OF TENELIGLIPTIN RESEARCH

While teneligliptin effectively lowers hemoglobin A1c, still need to be clarify the direct trophic effects on β-cell function and mass restoration, or its potential immunomodulatory effects during and after drug administration. This is a crucial aspect, as it could pave the way for type 1 DM intervention trials aimed at better disease management and prevention.

Because teneligliptin is a relatively new DPP-4 inhibitor studied primarily in Asian populations, large-scale multiethnic trials are warranted. These trials should assess the effectiveness of teneligliptin compared to other DPP-4 inhibitors, both as monotherapy and in combination therapy with other DM medications. Additionally, studies should identify the genetic and/or clinical factors influencing patient responses to teneligliptin, enabling personalized treatment approaches, and assessing the causative factors for rare side effects reported with DPP-4 inhibitors. Exploring the potential benefits of teneligliptin in high-risk populations, such as prediabetics and those with metabolic syndrome, is also of interest, considering the current limited focus on patients with DM in clinical trials.

LONG-TERM STUDIES AND INTERNATIONAL COLLABORATION

Long-term follow-up studies utilizing proteomics and metabolomics are necessary to understand the impact of teneligliptin on long-term complications such as neuropathy, nephropathy, and liver disease. Finally, we believe that the involvement of international societies and organizations would be highly beneficial. This collaboration could lead to the implementation of guidelines and a consensus for safer use of teneligliptin and other DPP-4 therapies in managing DM and its associated complications.

CONCLUSION

Zhang *et al*[13] suggested that teneligliptin, a drug for diabetes, may have therapeutic potential in DCM; however, further research is required. In mice, teneligliptin improves heart function and reduces damage markers, potentially by inhibiting inflammatory pathways. Human trials are necessary to confirm these findings and determine the optimal dose of teneligliptin for DCM. Addressing these future directions will bridge the gap between this preclinical study and clinical applications, allowing researchers to explore the full potential of teneligliptin for DCM and potentially broaden diabetes management.

FOOTNOTES

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EDITORIAL

Diabetic cardiomyopathy: Importance of direct evidence to support the roles of NOD-like receptor protein 3 inflammasome and pyroptosis

Lu Cai, Yi Tan, Md Shahidul Islam, Michael Horowitz, Kupper A Wintergerst

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Abstract

Recently, the roles of pyroptosis, a form of cell death induced by activated NOD-like receptor protein 3 (NLRP3) inflammasome, in the pathogenesis of diabetic cardiomyopathy (DCM) have been extensively investigated. However, most studies have focused mainly on whether diabetes increases the NLRP3 inflammasome and associated pyroptosis in the heart of type 1 or type 2 diabetic rodent models, and whether various medications and natural products prevent the development of DCM, associated with decreased levels of cardiac NLRP3 inflammasome and pyroptosis. The direct link of NLRP3 inflammasome and associated pyroptosis to the pathogenesis of DCM remains unclear based on the limited evidence derived from the available studies, with the approaches of *NLRP3* gene silencing or pharmaceutical application of *NLRP3* specific inhibitors. We thus emphasize the requirement for more systematic studies that are designed to provide direct evidence to support the link, given that several studies have provided both direct and indirect evidence under specific conditions. This editorial emphasizes that the current investigation should be circumspect in its conclusion, *i.e.*, not overemphasizing its role in the pathogenesis of DCM with the fact of only significantly increased expression or activation of NLRP3 inflam-

masome and pyroptosis in the heart of diabetic rodent models. Only clear-cut evidence-based causative roles of NLRP3 inflammasome and pyroptosis in the pathogenesis of DCM can help to develop effective and safe medications for the clinical management of DCM, targeting these biomarkers.

Key Words: Diabetic cardiomyopathy; Nucleotide oligomerization domain; NOD-like receptor protein 3 inflammasome; Cardiac cell death; Pyroptosis

Core Tip: The involvement of the NOD-like receptor protein 3 (NLRP3) inflammasome and pyroptosis in the pathogenesis of diabetic cardiomyopathy (DCM) has been extensively explored. However, most studies focused on whether diabetes causes NLRP3 inflammasome activation and pyroptosis in the diabetic heart, as well as the potential of medications and natural products to mitigate DCM progression along with reducing NLRP3 inflammasome expression and pyroptosis. Few studies directly investigated the roles of NLRP3 inflammasome and pyroptosis in the development of DCM, utilizing appropriate approaches, such as *NLRP3* gene silencing or pharmaceutical *NLRP3* inhibitors. Therefore, this aspect of investigation is an urgent need, as stated in this editorial.

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INTRODUCTION

Deaths attributable to cardiovascular diseases (CVDs) in the United States remain on the rise in the last decade[1]. One of the CVDs, diabetic cardiomyopathy (DCM), defined as a specific diabetes-induced cardiac disease, was reported more than 50 years ago[2]. However, its underlying mechanisms are not fully understood, despite numerous studies that have been conducted[2,3]. Cardiomyopathy refers to a group of diseases that affect the heart muscle, leading to cardiac structural remodeling and dysfunction. Emerging evidence suggests that cardiomyocyte death is key to the pathogeneses of cardiomyopathy[4,5]. Cardiac cell death, particularly cardiomyocyte death, leads to a reduction in contractile units and impairment of cardiac function, while also triggering cardiac inflammation and remodeling, all of which are critical to the development and progression of cardiomyopathy[4,5]. In a recent issue of this journal, Zhang *et al*[6] reported an important study, showing the preventive effects on the development of DCM by teneligliptin through the inhibition of NPRLP3, one of the inflammasomes (macromolecular protein complexes) that plays an important role in the immune system. In this study, mice were induced with type 1 diabetes mellitus (T1DM) by multiple small dose i.p. injections of streptozotocin (STZ) and were treated with teneligliptin. The authors observed a significant prevention of cardiac remodeling and dysfunction in T1DM mice with teneligliptin compared to those without treatment. Moreover, NOD-like receptor protein 3 (NLRP3) inflammasome activation and increased release of interleukin-1β (IL-1β) in the heart of diabetic mice were also inhibited by teneligliptin treatment. Accordingly, this study establishes the association of the prevention of DCM with the inhibition of NLRP3, but does not provide additional insights into the causative role of the NLRP3 inflammasome in the pathogenesis of DCM.

EVIDENCE NEEDED TO SUPPORT THE CAUSATIVE ROLE OF THE NLRP3 INFLAMMASOME AND PYROPSOSIS IN THE PATHOGENESIS OF DCM

As discussed by the authors[6], the NLRP3 inflammasome, consisting of NLRP3 (pyrin domain-containing NOD-like receptor protein 3), CARD-containing apoptosis-associated speck-like protein (ASC), and effector protein Caspase-1, has been extensively studied[6,7]. NLRP3 is expressed predominantly in macrophages. As a component of the inflammasome, activated NLRP3 undergoes self-oligomerization and assembles with ASC and the protease caspase-1 to form the NLRP3 inflammasome to trigger the immune response. This process results in the production of the active form of the caspase-1 p10/p20 splicer and induces the conversion of the proinflammatory cytokines IL-1β and IL-18 from their immature to their active forms, inducing so-called pyroptosis, a rapid, inflammatory form of lytic programmed cell death[6,7]. It is now well-accepted that the NLRP3 inflammasome plays important roles in the pathogenesis of both T1DM and type 2 diabetes mellitus (T2DM) and is linked to a number of the complications associated with diabetes[8]. As a key feature of diabetes, hyperglycemia was found to be able to activate NLRP3 inflammasome-mediated pyroptosis in multi-organs, including the heart[9,10]. These findings suggest that the *NLRP3* inflammasome may be involved in the development of DCM.

Zhang *et al*[6], did not provide direct evidence to confirm the capacity of specific inhibition of NLRP3 to prevent DCM, but instead cited several publications that discuss the crucial roles of NLRP3 (see their citations). However, these citations are of studies showing associations; in another words, not providing definitive evidence as to whether NLRP3 plays a causative role in the development of DCM or not. This is a general phenomenon in the current literature. Many publications have only cited studies that do not provide clear evidence to support the role of NLRP3 inflammasome and pyroptosis in the induction of DCM. For example, such studies demonstrated protective or preventive effects of different medications or natural compounds on the pathogenesis of DCM, accompanied by inhibition of cardiac inflammatory responses, including markers of the NLRP3 inflammasome and pyroptosis. Accordingly, they concluded that the protective or preventive effects of DCM are mediated by the inhibition of NLRP3 and/or pyroptosis (references are not cited due to page limitations). Therefore, it is appropriate to summarize the direct evidence to support the roles of *NLRP3* inflammasome and pyroptosis in the pathogenesis of DCM as described below.

In 2014, an important study was reported by Luo *et al*[11], in which the silencing of *NLRP3* gene ameliorated the development of DCM in a T2DM rat model. In their study, rats induced with T2DM by HFD/STZ exhibited severe metabolic disorder, cardiac inflammation, remodeling along with activated NLRP3, ASC, pro-caspase-1, cleaved caspase-1, mature IL-1β, and cell death (pyroptosis). These diabetic effects were markedly attenuated by *NLRP3* gene silencing therapy, supporting the potential role of activation of NLRP3 inflammasome in the pathogenesis of DCM. Following this study, the pharmaceutical application of *NLRP3* specific inhibitors to prevent diabetic complications was explored. For example, administration of the *NLRP3* specific inhibitor MCC950, the most widely used *NLRP3* specific inhibitor in preclinical and clinical studies[12], in STZ-induced diabetes in ApoE knockout mice resulted in the prevention of diabetes-induced atherosclerosis[13].

In terms of the heart, Yin *et al*[14] treated cultured cardiac H9C2 cells with palmitate as a commonly used fatty acid to mimic *in vivo* lipotoxicity in the diabetic condition. They showed the increased cardiac cell death by palmitate, when it was attenuated by the direct application of MCC950 into the medium, along with decreased gene and protein expression levels of NLRP3, ASC, Caspase-1, and GSDMD. Furthermore, in a mouse model of stroke-induced systemic inflammation with cardiac dysfunction, ischemic stroke-induced cardiac remodeling and dysfunction were more severe in mice with T2DM induced by HFD/STZ compared to mice without T2DM. M1-polarized macrophage infiltration and NLRP3 inflammasome activation in the heart following diabetic stroke are also more severe compared to stroke in non-diabetic mice. However, these effects are attenuated by the NLRP3 inflammasome inhibitor CY-09[15]. It is known that obesity increases the risk of cardiac fibrosis. Deng *et al*[16] recently reported that mice with HFD-induced obesity had more severe myocardial fibrosis than control mice, both in normal and ischemia/reperfusion conditions. However, the cardiac fibrosis could be attenuated by a NLRP3 inhibitor MCC950, supporting the concept that the heart is susceptible to fibrosis with obesity through activation of the NLRP3 inflammasome[16].

CONCLUSION

These pieces of evidence summarized above support, to a certain extent, a critical role of the NLRP3 inflammasome and associated pyroptosis in the development of DCM[11,13-16]. However, the relevant studies remain limited in terms of the experimental models of diabetes used, and DCM's typical pathological changes and dysfunction examined, gene silencing efficiency, and the NLRP3 inflammasome specific inhibitor types, doses, and times of administration at early or late stage of diabetes. Accordingly, more systematic studies with direct evidence are urgently needed to support the direct link between NLRP3 inflammasome, associated pyroptosis and DCM. At this time, it is appropriate to be circumspect in relation to its role in the pathogenesis of DCM despite the fact that several medications and natural products that are able to inhibit *NLRP3* inflammasome and associated pyroptosis, including the teneligliptin used in the study of Zhang *et al*[6], also prevent the pathogenesis of DCM[10,17-20]. We need to determine that these medications and natural products do not protect the heart from diabetes-associated damage *via* other mechanisms and that the increased expression/activation of NLRP3 inflammasome and pyroptosis-related markers that were attenuated do not represent non-specific responses. It is only when an unequivocal confirmation of their causative roles in the pathogenesis of DCM is provided, then these can be specifically targeted to develop effective and safe medications for clinical use. Zhang *et al*[6] have performed an important study and this editorial serves as a timely reminder in relation to the pivotal importance of direct, rather than indirect, evidence in this regard.

FOOTNOTES

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EDITORIAL

Diabetes and susceptibility to COVID-19: Risk factors and preventive and therapeutic strategies

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Abstract

Coronavirus disease 2019 (COVID-19) is a highly infectious disease caused by a novel human coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Diabetes is a well-known risk factor for infectious diseases with high prevalence and increased severity. Here, we elucidated the possible factors for the increased vulnerability of diabetic patients to SARS-CoV-2 infection and the more severe COVID-19 illness. The worsened prognosis of patients with both COVID-19 and diabetes may be attributable to host receptor angiotensin-converting enzyme 2-assisted viral uptake. Moreover, insulin resistance is often associated with impaired mucosal and skin barrier integrity, resulting in microbiota dysbiosis, which increases susceptibility to viral infections. It may also be associated with higher levels of pro-inflammatory cytokines resulting from an impaired immune system in diabetics, inducing a cytokine storm and excessive inflammation. This review describes diabetes mellitus and its complications, explains the risk factors, such as disease characteristics and patient lifestyle, which may contribute to the high susceptibility of diabetic patients to COVID-19, and discusses preventive and therapeutic strategies for COVID-19-positive diabetic patients.

Key Words: Diabetes mellitus; SARS-CoV-2; COVID-19; Susceptibility; Prevention; Treatment

Core Tip: This paper hypothesizes that the worsening prognosis of diabetic patients with coronavirus disease 2019 (COVID-19) is attributable to host receptor angiotensin-converting enzyme 2-assisted viral uptake. Insulin resistance is often associated with impaired mucosal and skin barrier integrity, resulting in microbiota dysbiosis, which increases susceptibility to viral infections. It may also be associated with higher levels of pro-inflammatory cytokines resulting from an impaired immune system in diabetic patients, which induces a cytokine storm and excessive inflammation. This review discusses the possible factors contributing to the increased susceptibility of diabetic patients to severe acute respiratory syndrome coronavirus 2 infection and the more severe COVID-19 disease and preventive and therapeutic strategies for COVID-19 in diabetic patients.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by a novel human coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 3 March 2024, 774834254 confirmed COVID-19 cases and 7037007 deaths have been reported globally[1]. According to the specification of the Centers for Disease Control and Prevention, the high-risk groups include people with chronic diseases such as type 2 diabetes, chronic lung, severe heart, and chronic kidney diseases, and obesity, as well as pregnant women[2]. In 2019, the prevalence of diabetes among adults aged 20-79 years worldwide was 9.3% (463 million) and was projected to continue to increase[3]. Diabetes has been a well-known risk factor for infectious diseases, especially increasing the risk of infection and death in severely ill patients [4].

A recent review published in the *World Journal of Diabetes* by Shukla *et al*[5] studied and explored the complex link between type 2 diabetes and COVID-19, revealing that reduced ACE2 expression in patients with diabetes mellitus (DM) enhances the activity of renin-angiotensin system, leading to inflammation and fibrosis. Moreover, it was highlighted that people with DM had a higher mortality and higher susceptibility to COVID-19 than those without the disease[5].

Therefore, improving the prognosis of DM and COVID-19 patients requires early diagnosis, prompt treatment, and stringent management. In order to manage patients with comorbid diseases effectively, this review describes the severity of DM, presents an epidemiological analysis of patients with DM comorbid with COVID-19, analyzes the possible factors for the high susceptibility of patients with DM to COVID-19, and examines the preventive and therapeutic strategies of DM patients with COVID-19. These findings could aid in the development of new potential treatments for COVID-19.

METHODOLOGY

Articles that were published in English were retrieved by a computerized search in the PubMed and Google Scholar databases. The search period was from database creation to March 2024. Keywords included "COVID-19", "coronavirus therapy", "SARS-CoV-2", "diabetes", "angiotensin-converting enzyme", "insulin resistance", and "immune system". Further references were added in the final manuscript based on consensus among all authors by hand-searching in the relevant literature.

DM AND ITS COMPLICATIONS

DM is a hyperglycemia disease caused by defective insulin secretion and/or action. DM-related chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of various organs, including the eyes, nerves, heart, kidneys, and blood vessels[6]. Nephropathy, cardiovascular disease, retinopathy, and diabetic foot are common complications of DM. Diabetic nephropathy is a serious microvascular complication of type 1 or type 2 DM that damages the kidney filtration system and can lead to life-threatening kidney failure in severe cases. Interstitial fluid glucose accumulation is the basic pathogenesis of blood glucose elevation in DM patients, and its fluctuations in glucose levels with daily hypoglycemia and hyperglycemia are considered important in driving DM-related pathologies[7]. Changes in glucose levels lead to transcriptional changes in renal tubular cells, alterations in the renal extracellular matrix, and metabolic mitochondrial rewiring, which are early indicators of kidney disease that are triggered by hyperglycemia. Furthermore, kidney disease still occurs in DM patients even with strict blood glucose control, suggesting that oxidative stress and other insults, such as lipotoxicity, may also play a key role in its pathogenicity[8].

Cardiovascular disease is a widespread disease of cardiovascular system dysfunction, which is a major complication that contributes to type 2 DM-related deaths. High blood glucose levels in diabetics could induce the production of advanced glycosylation end products (AGEs) which attach to proteins or lipids in a non-enzymatic manner, altering their

function and inducing atherosclerosis[9]. Diabetic retinopathy is another common complication of DM, and almost all patients with DM eventually develop this disease. The dysfunction of the retinal cells involved in diabetic retinopathy includes the endothelial cells of the retinal microvessels and pericytes that lie beneath the endothelial cells[10]. One of the earliest features of diabetic retinopathy is pericyte loss. The integrity of the tight junction between endothelial cells is compromised by hyperglycemia, oxidative stress, and AGEs. These factors also cause pericyte detachment and apoptosis.

EPIDEMIOLOGIC ANALYSIS OF DM AMONG COMORBIDITIES IN COVID-19 PATIENTS

DM is a known risk factor for infectious diseases with high prevalence and increased severity. According to epidemiologic studies, DM has become one of the most common comorbidities associated with COVID-19 severity and mortality [11]. A report from Wuhan, China suggests that diabetics are overrepresented among deceased patients due to COVID-19 [12]. A study from Poland evaluated the impact of the COVID-19 pandemic on DM-related hospitalizations in different age groups and reported an increase of 66.7% and 48.5% in in-hospital mortality in DM patients, respectively[13].

Hospitalization mortality increases with age. Although increasing age is a consistent risk factor for increased COVID-19 severity and mortality, there is growing skepticism that this may not be a simple additive effect of age-related risk in patients with DM. In fact, a recent study has demonstrated that younger individuals with COVID-19 and type 2 DM have significantly higher mortality rates than older individuals[14]. In addition, COVID-19 infection induces acute hyperglycemic crisis and worsens the prognosis of patients with poorly controlled DM[15]. Therefore, achieving glycemic control is critical to reduce the risk of complications and death after COVID-19 infection.

RISK FACTORS FOR HIGH SUSCEPTIBILITY TO COVID-19 IN DM PATIENTS

Possible risk factors for high susceptibility to COVID-19 in diabetic patients are summarized in Figure 1.

Expression of angiotensin-converting enzyme 2

As an enzymatic functional receptor on the cell surface, angiotensin-converting enzyme 2 (ACE2) plays an important role in the renin-angiotensin-aldosterone system (RAAS). It exerts most of the RAAS physiological and pathophysiological effects, and genetically controls cardiovascular function and injury to several organs, including the lungs, liver, and kidney[16]. SARS-CoV-2 disrupts ACE/ACE2 homeostasis and activates RAAS, eventually leading to COVID-19 progression, especially in patients with comorbidities including hypertension, DM, and cardiovascular disease. In the pancreas, ACE2 antagonizes angiotensin II-mediated oxidative stress and apoptosis in islet cells, exerting islet protective functions. When the body is infected with neocoronaviruses, they reduce ACE2 expression by binding to it or directly damaging ACE2-expressing pancreatic islet β-cells, which ultimately leads to glucose metabolism disorders and acute hyperglycemia[17]. In human kidney-like organs, diabetic disease has recently been revealed to induce ACE2 expression, and single-cell analyses of 436 patients have suggested that increased ACE2 expression in the lungs and kidneys may present an increased risk of SARS-CoV-2 infection[18]. Elevated glucose levels may directly promote SARS-CoV-2 replication and pro-inflammatory cytokine production. Furthermore, SARS-CoV-2 facilitates the shift in monocyte metabolism to aerobic glycolysis, thereby sustaining SARS-CoV-2-induced monocyte responses and their replication.

In addition to the imbalance in ACE2 expression after SARS-CoV-2 infection, cellular metabolism in renal-like organs contributes directly to the increasing viral load. Cells isolated from renal biopsies of DM patients show altered mitochondrial respiration and glycolysis, leading to an increased prevalence of SARS-CoV-2 infection. By contrast, dichloroacetate could reduce SARS-CoV-2 infection in patient cells as an aerobic glycolysis inhibitor[19]. Shukla *et al*[5] emphasized the potential of ACE2 receptors in the kidney as a treatment and prevention target of diabetic nephropathy, which was a microvascular complication induced by a long-term hyperglycemic environment, manifested by proteinuria and deterioration of the glomerular filtration rate. However, there is concern that ACE2 overexpression leads to a more rapid viral uptake, which may exacerbate lung injury and result in fatal outcomes in COVID-19 patients[5].

Insulin resistance

In cases of overnutrition, more insulin is secreted under the influence of hyperglycemia, thereby leading to insulin resistance, which may be an aberrant immune response caused by the damaged insulin/IGF signaling pathway in the metabolic organs. This signaling pathway is also impaired by interferon regulator 1 produced through SARS-CoV-2 infection, leading to the development of adverse outcomes, such as disease exacerbation, cell death, and metabolic disorders in COVID-19 patients[20]. Obesity-induced chronic activation of pro-inflammatory signaling pathways (*e.g.*, autocrine/paracrine cytokine signaling and endocrine cytokine signaling) may lead to vascular and metabolic problems and decreased insulin sensitivity, which may be one of the mechanisms of SARS-CoV-2 infection in DM patients[21]. Hyperglycemic states lead to a severe clinical course of COVID-19, which subsequently exhibits deleterious effects on glucose metabolism, and may lead to new-onset DM. In addition, insulin resistance is often associated with impaired mucosal and skin barrier integrity, which may exacerbate systemic inflammation through microbial translocation[22]. One study examined the relationship between the pre-diabetic microbiome and host health by characterizing microbiomes, and showed that insulin-resistant patients exhibited reduced fecal microbiome richness and increased susceptibility in skin microbiome to opportunistic pathogens[23]. Thus, insulin resistance results in microbiota dysbiosis that increases host susceptibility to viral infections.

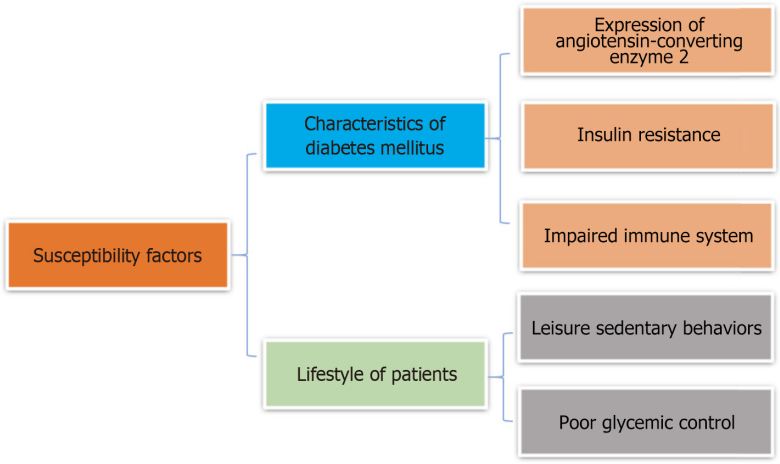

Figure 1 Possible risk factors for high susceptibility to coronavirus disease 2019 in diabetic patients.

Impaired immune system

The key components of innate immunity (*e.g.*, macrophages and neutrophils) are activated by SARS-CoV-2 infection. Subsequently, immune cells in the adaptive immune system (*e.g.*, antibody-producing B and T cells) are also activated following the release of multiple chemokines and cytokines. However, in severe cases, the over-activation of immune cells produces a cytokine storm, and thus causes damage to the organs in these patients, leading to a poor disease prognosis[24]. Elevated blood glucose levels also produce covalent glycoconjugates with a variety of proteins through non-enzymatic glycosylation, which can impair humoral immunity and increase susceptibility to SARS-CoV-2 infection [25]. Elevated levels of adipokines of adipose-tissue origin, interferon, and tumor necrosis factor a in DM patients may impair immune responses to SARS-CoV-2[26]. Moreover, DM patients have an impaired ability to clear SARS-CoV-2 from their circulation[27].

In addition, obesity, as one of the recognized risk factors for type 2 DM, can also be a major trigger of systemic low-level inflammation. Increased responses of the immune system, which are associated with obesity, can lead to metabolic disorders in insulin-sensitive tissues, further aggravating insulin resistance and forming a vicious circle. Subsequently, a compromised immune system and various metabolic imbalances increase patient susceptibility to SARS-CoV-2. DM leads to decreased immune function, increased production of pro-inflammatory cytokines, and a thrombotic state, thus increasing the severity of comorbid COVID-19 in patients with DM. Varga *et al*[28] found that endothelial cells may be directly infected by SARS-CoV-2, which is followed by necrosis, eventually showing hypercoagulable states[28]. This may be related to the increased incidence of thrombotic events in COVID-19 patients. The presence of increased pro-inflammatory cytokines in DM patients is associated with a "cytokine storm" of excessive inflammation in response to the virus. These responses may produce a fragile environment in the body, leading to exacerbation of COVID-19 symptoms, such as acute respiratory distress syndrome and severe pneumonia, which can increase mortality in COVID-19 patients[29].

RELATIONSHIP BETWEEN LIFESTYLE AND COVID-19 SUSCEPTIBILITY IN DM PATIENTS

Leisure sedentary behaviors

Owing to the COVID-19 pandemic, strict measures such as "lockdown" were adopted in many regions. People had to spend less time outdoors and more sedentary time at home. A lack of physical activity may be associated with an increased risk of developing various chronic diseases[30]. A cohort study of adults in the United Kingdom has suggested that unhealthy lifestyle, such as physical inactivity and smoking, increases susceptibility of SARS-CoV-2 infection[31]. Chen *et al*[32] applied a Mendelian randomization method to assess the potential causal effects of sedentary behavior on COVID-19 susceptibility, hospitalization, and severity characteristics. The results suggest that those with obesity or type 2 DM, especially in older people, comprise a susceptible subgroup to sedentary behavior during a pandemic. Metabolic disorders and smoking behavior may also contribute to COVID-19 susceptibility associated with recreational sedentary behavior.

Poor glycemic control

Maintaining healthy blood glucose levels is beneficial for DM patients to reduce the chances of hospitalization and severity and mortality in COVID-19 DM patients. In a retrospective study of 952 COVID-19 patients comorbid with type 2 DM, a stable blood glycemic control between 3.9 mmol/L and 10.0 mmol/L reduced patient mortality by a factor of 10 [33]. A study in Saudi Arabia showed that less than 15% of patients with type 2 DM were able to maintain optimal glycemic control[34]. Regarding the factors associated with adherence to diabetic dietary guidelines, there was no association between educational attainment and dietary adherence. Best dietary adherence was found to exist in patients diagnosed for 3–5 years. In DM patients, poor glycemic control leads to a higher susceptibility to the effects of COVID-19

[35]. Overall, fluctuations in blood glucose levels and their complications make it more difficult to treat viral infections in DM patients.

STRATEGIES FOR PREVENTION AND TREATMENT

Prevention strategies

Vaccination is an important measure to prevent the spread of the SARS-CoV-2 virus and the emergence of new variants [36]. The Pfizer (BioNTech) vaccination was reportedly > 95% effective in reducing COVID-19 in people who had never been infected. On the other hand, Moderna's vaccine was 94.1% effective in preventing the occurrence of symptomatic infections in people who had not been previously infected with COVID-19[37]. In addition to general prophylaxes for COVID-19, including maintaining social distancing, mask-wearing, proper hand hygiene, and vaccinations[38], other prevention strategies are required to protect DM patients with COVID-19. First, they should be advised to adopt healthy lifestyles by reducing sedentary behaviors, promoting appropriate exercise, and maintaining adherence to diets and tight control of blood glucose levels. In addition, increased education on hyperglycemia is an important step in patient management. Various digital solutions can be used to disseminate information, educate persons with disabilities, track individuals, monitor their health, and help them to take care of themselves. In Europe, cooperation between governments and DM associations has enabled the effective implementation and expansion of telemedicine services, thus ensuring continuity of care for people with DM[39]. During the Chinese COVID-19 epidemic, a wide range of online services for blood glycemic control were implemented for patients with DM and the general population. Free educational videos and e-books on DM self-management and COVID-19 prevention were also made available to the public through the WeChat mobile application[40].

Treatment strategies

Insulin therapy is the preferred treatment for COVID-19 patients with severe hyperglycemia, especially for heavy and critically ill patients. Insulin therapy exerts a powerful anti-inflammatory effect during critical illness and improves blood glucose management in hospitalized patients[41]. Although there is uncertainty about the use or discontinuation of some glucose-lowering medications, controlling blood glucose levels is necessary.

Sodium-glucose cotransporter protein-2 (SGLT 2) inhibitors could not only treat type 2 DM but also exhibit anti-inflammatory effects and reduce cardiovascular and renal complications[42]. However, the use of SGLT 2 inhibitors during COVID-19 infection may cause decreased blood volume due to vomiting and anorexia, as well as diabetic ketoacidosis triggered by the direct cytolytic effect of the virus on β-cells. These factors further lead to reduced endogenous insulin secretion and increased inflammatory response and interleukin-6 levels[43].

Dipeptidyl peptidase-4 inhibitors are transmembrane glycoproteins that play an important role in glucose homeostasis. They may have therapeutic benefits in COVID-19 and may reduce the production of pro-inflammatory cytokines[44]. Glucagon-like peptide-1 receptor agonists have anti-inflammatory effects on mild inflammation and help reduce weight in obese individuals, a condition associated with chronic inflammation and impaired immune response[45]. However, a risk of gastrointestinal side effects, such as nausea, vomiting, and aspiration, have been presented[46]. Although metformin has anti-inflammatory properties and can reduce inflammatory biomarker expression, its immunomodulatory role in the context of COVID-19 remains unclear. Crouse *et al*[47] found that metformin may improve the prognosis of patients hospitalized with type 2 DM and COVID-19 by reducing mortality. However, it carries a risk of lactic acidosis and is not indicated for critically ill patients. Li *et al*[48] showed that thiazolidinediones were the peroxisome proliferator-activated receptor-γ agonists and could ameliorate insulin resistance and have anti-inflammatory and anti-atherosclerotic properties[48]. Unfortunately, they are associated with exacerbation of edema and heart failure and are not suitable for critically ill patients[49].

The therapeutic drugs for COVID-19 in DM patients and their properties and risks are shown in Table 1.

CONCLUSION

To date, there is still no specific remedy for COVID-19 in DM patients; therefore, early diagnosis, timely treatment, and strict management of patients with DM and COVID-19 are important to improve their prognoses. Recent studies have reported that green tea polyphenols possess antiviral activity against SARS-CoV-2, as well as antibacterial, antioxidant, and anti-inflammatory effects[50]. Since drug improvement and vaccine progression require a significant investment of time, alternative strategies also need to be urgently tested. The potential effects of various drugs on the main SARS-CoV-2 protease have been demonstrated through combined computerized and biochemical studies[51]. Seven promising medicines, namely, sapanisertib, ornidazole, napabucasin, lenalidomide, daniquinone, indolimod, and salicylamide were identified through virtual screening against the major proteases of SARS-CoV-2 and are considered essential for the treatment of COVID-19[52]. However, extensive *in vivo* and *in vitro* studies are warranted to assess each drug's activity. In addition, numerous studies have reported that nutritional therapeutic measures, including natural dietary supplements, vitamins, minerals, micronutrients, probiotics, and hydrogen therapy[53] could be promising and effective adjunctive treatments for COVID-19 owing to their antioxidant, antiviral, anti-inflammatory, and positive immunomodulatory properties[54]. For patients with COVID-19 comorbid DM, tailored treatment strategies and optimal glycemic control should be developed based on specific clinical classification, comorbidities, and other influencing factors. All hospitalized

Table 1 Medication-related information

Therapeutic drug	Anti-inflammatory properties	Risks
Insulin	Yes	Risk of hypoglycemia
SGLT-2 inhibitors	Yes	Risk of euglycemic diabetic ketoacidosis
DPP-4 inhibitors	Yes	Relatively safe
GLP-1 RA	Yes	Gastrointestinal symptoms
Metformin	Yes	Risk of lactic acidosis
Pioglitazone	Yes	Risk of fluid retention and heart failure

DPP-4: Dipeptidyl peptidase-4; GLP-1RA: Glucagon-like peptide-1 receptor agonist; SGLT-2: Sodium-glucose cotransporter-2.

patients should control their blood glucose levels to monitor the disease progression and avoid exacerbations. In critically ill patients, early recognition and proper management of adverse drug reactions can prevent the worsening of symptoms. The emphasis on preferred insulin therapy and the development of individualized glycemic management strategies are important. Optimized glycemic management in terms of glucose monitoring, lifestyle, and hyperglycemia education is required to prevent adverse prognoses and improve disease regression. Further epidemiological, clinical, and fundamental studies on the possible factors responsible for the high susceptibility of DM patients to COVID-19 in terms of the characteristics of DM and lifestyle, and preventive and therapeutic strategies for COVID-19 in DM patients, are urgently needed in the future.

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FOOTNOTES

Author contributions: Wang MK and Yang JS conceptualized and designed the research; Liu JW, Huang X, Wang MK, and Yang JS wrote and revised the paper; All the authors have read and approved the final manuscript. Liu JW conducted the literature search, organized the literature, and prepared the first draft of the manuscript. Huang X was responsible for organizing the literature on risk factors and treatment strategies related to the susceptibility of diabetic patients to COVID-19, and drew the figure. Both authors have made crucial and indispensable contributions towards the completion of the project and thus qualified as co-first authors of the paper. Both Wang MK and Yang JS conceptualized, proposed, and supervised the whole process of this paper, and played important and indispensable roles in the manuscript preparation and revision as the co-corresponding authors. Wang MK applied for and obtained the funds for this research project. Wang MK conceptualized, designed, and supervised the whole process of the project. He searched the literature, and revised and submitted the early drafts of the manuscript. Yang JS was instrumental and responsible for the comprehensive literature search, preparation, and submission of the current version of the manuscript. This collaboration between Wang MK and Yang JS is crucial for the publication of this manuscript and other manuscripts still in preparation.

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Periodontal disease: A silent factor in the development and progression of diabetic retinopathy

Sarah Monserrat Lomelí Martínez, Irán Cortés Trujillo, Melissa Martínez Nieto, Ana Esther Mercado González

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Abstract

The global increase in the prevalence of type 2 diabetes mellitus (T2DM) and its complications presents significant challenges to public health. Recently, periodontal disease (PD) was recognized as a factor that is likely to influence the progression of T2DM and its complications due to its potential to exacerbate systemic inflammation and oxidative stress. In this editorial, we comment on the article published by Thazhe Poyil *et al* in the very recent issue of the *World Journal of Diabetes* in 2024, which investigated the correlation between PD and diabetic retinopathy (DR) in T2DM patients, with emphasis on the association between periodontal swollen surface area, glycated hemoglobin (HbA1c), interleukin-6 (IL-6), and lipoprotein (a). The findings by Thazhe Poyil *et al* are significant as they demonstrate a strong link between PD and DR in T2DM patients. This correlation highlights the importance of addressing periodontal health in diabetes management to potentially reduce the risk and severity of DR, a complication of diabetes. The integration of periodontal evaluation and treatment into diabetes care protocols may lead to improved glycemic control and better overall outcomes for T2DM patients . A few studies have established an interconnection between PD and diabetic complication, specifically DR, in T2DM patients, which we aim to highlight in this editorial. Emphasis was placed on the different mechanisms that

suggest a bidirectional relationship between PD and T2DM, where the presence of periodontal inflammation negatively influenced glycemic control and contributed to the development and progression of DR through shared inflammatory and vascular mechanisms. This article highlights the importance of collaboration amongst diabetes specialists, ophthalmologists, periodontists, and public health professionals to advance the prevention, early detection, and treatment of PD and DR. This will improve the health and quality of life of T2DM patients. Moreover, the editorial highlights the need for further research on the specific molecular and immunological mechanisms that underlie the link between periodontitis and DR, with identification of common inflammatory biomarkers and signaling pathways. This is expected to facilitate effective direction of therapeutic objectives, thereby improving the management of diabetes and its complications through integrated care that incorporates oral health.

Key Words: Type 2 diabetes mellitus; Periodontal disease; Periodontitis; Diabetic retinopathy; Editorial

Core Tip: In this editorial, we commented on the observational study by Thazhe Poyil *et al* published in the recent issue of the *World Journal of Diabetes* in 2024, in which the correlation between periodontal disease (PD) and diabetic retinopathy (DR) in patients with type 2 diabetes mellitus (T2DM) was investigated. We discussed some of the most notable studies, with emphasis on the different mechanisms that suggest a bidirectional relationship between PD and T2DM, where the presence of periodontal inflammation negatively influenced glycemic control and contributed to the development and progression of DR through shared inflammatory and vascular mechanisms.

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INTRODUCTION

The surge in diabetes mellitus (DM) cases over the last three decades has escalated into a critical global health challenge, with DM emerging as a leading cause of morbidity and mortality worldwide. Presently, over 420 million people are grappling with type 1 or type 2 diabetes mellitus (T2DM), a figure resulting from quadrupling of the value in 1980. It has been projected to surpass 500 million by the end of the next decade. The factors that contribute to this surge in DM are unhealthy dietary patterns, sedentary lifestyles, obesity, and genetic predispositions[1]. The chronic complications of DM encompass macrovascular issues, which are the foremost contributors to DM-related mortality, while microvascular complications significantly impact quality of life of DM patients. Glycemic control and blood pressure control have demonstrated efficacy in mitigating certain microvascular complications, notably ocular lesion, *i.e.* diabetic retinopathy (DR). In contrast, factors such as smoking, alcohol consumption, hyperlipidemia, and periodontitis heighten the risk of DR[2].

DR affects the retinal microvasculature, and it primarily correlates with glycemic control, duration of diabetes, and hypertension. This complication severely compromises vision, and it has emerged globally as a leading cause of blindness. DR manifests in two stages: non-proliferative DR (characterized by capillary hyperpermeability, macular edema, ischemia, hemorrhage, and microaneurysms), and proliferative DR (an advanced stage marked by retinal neovascularization, vitreous hemorrhage, and fibrovascular proliferation). Although various treatments are used to delay DR progression, it has been shown that the control of hemoglobin (HbA1c) levels and management of hypertension are effective in preventing or impeding accelerated development of the disease[3,4].

Periodontal disease (PD) is a prevalent oral disease that affects 20%-50% of the population, and it is ranked amongst the top common conditions in the world[5]. There has been a significant surge in the incidence of PD, a situation that has earned it global public health recognition. Although the pathogenesis of PD is multifactorial, the primary cause stems from formation of pathogenic bacterial biofilm. This results in dense immunoinflammatory infiltrates that damage soft tissues and, in severe cases, lead to tooth detachment due to loss of periodontal support[6]. Beyond local symptoms such as inflammation and pain, PD-associated bacterial infections release proinflammatory cytokines such as interleukin 1β (IL-1β), IL-6, C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), prostaglandin E2, and receptor activator of nuclear factor-κB ligand as autoimmune responses, all of which contribute to systemic health issues[7]. The association of PD with non-oral diseases such as DM, cardiovascular disorders, and certain cancers underscores its systemic impact[8,9]. Individuals with T2D who have severe PD have 3.2-fold higher risk of mortality than those with mild or no periodontitis[5]. This indicates that individuals with T2DM are more susceptible to developing PD and are likely to experience more severe forms of periodontitis than non-diabetic individuals[10]. Moreover, the raised levels of proinflammatory cytokines in diabetic patients may reach the gum and exacerbate existing PD, thereby indicating the likelihood of a

bidirectional relationship between periodontitis and diabetes mellitus[11].

The present editorial emphasizes the critical need to understand the interplay amongst PD, DM, and DR development, with highlights on how periodontal health profoundly influences diabetic complications. It advocates for a comprehensive, multidisciplinary approach to public health assessment, in recognition of the systemic implications and interconnected pathways amongst PD, DM, and DR.

ASSOCIATION BETWEEN DR AND PD

The correlation between PD and the onset of DR may be elucidated through several mechanisms. The association of PD with increased levels of IL-6, CRP, and fibrinogen contributes to heightened insulin resistance. Furthermore, oxidative stress induced by PD exacerbates tissue damage and cellular demise. Studies have shown that PD triggers gradual increases in the level of vascular endothelial growth factor (VEGF) within the gingival crevicular fluid[12]. Periodontitis leads to atherosclerosis, resulting in retinal hypoxia and the formation of fragile, leaky vessels, and ultimately retinal detachment. Additionally, systemic inflammation markers such as C-reactive protein, TNF-α, IL-1b, and IL-6 are associated with altered lipid homeostasis and increased levels of adipose tissue macrophages. This leads to higher lipid concentrations in the bloodstream and ectopic fat deposits in the endothelium, potentially triggering retinal hypoxia and DR[13]. However, this connection has not been completely validated, despite individual studies suggesting an association between diabetic DR and periodontitis.

In the recent 2024 issue of the *World Journal of Diabetes*, Thazhe Poyil *et al*[14] published an interesting paper titled "Correlation of periodontal inflamed surface area with glycated hemoglobin, interleukin-6 and lipoprotein(a) in type 2 diabetes with retinopathy". This cross-sectional study analyzed the correlation between PD and DR in T2DM patients. Eighty T2DM patients were included in the study (40 patients with DR and 40 without retinopathy). The periodontal parameters evaluated were Plaque Index (PI), percentage of sites with bleeding on probing (BOP), probing pocket depth, gingival recession, clinical attachment loss (CAL), periodontal inflamed surface area (PISA), and systemic parameters such as glycosylated HbA1c, IL-6, and lipoprotein (a) [(Lp (a)]. The results showed that the proportion of periodontitis was higher in T2DM with DR (47.5%) than in T2DM without DR (27.5%), with a significant difference in the severity of PD between both groups ($P = 0.05$). Periodontitis severity, CAL, PISA, IL-6, and Lp (a) were higher in the T2DM group with DR. A significant difference was observed in the mean percentage of sites with BOP between T2DM with DR (69%) and T2DM without DR (41%). Moreover, HbA1c was positively correlated with CAL ($P = 0.001$) and PISA ($P \leq 0.001$) in the studied subjects. Additionally, there were positive correlations between PISA and IL-6 ($P < 0.0001$); PISA and Lp (a) ($P < 0.001$); CAL and IL-6 ($P < 0.0001$), and CAL and Lp (a) ($P < 0.001$). The authors proposed that in view of the bidirectional link between periodontitis and DM, it is most likely that the presence of DR contributed to the severity of periodontal destruction and that periodontitis might have influenced DR progression.

In a cross-sectional study conducted by Tandon *et al*[15] on 213 South Indian patients diagnosed with T2DM, 66.2% of the population had DR, while approximately 91% had PD. The presence of moderate-to-severe PD increased the risk of DR by 1.6-fold. Patients with proliferative DR had significantly higher gingival plaque indices than those with non-proliferative DR or those without DR. These findings established a significant association between the presence and severity of DR and PD in patients with T2DM. This correlation implies that recognizing the link between these two conditions could help identify potentially sight-threatening retinopathy in diabetic patients who visit the dental clinic with PD.

On the other hand, in an interesting cross-sectional study on the association between PD and DR, Veena *et al*[12] investigated 200 adult T2DM patients with DR of varying severity. The severity of PD was assessed using clinical parameters, and HbA1c and serum creatinine levels were measured before DR treatment. The authors found a statistically significant association between diabetes duration and the severities of DR and PD. The severity of PD was directly correlated with the severity of DR, with higher plaque and gingival indices in patients with proliferative DR. A significantly higher association of HbA1c level was found between the group with DR and the group without DR. This is consistent with the study by Thazhe Poyil *et al*[14], which indicated worse glycemic control in the presence of DR. The study by Veena *et al*[12] suggests the likelihood of a plausible relationship between DR and PD, which highlights the importance of prevention and control of PD as an integral part of diabetes management strategies. In addition, there were significant associations between serum creatinine levels and DR and PD severity, unlike in the studies by Tandon *et al*[15] and Thazhe Poyil *et al*[14], in which kidney function was not evaluated.

It is important to highlight that Thazhe Poyil *et al*[14] measured PISA (which estimates periodontal inflammatory load), as well as levels of IL-6 and Lp (a). Lp (a) and IL-6 were positively correlated with PISA and PD. This contrasts with the studies by Tandon *et al*[15] and Veena *et al*[12], in which these inflammation and lipid markers were not evaluated. Despite the methodological differences in sample sizes, with Veena *et al*[12] having a larger sample size ($n = 200$) than Thazhe Poyil *et al*[14] ($n = 80$) and Tandon *et al*[15] ($n = 213$), the periodontal and systemic parameters evaluated in the three studies indicated a significant association between the presence and severity of PD and DR in T2DM patients. This association could be attributed to shared inflammatory mechanisms. The more severe the DR, the higher the proportion and severity of PD. These findings suggest that incorporating periodontal therapy into comprehensive diabetes management would be beneficial in improving glycemic control and preventing the progression of diabetic complications.

FUTURE PERSPECTIVES

The study by Thazhe Poyil *et al*[14] posits the existence of a bidirectional link between periodontitis and T2DM. This suggests that DR contributes to increased periodontal destruction, and *vice versa*. These findings are noteworthy as they underscore inflammation as a common component in the pathogenesis of periodontitis and DR. Moreover, the findings emphasize the importance of dental care in the management of patients with T2DM, especially those having complication with DR. These findings pave the way for several crucial avenues for future research in diabetes management.

It would be beneficial to integrate research projects with adequate longitudinal design and appropriate adjustments for confounding factors in order to identify the specific molecular and immunological mechanisms that underlie the bidirectional link between periodontitis and DR. The need for studies that determine common inflammatory biomarkers, signaling pathways, and epigenetic changes connecting these conditions should be emphasized. This will allow for effective direction of therapeutic objectives.

The bidirectional link between periodontitis and DM suggests that good periodontal health must be considered for the adequate management of diabetes[16,17]. However, more research is necessary to evaluate whether periodontal health has the potential to improve glycemic control and prevent the progression of DR to some extent. Additionally, there is a need to study the benefit of incorporating periodontal evaluation into DR diagnosis and management guidelines. This would contribute to identifying patients at higher risk of DR. An interesting aspect would be to evaluate the impact of periodontal treatment on the long-term outcomes of DR such as disease progression, need for laser treatment, and risk of vision loss. These will provide a more solid foundation for the potential advantages of periodontal management in diabetes patients.

Oral dysbiosis, inflammation, and destruction of the periodontium are characteristics of periodontitis[16]. Recent studies suggest that oral dysbiosis generated by periodontitis may result in chronic and repetitive discharge of periodontal microbes and their byproducts into the bloodstream, leading to systemic inflammation and creating or exacerbating insulin resistance and diabetes complications[16]. However, more research is needed to fully understand these links. In this sense, it would be important to investigate the role of the oral microbiota in the relationship between periodontitis and diabetes complications, including DR.

A multidisciplinary approach that integrates diabetes specialists, ophthalmologists, periodontists, and public health experts should be adopted for the management of diabetes and its complications. The implementation of this comprehensive management will ensure better prevention strategies, as well as early diagnosis and treatment of periodontitis and DR, thereby ultimately improving treatment outcomes and quality of life for patients. Similarly, it would be beneficial to promote the development of educational strategies for raising awareness among health professionals and patients, especially on the importance of oral health in the management of diabetes and the prevention of diabetes complications.

However, there may be challenges and barriers to effective integration of periodontal health into the management of DM. One of the main challenges is the lack of awareness amongst patients and health professionals about the importance of periodontal health and its impact on DM, and *vice versa*. In addition, limited availability of resources, especially in low-resource areas, makes it difficult to embark on regular periodontal health assessments and necessary interventions. For comprehensive management, there is a need for collaboration amongst dentists, endocrinologists, diabetologists, and other health professionals. However, this may be limited by lack of effective referral systems or communication between different specialties. In addition, patients may not perceive PD as a priority, especially if they are focused more on another aspect of diabetes such as blood glucose control. Thus, they may not follow recommendations for periodontal treatment, which, apart from being expensive, is not always covered by health insurance, thereby limiting access for some patients. Addressing these challenges requires a collaborative, multidisciplinary approach that prioritizes education of the patient, healthcare integration, and accessibility to affordable treatments.

Ultimately, more research is essential to understand the pathophysiological mechanisms that connect the complex relationship between EP and DR while unravelling the clinical implications. Additionally, it is necessary to ensure collaboration amongst health professionals, social workers, and community organizations to develop a comprehensive approach that addresses the needs of DM patients.

CONCLUSION

There is a significant association between periodontitis severity and the presence and severity of DR, indicating that patients with T2DM and DR experience greater PD burden. This suggests that, not only is periodontitis more prevalent and severe in these patients, but it may also play a role in the progression of DR through shared inflammatory and vascular mechanisms. These findings highlight the importance of adopting a multidisciplinary approach to the management of DM by incorporating the evaluation and treatment of periodontitis as essential components of comprehensive care. Integrating dental care into the management of DM may offer significant opportunities to improve glycemic control and mitigate the risk and progression of DM-related complications. Future research is needed to further investigate the underlying mechanisms linking periodontitis and DM, including the identification of common inflammatory biomarkers and signaling pathways. This is expected to facilitate the development of more effective therapeutic strategies targeting these shared pathological processes, thereby improving health outcomes for T2DM patients. The integration of dental care into the management of DM not only promises to improve glycemic control and mitigate the progression of its complication, but also represents a transformative opportunity for enhancing public health outcomes and quality of life of T2DM patients. These advantages highlight the importance of a multidisciplinary approach in the

treatment of this complex disease.

FOOTNOTES

Author contributions: Lomelí Martínez SM, Cortés Trujillo I, Martínez Nieto M, and Mercado González AE contributed equally to the preparation of this manuscript; Lomelí Martínez SM and Cortés Trujillo I conceptualized the study; Lomelí Martínez SM, Cortés Trujillo I, Martínez Nieto M, and Mercado González AE performed literature searches; Lomelí Martínez SM, Cortés Trujillo I, Martínez Nieto M, and Mercado González AE wrote the preliminary draft; Lomelí Martínez SM and Cortés Trujillo I critically reviewed and approved the manuscript.

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EDITORIAL

Diabetic cardiomyopathy: Emerging therapeutic options

Cornelius James Fernandez, Sahana Shetty, Joseph M Pappachan

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Abstract

Diabetic cardiomyopathy (DbCM) is a common but underrecognized complication of patients with diabetes mellitus (DM). Although the pathobiology of other cardiac complications of diabetes such as ischemic heart disease and cardiac autonomic neuropathy are mostly known with reasonable therapeutic options, the mechanisms and management options for DbCM are still not fully understood. In its early stages, DbCM presents with diastolic dysfunction followed by heart failure (HF) with preserved ejection fraction that can progress to systolic dysfunction and HF with reduced ejection fraction in its advanced stages unless appropriately managed. Apart from prompt control of DM with lifestyle changes and antidiabetic medications, disease-modifying therapy for DbCM includes prompt control of hypertension and dyslipidemia inherent to patients with DM as in other forms of heart diseases and the use of treatments with proven efficacy in HF. A basic study by Zhang *et al*, in a recent issue of the *World Journal of Diabetes* elaborates the potential pathophysiological alterations and the therapeutic role of teneligliptin in diabetic mouse models with DbCM. Although this preliminary basic study might help to improve our understanding of DbCM and offer a potential new management option for patients with the disease, the positive results from such animal models might not always translate to clinical practice as the pathobiology of DbCM in humans could be different. However, such experimental studies can encourage more scientific efforts to find a better solution to treat patients with this enigmatic disease.

Key Words: Diabetic cardiomyopathy; Diabetic cardiomyopathy; Heart failure; Pathobiology; Teneligliptin

Core Tip: Diabetic cardiomyopathy (DbCM) is a common and underrecognized complication of diabetes mellitus. The pathobiological mechanisms of DbCM are not fully elusive and the therapeutic options are not adequate. Stages of DbCM include diastolic dysfunction followed by heart failure (HF) with preserved ejection fraction, systolic dysfunction and HF with reduced ejection fraction in its order of progression. Zhang *et al*, in a recent issue of the *World Journal of Diabetes,* demonstrate the potential pathophysiological changes of DbcM and explore the therapeutic potential of teneligliptin in mouse models of DbCM. Such experimental models might harness better research efforts to find an appropriate therapeutic strategy for DbCM in the near future.

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INTRODUCTION

Heart disease is the most common cause of mortality in patients with diabetes mellitus (DM). Diabetic cardiac diseases comprise ischemic heart disease (IHD), cardiac autonomic neuropathy (CAN) and diabetic cardiomyopathy (DbCM)[1]. Although the first 2 forms are well-known, DbCM is not that familiar to most clinicians. The risk of hospitalization for heart failure (HF) is two-fold higher in those with DM than in nondiabetics, and diabetes is identified as an independent risk factor for worse clinical HF outcomes, including a greater decline in left ventricular ejection fraction over time[2]. DbCM, the most important cause of both diastolic and systolic HF in patients with DM, is associated with significantly higher morbidity and mortality risk.

Although there are very good treatment options for diabetics with IHD resulting from coronary artery disease (CAD) to improve the clinical and mortality outcomes, the treatment options for patients with DbCM are mostly symptom control measures. The main reason is the lack of therapeutic avenues targeting the pathobiological mechanisms involved in the development of DbCM. Therapeutics targeting the pathophysiological abnormalities of DbCM are being explored now. The therapies addressing the underlying aetiopathogenesis with the potential to reduce adverse cardiovascular outcomes and mortality are the need of the hour. A basic study by Zhang *et al*[3] in the recent issue of the *World Journal of Diabetes* that reveals the beneficial effect of teneligliptin in improving the disease process in mouse models of DbCM, is an example of treatment options targeting the pathobiological processes of DbCM. This editorial briefly discusses the pathobiology and emerging therapeutic options for patients with DbCM.

DISEASE EPIDEMIOLOGY

DbCM is defined as the structural, functional, and metabolic myocardial changes that result in HF in the absence of CAD, valvular heart disease and conventional cardiovascular risk factors such as hypertension and dyslipidaemia[4]. The resultant HF can either be HF with preserved ejection fraction (HFpEF; approximately 2/3rd of DbCM cases) or HF with reduced ejection fraction (HFrEF; approximately 1/3rd of DbCM)[4]. The prevalence of DbCM among community-dwelling adults with diabetes varied between 67.0% when the least restrictive criteria for diagnosis were used to 11.7% when the most restrictive criteria were used[5]. DbCM is associated with a high risk of incident HF with the highest risk when the most restrictive criteria are used [hazard ratio (HR): 2.55; 95% confidence interval (CI): 1.69-3.86] and the lowest risk when the least restrictive criteria are used (HR: 1.99; 95%CI: 1.50-2.65)[5]. These figures clearly explain the higher prevalence of incident HF at any point in time among patients with DM.

PATHOBIOLOGY OF DbCM

Although not fully elusive, multiple metabolic, structural, and functional alterations have been identified in the etiopathogenesis of DbCM. The pathobiological changes associated with the onset and progression of DbCM include chronic hyperglycemia, insulin resistance, hyperinsulinemia, alterations in the pathways controlling intracellular myocardial energy metabolism, increased myocardial fatty acid oxidation (and the consequent mitochondrial dysfunction); ceramide and diacylglycerol accumulation (with cardiomyocyte lipo-apoptosis), increased reactive oxygen species formation (cardiac oxidative stress), increased generation of advanced glycation end products (AGE) (with nuclear factor-κB

mediated inflammation), impaired calcium handling and apoptosis (from mitochondrial dysfunction, and endoplasmic reticulum stress), altered mitochondrial bioenergetics, overactivation of the renin-angiotensin-aldosterone systems (RAAS), impaired nitric oxide and endothelium-derived hyperpolarising factor-mediated vasodilation (leading to microvascular dysfunction), and exosome dysregulation (cardiomyocyte apoptosis)[1,4,6]. Structural changes associated with the pathogenesis of DbCM include myocardial hypertrophy, fibrosis, ischemia, and adverse cardiac remodelling[1,4,6]. Functional changes associated with the pathogenesis of DbCM include the development of diastolic dysfunction, HFpEF, systolic dysfunction, and subsequently HFrEF[1,4,6].

DIAGNOSTIC ASPECTS

The clinical features, structural changes, functional changes, and biomarkers are not specific enough to accurately diagnose DbCM. Cardiac remodelling is an important feature of DbCM, and various imaging tools used to diagnose cardiac remodelling include echocardiography, cardiac computed tomography (CT), magnetic resonance imaging (MRI), and nuclear imaging techniques[7]. These non-invasive tools can detect changes in myocardial mass, wall thickness and perfusion as indicators of diastolic dysfunction which is the key feature of early stages of DbCM[7].

While 2-D echocardiography can provide details regarding structural changes of DbCM, tissue doppler imaging can provide details regarding functional changes (E/e' velocity and mitral annular velocity)[7,8]. Dilated left atrium (LA) is an early feature that supports the diagnosis of DbCM. Apart from the LA volume index, 2-D speckle tracking echocardiography provides a measurement of the LA contractile strain rate. Contrast-enhanced cardiac MRI can provide details regarding structural changes (myocardial fibrosis, steatosis, and left ventricular mass), functional changes (diastolic and systolic function), and metabolic changes (MR spectroscopy for myocardial triglyceride content and high-energy phosphate metabolism). In addition to providing a more accurate measurement of LA size over echocardiography, the cardiac MRI can evaluate LA ejection fraction. Similarly, cardiac CT can measure LA total emptying fraction (LATEF) as a measure of global LA function. As CAN is an independent risk factor of DbCM, decreased ^{123}I-MIBG (Iodine-123 metaiodobenzylguanidine) activity can be seen in patients with DbCM[7].

Although the most definitive evidence of diastolic dysfunction is obtainable from cardiac catheterisation studies, it is rarely needed due to the availability of various highly sensitive and specific non-invasive tests. Investigational biomarkers of structural changes are matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (for myocardial fibrosis); and of functional changes include mi-RNA (for contractile function), procollagen 3 N-terminal peptide and troponin (for LV dysfunction), and BNP (brain natriuretic peptide) for LV diastolic and systolic function[1]. Figure 1 shows the metabolic, functional and structural changes in the heart that lead to DbCM.

PROGNOSIS

Initial stages of DbCM are clinically silent and are characterized by structural and functional changes including myocardial hypertrophy, myocardial interstitial fibrosis, and myocardial stiffness[9]. These changes are associated with subclinical diastolic dysfunction, which later evolves into HFpEF and eventually systolic dysfunction accompanied by HFrEF. Early detection and treatment of DbCM are important for preventing the onset and progression of myocardial fibrosis, the irreversible stage of the disease.

THERAPEUTIC INTERVENTIONS

Reversing adverse cardiac remodelling of DbCM is challenging and often needs a multi-faceted approach. The best approach to reverse the cardiac remodelling of DbCM is through lifestyle modifications (regular exercise, restricted fat and refined carbohydrate consumption aiming to improve the body weight and metabolic functions), and through drugs that improve systemic and tissue level insulin sensitivity, myocardial glucose uptake and myocardial function[10,11]. For example, metformin acting *via* the adenosine monophosphate-activated protein kinase (AMPK) pathway and thiazolidinediones acting *via* peroxisome proliferator-activated receptor gamma(PPAR-γ) modulate insulin sensitivity and myocardial metabolism. In addition, optimising the cholesterol levels and blood pressure is also important[10,11].

Apart from glycaemic control and weight reduction, the glucagon-like peptide 1 receptor agonists exhibit antifibrotic properties by activating the AMPK pathway, reducing the endoplasmic reticulum stress, and inhibiting the expression of type I/III collagen and MMPs, thereby preventing the development of DbCM. Dipeptidyl peptidase-4 (DPP-4) inhibitors could also prevent myocardial hypertrophy and diastolic dysfunction by inhibiting oxidative stress and myocardial fibrosis[10,11].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are also promising drugs in the treatment of DbCM. They enhance sodium excretion and decrease cardiac preload and afterload, thereby improving cardiac output. SGLT2 inhibitors inhibit myocardial fibroblast activation by blocking the transforming growth factor-β (TGF-β)/SMAD signalling pathway[10,11]. SGLT2 inhibitors inhibit the cytosolic sodium-hydrogen (Na^+-H^+) pump, decrease intracellular calcium and thereby myocardial injury, hypertrophy, and fibrosis. Cardio-protection by SGLT2 inhibitors is also mediated by raised β-hydroxybutyric acid production (increasing the mitochondrial metabolism) and uric acid excretion[10,11].

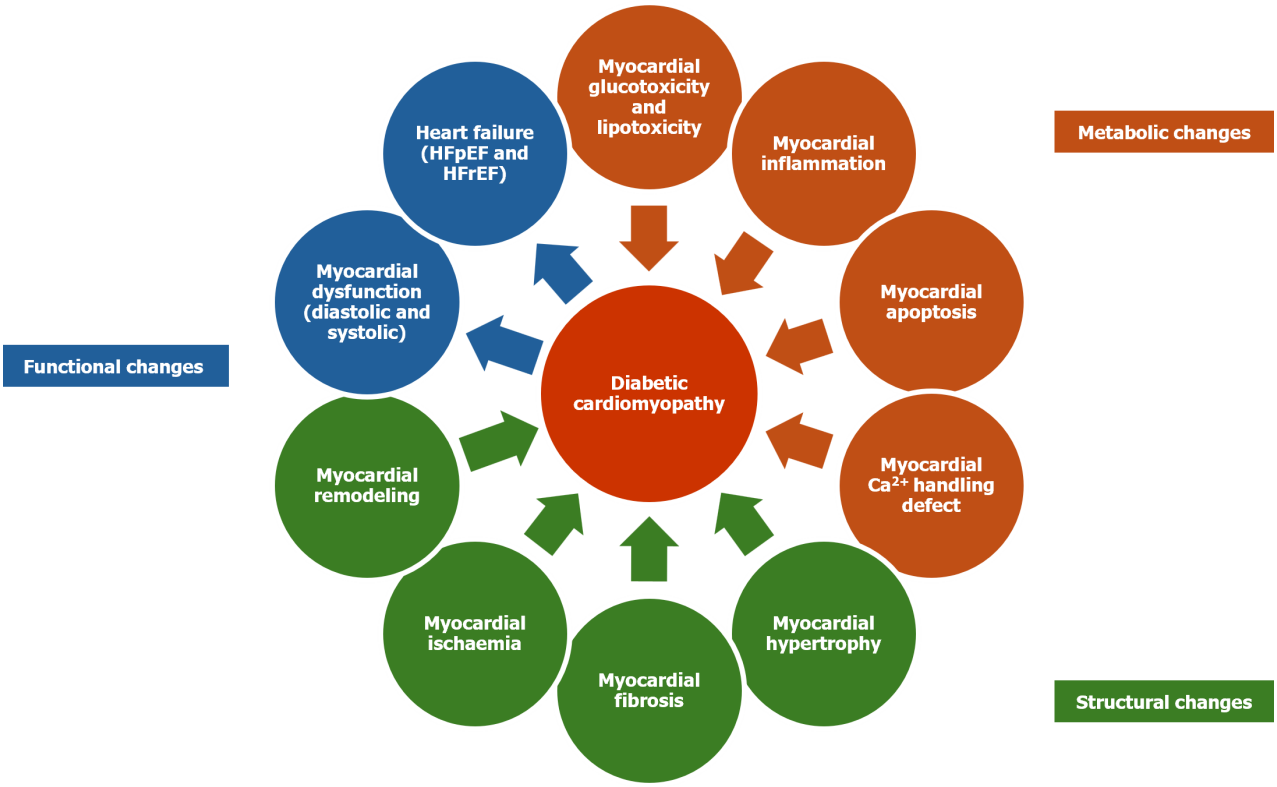

Figure 1 The metabolic, structural, and functional changes in the heart leading to the development of diabetic cardiomyopathy. Ca^{2+}: Calcium; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction.

Drugs with proven efficacy for HFrEF to reverse LV remodelling *via* inhibition of RAAS such as angiotensin convertase enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists may also be of value in the treatment of HFpEF mediated by DbCM by inhibiting the development of inflammatory and fibrotic responses[10,11]. Similarly, β-blockers reduce myocardial oxygen consumption, improve cardiac metabolism, and reduce myocardial hypertrophy, apoptosis, and fibrosis. Spironolactone could reduce myocardial oxidative stress, inflammatory response, and fibrosis[10, 11].

As multiple mechanisms are involved in the pathogenesis of DbCM, the current treatments are not fully effective in the prevention and treatment of DbCM. Many potential therapies are in the preliminary stages of development including sphingosine-1-phosphate modulators, AGE inhibitors, aldose reductase inhibitors, N-acetylcysteine, nicorandil, Nesfatin-1, Schisandrin B, Paeonol, Piceatannol, and flavonoids which are tailored towards different specific pathways[10-12]. On the other hand, gene therapy and stem cell therapy could intervene in the root cause of the disease and might achieve long-term beneficial effects with the potential to reverse myocardial fibrosis[10].

TENELIGLIPTIN AS A POTENTIAL THERAPEUTIC AGENT IN DbCM

Various types of programmed cell death (apoptosis, pyroptosis, necroptosis, and ferroptosis) are involved in the clearance of cells that lost their function, have an infection, or are affected by neoplasms. Pyroptosis, a highly inflammatory mode of apoptosis, is involved in the pathogenesis of atherosclerosis, ischemia-reperfusion injury, and DbCM. Chronic hyperglycaemia-induced reactive oxygen species (ROS) formation activates NOD-like receptor protein 3 (NLRP3) inflammasome which in turn activates downstream molecules to cause membrane rupture characteristic of pyroptosis and produce pro-inflammatory cytokines[13]. As NLRP3 inflammasome participates in the cardiomyocyte pyroptosis of DbCM, NLRP3 is upregulated in DbCM and the level of NLRP3 correlates with ROS generation. By inhibiting ROS generation, NLRP3 gets inactivated. Thus, NLRP3 is a critical target for treating DbCM. Many diabetic medications including metformin, thiazolidinediones, SGLT2 inhibitors and DPP-4 inhibitors improve DbCM by inhibiting the NLRP3 inflammasome activation[13].

The experimental study by Zhang *et al*[3] in a recent issue of the *World Journal of Diabetes* uncovers the potential benefits of the DPP-4 inhibitor teneligliptin in the prevention of DbCM in a diabetic mouse model. The following observations were made by the researchers: In streptozotocin-induced diabetic mice, teneligliptin could ameliorate myocardial hypertrophy and improve heart function. The study also observed that the drug could reduce the activation of NLRP3 inflammasome and the resultant myocardial injury. Finally, the drug increased the AMPK phosphorylation in the hyperglycaemia-induced cardiomyocytes and its beneficial effects on hyperglycaemia-induced cardiomyocytes were abolished by AMPK inhibition, suggesting that the effect of teneligliptin on DbCM is also regulated by AMPK.

This study also sheds light on one of the pathobiological mechanisms of DbCM and the great potential for the prevention and management of this dreaded disease with a readily available therapeutic option used for treating patients with type 2 DM for several years. The study would evoke enthusiasm among researchers in further evaluating the potential role of other DPP-4 inhibitors in the prevention and management of DbCM. However, we should be mindful of the lack of significant benefits of several translational experimental models in our day-to-day clinical practice. Therefore, the small experimental data published by Zhang *et al*[13] needs further validation in larger laboratory models and human beings to ensure the reproducibility of the positive results to compile clearer scientific evidence.

Moreover, we should also consider the reports on higher hospitalisation risk with some of the DPP-4 inhibitors in patients with advanced HF[14,15]. Real-world cohort studies including retrospective data analysis and randomised controlled trials investigating the use of DPP-4 inhibitors at various stages of DbCM may help us to explore the benefits and risks posed by these drugs shortly.

CONCLUSION

DbCM is a common, serious and underrecognized complication of DM, the pathobiological mechanisms of which are still not fully elusive. Present evidence suggests that several disease processes are involved in its causation and some available therapeutic avenues for prevention and management of DbCM with the currently available medications. However, there are still great knowledge gaps regarding the pathobiology and treatment options targeting DbCM. More research input is imperative for shedding light on our understanding of the mechanisms and management strategies of this disease. The study by Zhang *et al*[3], published in the *World Journal of Diabetes* is one such remarkable attempt. However, more experimental and clinical data with DPP-4 inhibitors in DbCM are necessary to affirm their study findings.

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FOOTNOTES

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EDITORIAL

Urgent call for attention to diabetes-associated hospital infections

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Abstract

In this editorial, we discuss the recent article by Zhao *et al* published in the *World Journal of Diabetes,* which highlights the importance of recognizing the risk indicators associated with diabetes mellitus (DM). Given the severe implications of healthcare-associated infections (HAIs) in hospitalized individuals- such as heightened mortality rates, prolonged hospitalizations, and increased costs- we focus on elucidating the connection between DM and nosocomial infections. Diabetic patients are susceptible to pathogenic bacterial invasion and subsequent infection, with some already harboring co-infections upon admission. Notably, DM is an important risk factor for nosocomial urinary tract infections and surgical site infections, which may indirectly affect the occurrence of nosocomial bloodstream infections, especially in patients with DM with poor glycemic control. Although evidence regarding the impact of DM on healthcare-associated pneumonias remains inconclusive, attention to this potential association is warranted. Hospitalized patients with DM should prioritize meticulous blood glucose management, adherence to standard operating procedures, hand hygiene practices, environmental disinfection, and rational use of drugs during hospitalization. Further studies are imperative to explore the main risk factors of HAIs in patients with DM, enabling the development of preventative measures and mitigating the occurrence of HAIs in these patients.

Key Words: Diabetes mellitus; Healthcare-associated infections; Nosocomial urinary tract infections; Surgical site infections; Nosocomial bloodstream infections

Core Tip: Diabetes mellitus (DM) is an important risk factor for nosocomial urinary tract infections and surgical site infections, which may indirectly affect the occurrence of nosocomial bloodstream infections, especially in DM patients with poor glycemic control. Diabetic patients should therefore pay more attention to the prevention of healthcare-associated infections, with a focus on the management of their blood glucose level.

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INTRODUCTION

Originally denoting infections linked to hospitalization (formerly termed nosocomial infections)[1], healthcare-associated infections (HAIs) now encompass infections occurring in hospital environments among patients or medical personnel. This includes infections emerging during hospitalization, as well as those arising within hospitals and after discharge (*e.g.* 48 hours post-hospitalization, within 30 days after receiving health care, or up to 90 days after undergoing certain surgical procedures), excluding infections existing pre-admission or present upon admission[2]. The prevalence of HAIs ranges from 3%-10%[3], presenting a significant challenge to healthcare workers (HCWs). HAIs can affect the treatment of primary diseases, increasing mortality and disability rates, as well as escalating the economic burden on patients' families and society, thereby straining healthcare systems worldwide, especially in developing countries[4]. HAIs yield worse outcomes than community-acquired infections, attributed to the compromised health status of hospitalized patients and the higher prevalence of multidrug-resistant (MDR) bacteria in hospital settings compared to community settings[5]. Severe HAIs often induce sepsis, estimated at 48.9 million cases globally in 2017 and remaining a leading cause of mortality worldwide[6]. In the recent issue of the *World Journal of Diabetes*, Zhao *et al*[7] presented an insightful article. Through an analysis of diabetes mellitus (DM) prevalence and risk factors among the elderly, the authors proposed that heightened attention to the abnormalities of related risk signs can aid in improving their overall health. This prompts consideration of HAI management in hospitalized patients with DM, given their higher risk of infection compared to the general population.

IS DM A RISK FACTOR?

The microenvironment shaped by DM triggers molecular changes in key components of the defense system, including neutrophils, natural killer cells, and macrophages, thereby affecting innate immunity[8]. Concurrently, hyperglycemia disrupts cytokine equilibrium, inhibiting the adaptive immune response against invading pathogens, and further increasing the susceptibility of diabetic patients to microbial infections[8]. Compared to the general population, patients with DM face an elevated risk of infection[9]. The severity of systemic microangiopathy in diabetic patients can lead to tissue damage, creating a favorable milieu for pathogenic bacteria due to the high sugar content, thereby fostering the growth of fungi and bacteria. Consequently, these wounds may serve as sites for secondary infections or facilitate the spread of infection to adjacent soft tissues or deeper into bones[10]. A significant proportion of hospitalized patients with DM present with preexisting infections upon admission, predominantly complex skin and soft tissue lesions. These patients carry common pathogens such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*), thereby increasing the likelihood of HAI occurrence, prolonged hospital stays, and increased susceptibility to adverse drug events[9,11]. Genito *et al*[12] revealed that co-infection with *S. aureus* and *Pseudomonas aeruginosa* in diabetic mice enhanced virulence potential. Meta-analyses corroborate the established association between HAIs and DM prevalence[13,14]. Notably, patients with severe DM, particularly those with elevated HbA1c levels, exhibit heightened susceptibility to intensive care unit (ICU) infections compared to non-diabetic patients and those with well-controlled blood glucose (Table 1). During the coronavirus disease 2019 (COVID-19) pandemic, patients with DM experienced a more severe disease course and heightened mortality rates[15]. Poor blood glucose management in patients with diabetes often precipitates recurrent infections, consequently escalating disability rates[10]. Therefore, heightened vigilance is imperative for preventing HAIs in hospitalized diabetic patients.

Nosocomial urinary tract infections

Nosocomial urinary tract infections (NUTIs) represent a prevalent form of nosocomial infection, with approximately 80% of cases attributed to catheter-associated urinary tract infections (CAUTIs)[2,16]. Between 15% and 25% of hospitalized patients undergo short-term indwelling urinary catheterization[17]. The insertion of urinary catheters, typically *via* the urethra or pubic bone, facilitates bacterial colonization of catheter surfaces or the formation of biofilms, thereby predisposing to infection[16]. Notably, the risk of CAUTIs increases with the duration of catheterization[2].

Patients with DM are at a heightened risk of developing NUTIs due to the high-sugar environment that favors the proliferation of bacteria[9,17]. The American College of Radiology has explicitly identified DM as a risk factor for acute pyelonephritis[18]. Evidence from a cross-sectional study conducted in Pakistan involving 1074 participants revealed a

Table 1 Research on diabetes mellitus and different HAIs

Type of HAIs	Primary disease	Results	Research type	Country	Ref.
NUTIs	-	The overall incidence of NUTIs in the diabetic group was significantly higher than in the non-diabetic group (13.67% *vs* 6.40%; $P = 0.004$)	Cross-sectional study	Pakistan	Ramrakhia *et al*[19]
	Undergoing surgery for colorectal cancer	DM with chronic complications is an independent risk factor of NUTIs	Retrospective study	United States	Kang *et al*[20]
	-	No significant association between DM and NUTIs	Retrospective study	France	Girard *et al*[21]
	-	DM was an independent risk factor for CAUTIs in elderly hospitalized patients	Case-control study	China	Shen *et al*[22]
HCAPs	-	A high mortality rate from HAPs was strongly correlated with DM	Prospective study	Egypt	Yakoub *et al*[30]
	-	The incidence of all types of pneumonia analyzed was significantly higher in patients with T2DM than in patients with non-T2DM	Retrospective study	Spain	Lopez-de-Andres *et al*[31]
	Acute cerebral infarction	HCAPs occurred in 80% of all patients with DM, which was significantly higher than that in non-DM patients (72.2%)	Retrospective study	China	Liu *et al*[32]
	Acute cerebral infarction	DM is not a risk factor for non-VAPs	Retrospective study	China	Yang *et al*[33]
	-	DM is not a risk factor for the development of HAPs or an increased mortality factor for HAP-related hospital complications	Meta-analysis	-	Vardakas *et al*[34]
	-	MDR bacteria-induced VAPs were not associated with DM	Meta-analysis	-	Hu *et al*[35]
	-	VAPs after cardiac surgery were not associated with DM	Meta-analysis	-	He *et al*[36]
SSIs	ACLR	DM may increase the risk of SSIs after ACLR	Meta-analysis	-	Zhao *et al*[40]
	HNC tumor resection	The risk of SSIs is more than 3 times higher in diabetic patients than in people without DM	Retrospective study	China	Gan *et al*[41]
	-	DM was an independent risk factor for SSIs for multiple surgical procedure types, and this association was highest for cardiac surgery compared with other types of surgeries	Meta-analysis	-	Martin *et al*[42]
	Noncardiac surgery	Glucose control in the first 24 hours after surgery was poor and the mean serum glucose concentrations of ≥ 150 mg/dL during this time were associated with increased rates of postoperative infectious complications	Meta-analysis	America	King *et al*[43]
NBSIs	-	A significant increase was noted in NBSIs and mortality in patients with DM, but DM was not an independent risk factor for NBSIs	Prospective observational study	Greece	Tsakiridou *et al*[37]
	-	Diabetic patients showed a 1.7-fold probability of developing ICU-acquired NBSIs compared to nondiabetic subjects	Prospective observational study	Greece	Michalia *et al*[48]
	-	CRBSIs in diabetic patients were 4.32 times higher than in non-diabetic patients	Prospective study	China	Jia *et al*[49]
	-	COVID-19 patients with DM were at higher risk of developing NBSIs	Descriptive cross-sectional study	India	Samantaray *et al*[50]
	-	DM was a predictor of shorter survival in patients with sepsis or septic shock	Prospective study	Germany	Schmidt *et al*[51]

HAIs: Healthcare-associated infections; DM: Diabetes mellitus; NUTIs: Nosocomial urinary tract infections; HCAPs: Health care-associated pneumonias; T2DM: Type 2 diabetes mellitus; VAPs: Ventilator-acquired pneumonias; MDR: Multidrug-resistant; SSIs: Surgical site infections; ACLR: Anterior cruciate ligament reconstruction; HNC: Head and neck cancer; HAPs: Hospital-acquired pneumonias; NBSIs: Nosocomial bloodstream infections; ICU: Intensive care units; CRBSIs: Catheter-related bloodstream infections; COVID-19: Coronavirus disease 2019.

significantly elevated incidence of NUTIs among diabetic individuals compared to the non-diabetic group[19]. In the study reported by Kang *et al*[20], DM with chronic complications was an independent risk factor for postoperative NUTIs in patients with colorectal cancer. Furthermore, Girard *et al*[21] retrospectively analyzed three prospective cohorts of 4669 older patients, including 4045 without catheterization, and reported a lack of significant association between DM and NUTIs, possibly attributed to the inclusion of all diabetic patients irrespective of catheterization status. However, a case-control study involving 7295 hospitalized patients with indwelling urinary catheters ≥ 60 years reported DM as an independent risk factor for CAUTIs[22]. Notably, the heightened risk of NUTIs may predominantly pertain to cases of complex insulin-dependent diabetes[21] and those related to *Trichosporon asahii*[23].

Crucial strategies for CAUTI prevention include minimizing unnecessary catheterizations and shortening catheterization durations[24]. Emphasis should also be placed on aseptic insertion, proper maintenance of medical devices, and adherence to established practices such as hand hygiene. A notable example is the implementation of comprehensive unit-based safety plans across 603 hospitals in the United States between 2011 and 2013, which led to a reduction in non-ICU catheter-related urinary tract infection rates through coordinated education efforts and resource distribution[25]. Future advancements in catheter materials hold promise for mitigating bacterial growth and CAUTI incidence.

Healthcare-associated pneumonias

Healthcare-associated pneumonias (HCAPs) represent a significant subset of HAIs with notable lethality. HCAPs are commonly categorized into hospital-acquired pneumonias (HAPs), occurring 48-72 hours post-admission, and ventilator-acquired pneumonias (VAPs), manifesting 48-72 hours post-endotracheal intubation[2]. Patients treated in ICU are at an increased risk of death not only due to their underlying conditions but also attributable to HAIs[1]. Moreover, ICU patients often require endotracheal intubation and invasive mechanical ventilation, with VAPs affecting approximately 9% to 27% of these patients[1]. The primary route of acquisition for non-ventilator HCAPs is bacterial, viral, and fungal aspiration[26]. The etiology of HAPs/VAPs varies geographically, influenced by ICU patient profiles, durations of hospitalization and ICU stays pre-onset, and risk factors for MDR pathogens[27]. In ICUs, gram-negative bacteria (GNB) predominantly cause bacterial pneumonia, often exhibiting high antibiotic resistance, while viral pneumonia is commonly attributed to influenza and respiratory syncytial virus[26,27].

Bacterial pneumococcal pneumonia is relatively common in patients with DM[28]. The adverse effects of DM on morbidity and mortality have been well-documented during influenza and COVID-19 epidemics[28,29]. A prospective longitudinal study in ICUs identified a strong correlation between DM and heightened mortality rates from HAPs[30]. A retrospective observational epidemiological study in Spain demonstrated a higher incidence of pneumonia in patients with type 2 DM (T2DM) compared to those without T2DM[31]. Another recent retrospective analysis of 1093 patients with acute cerebral infarction found that 80% of patients with DM had lung infections caused by GNB, which was significantly higher than those without DM (72.2%)[32]. However, the results are not conclusive. Another study, also from China, reported that DM was not a risk factor for pneumonia in elderly patients with acute ischemic cerebral infarction[33]. Similarly, Vardakas *et al*[34] reviewed 87 articles between January 1950 to April 2005 and found that DM was not a risk factor for the development of HAP or the increased mortality associated with HAP in hospitals. Moreover, Hu *et al*[35] conducted a meta-analysis of articles from Jan 1996 to Aug 2022, revealing that MDR bacteria-induced VAPs were not associated with DM. Similarly, another meta-analysis including 11 studies on VAPs after cardiac surgery concluded that the occurrence of VAPs was not associated with DM[36].

Evidence suggests that diabetic immune dysfunction alone may not suffice to precipitate clinically significant respiratory infections in the ICU. Multiple factors, including micro aspirations, illness severity, and mechanical ventilation duration, likely contribute to VAP occurrence[37]. For diabetic patients, prevention of aspiration, disinfection of respiratory apparatus, active hand washing, and reduction of GNB through oral care can reduce the occurrence of VAPs[2]. Factors such as infection control protocols, hospital environment, medication management, and invasive procedure utilization appear to play pivotal roles in HAP development in DM[34,37]. Vaccination emerges as an effective strategy for reducing pneumonia incidence in patients with DM.

Surgical site infections

Surgical site infections (SSIs) afflict 2%-11% of all surgical interventions[38], with patients' endogenous pathogens, including skin bacteria or gut bacteria in gastrointestinal surgeries, typically implicated[38]. In hospitalizations surpassing 5-7 days, exogenous and nosocomial flora are dominant, with *S. aureus* emerging as the most frequently isolated pathogen[38].

DM poses a notable risk factor for SSIs, attributed not only to its strong association with morbid obesity but also the effects of hyperglycemia and glycosylation on cell-mediated immunity[39]. Numerous studies have been published on the effect of diabetes on the increased incidence of SSIs and the potential relationship between hyperglycemia and SSIs. Zhao *et al*[40] conducted a meta-analysis of 23 studies, which focused on risk factors for SSIs after anterior cruciate ligament reconstruction, with moderate evidence from nine studies affirming the effect of DM on SSI. In a retrospective analysis of 632 patients with head and neck cancer who underwent surgery, Gan *et al*[41] reported that the risk of SSIs in diabetic patients increased by more than three times compared with those without DM. Similarly, Martin *et al*[42] summarized the independent association between DM and SSIs across multiple surgical procedures, encompassing 90 published studies between December 1985 and July 2015, and found that DM was an independent risk factor for SSIs for multiple surgical procedure types, with cardiac surgery exhibiting the highest association.

King *et al*[43] retrospectively analyzed 55408 individuals with DM who underwent various noncardiac surgeries and found that poor glycemic control in the first 24 hours after surgery, during which mean serum glucose concentrations of over 150 mg/dL were associated with an increased incidence of postoperative infectious complications. It is strongly recommended to implement perioperative glycemic control measures, maintaining perioperative plasma glucose levels

below 11.1 mmol/L in both diabetic and non-diabetic patients[39]. Moreover, preoperative risk factors can be mitigated through smoking cessation, addressing malnutrition, and judicious antibiotic therapy. Rigorous preoperative skin antiseptic and appropriate surgical preparation are imperative for SSI prevention[2,38].

Nosocomial bloodstream infections

Nosocomial bloodstream infections (NBSIs) constitute a grave bacterial infection with significant morbidity and mortality, frequently associated with intravascular devices, of which central venous catheters account for 72%[2]. The causative microorganisms of NSSIs are mainly coagulase-negative *Staphylococcus*, *S. aureus*, gram-negative *Bacilli*, and *Candida*[44,45]. The occurrence of NBSIs correlates with factors such as underlying diseases, catheter type and cleanliness, infused fluid type, and HCWs procedures (*e.g.*, time of catheter placement, frequent catheter manipulation, and catheter location)[45].

Patients with DM experience prolonged abnormal glucose metabolism, fostering a state of negative nitrogen balance characterized by decreased anabolism and increased catabolism, which puts the body in a state of negative nitrogen balance. Concurrently, reduced immunoglobulin synthesis diminishes immunity, augmenting infection susceptibility. Extensive antibiotics used during treatment can disrupt the microbial balance in the body, further increasing the risk of infection. While previous studies have suggested that DM is a risk factor for NBSIs[45-47], two prospective observational studies from Greece have presented conflicting evidence regarding DM's independent association with NBSIs, yet consistently emphasize a significant association between DM and BSI[37,48]. Similarly, in China, a prospective survey of 2605 ICU patients reported that the incidence of catheter-related BSIs in diabetic patients was 4.32 times higher than that in non-diabetic patients[49]. Furthermore, a recent descriptive cross-sectional study among patients with COVID-19 revealed that patients with DM were at a higher risk of developing NBSIs[50]. A prospective registry study in Germany collected data on the long-term survival of 1975 patients with sepsis, identifying DM as a predictor of shorter survival [51].

Prevention strategies for NBSIs include stringent infection control measures (hand hygiene, environmental disinfection), meticulous preparation before line insertion (skin preparation), adherence to correct operation protocols, and HCW education (*e.g.*, aseptic operation, removal of unnecessary catheters, insertion site of catheters, immediate replacement of moistened or detached catheter dressings, and timely removal of catheters)[2,52].

PREVENTION OF HAIs IN HOSPITALIZED DIABETIC PATIENTS

Vulnerable populations such as the elderly (> 60 years of age) and infants (< 1 year of age) face heightened susceptibility to HAIs, with underlying diseases, illness severity, and autoimmune status further increasing their susceptibility[2]. A retrospective study from China involving 472 patients with non-Hodgkin lymphoma reported that hospitalization duration, clinical stage, bone marrow infiltrate presence, and peripheral blood neutrophil count were independent risk factors[53]. Another case-control study from China found that different ABO blood types may be susceptible to certain types of HAIs[54]. Notably, in a vicious cycle, HAIs increase the length of hospital stay and prolonged hospitalization increases the risk of exposure to microorganisms in the hospital environment[5]. Microorganisms persist in the environment for a few hours or even months, making the hospital environment a significant contributor to the transmission of hospital pathogens to patients, especially from high-touch infected surfaces such as bedside tables, bed rails, faucets, or doorknobs[55]. In the ICU, the patient's oxygen masks, ventilators, and bed linen are the most contaminated[56]. Additionally, HCWs are susceptible to microbial contamination of their hands and/or gloves, as well as mobile phones used in their daily work, due to their frequent contact with the environmental surfaces of patient's rooms [57]. Additionally, water reservoirs in aqueous medical devices also provide a favorable environment for the growth of microorganisms such as non-tuberculous mycobacteria[58]. For instance, dental waterlines can be contaminated not only by microorganisms in the aquatic environment but also by bacteria from the patient's oral cavity, posing a risk of infection to both HCWs and patients[59]. Moreover, invasive procedures, nasogastric tubes, urinary and vascular catheters, central venous catheters, antibiotic abuse, and immunosuppressant use are other risk factors for HAIs[2,5,60]. Notably, the most common interventions in the ICU are endotracheal intubation and tracheostomy, as well as mechanical ventilation[61].

Approximately 20%-50% of HAIs occur in the ICU, mainly attributed to the patients' severe immunocompromised state. The emergence of MDR organisms has reduced the efficacy of antibiotics in treating many common infectious diseases, contributing to increased HAI-related mortality rates[2]. Additionally, the relatively enclosed hospital environment, dense population, and inadequate ventilation pose challenges for infection control[62].

Risk factors for diabetes-associated hospital infections are summarized in Figure 1. These infectious factors can be intervened with strict prevention and control measures. In 2015, the United States Centers for Disease Control and Prevention conducted a point-prevalence survey of 12299 patients from 199 hospitals, wherein 3.2% of hospitalized patients were reported to develop HAIs, which was a decrease from 4% in 2011. This decrease could be attributed to the effective control of surgical site and urinary tract infections[63]. Pathogens can also be transmitted through the hands of HCWs or contaminated objects/aerosols. In a hospital setting, the operating room, ICU environment, and related equipment have strict hygiene requirements, and strict adherence to these requirements can reduce the rate of HAIs[56]. HCWs should pay attention to hand hygiene and the standardized use of medical devices. Additionally, health education for patients can encourage self-management and behavioral changes in the hospital wards. Furthermore, especially in patients with DM, glycemic control and nutritional support during hospitalization can be beneficial for HAI prevention. Nutritional support, such as natural dietary supplements, vitamins, minerals, trace elements, and probiotics in

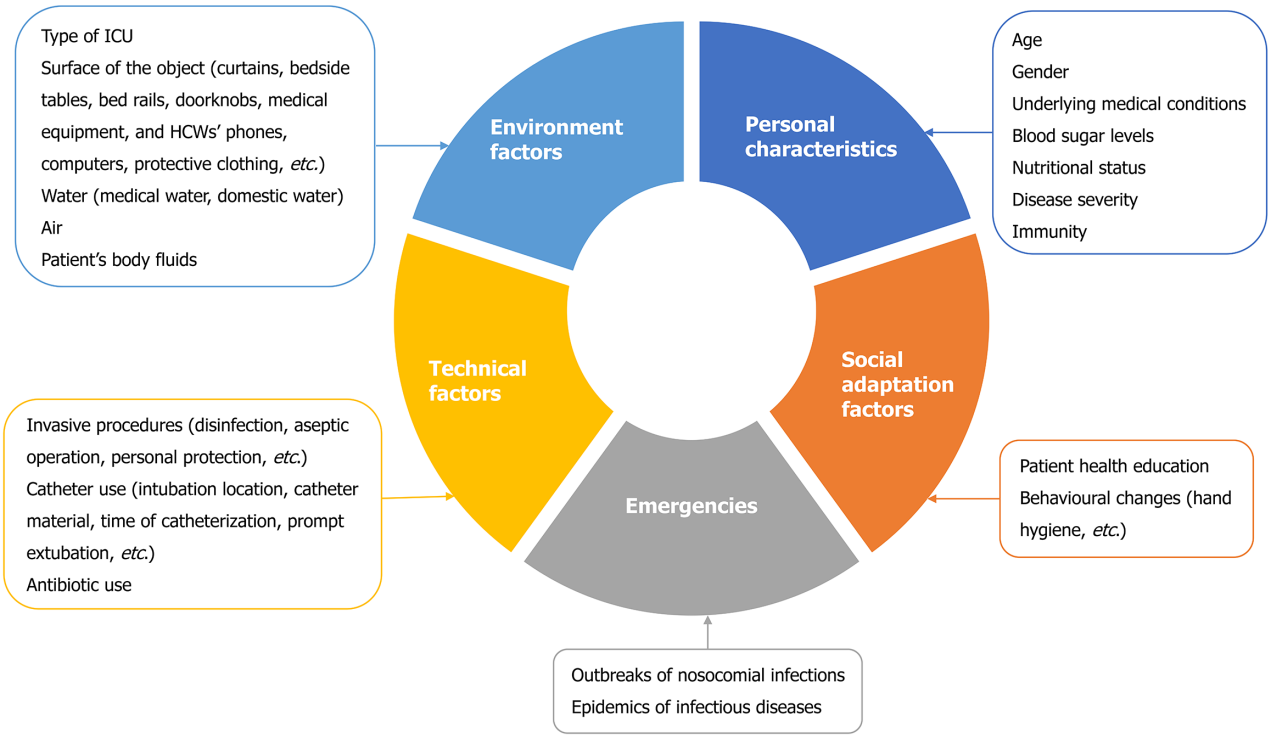

Figure 1 Risk factors for healthcare-associated infections in patients with diabetes mellitus. ICU: Intensive care units; HCWs: Health-care workers.

appropriate doses, not only improves blood sugar control but also enhances immune regulation[64].

CONCLUSION

Hospitalized patients with DM exhibit weakened skin and mucosal barriers, decreasing the body's ability to synthesize immunoglobulins and antibodies, rendering them more susceptible to pathogenic bacterial invasion and infection. Elevated blood glucose concentrations further exacerbate microbial reproduction and provoke inflammatory responses. While the current evidence falls short of conclusively establishing DM's role in HCAPs, it has unequivocally identified DM as a high-risk factor for NUTIs and SSIs, which may indirectly impact the occurrence of NBSIs. Further studies are required to improve our understanding of the role of DM and glycemic control in HAIs, so as to refine the guidelines for blood glucose control synchronously. Undoubtedly, heightened attention to HAI prevention, especially blood glucose management, is imperative for patients with DM.

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FOOTNOTES

Author contributions: Wang MK and Yang JS conceptualized, designed, and revised the manuscript; Yu XL drafted the manuscript; Zhou LY draw the table and graph; Huang X, Li XY and Pan QQ collected the literature; All authors have read and approved the final manuscript. Both Wang MK and Yang JS have conceptualized, proposed, designed, and supervised the whole process of the article, and played important and indispensable roles in the manuscript preparation and revision as the co-corresponding authors. Wang MK applied for and obtained the funds for this research project. Wang MK conceptualized, designed, revised and supervised the whole process of the project. Yang JS was instrumental, conceptualized, and revised the manuscript. This collaboration between Wang MK and Yang JS is crucial for the publication of this manuscript and other manuscripts still in preparation.

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REVIEW

Bariatric surgery and diabetes: Current challenges and perspectives

Yan-Fei He, Xiao-Dong Hu, Jun-Qiang Liu, Hu-Ming Li, Shuang-Feng Lu

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Abstract

Diabetes mellitus (DM) and obesity have become public issues of global concern. Bariatric surgery for the treatment of obesity combined with type 2 DM has been shown to be a safe and effective approach; however, there are limited studies that have systematically addressed the challenges of surgical treatment of obesity combined with DM. In this review, we summarize and answer the most pressing questions in the field of surgical treatment of obesity-associated DM. I believe that our insights will be of great help to clinicians in their daily practice.

Key Words: Bariatric surgery; Diabetes mellitus; Obesity; Metabolic surgery; Challenge

Core Tip: Obesity and diabetes are now public problems that threaten the health of people worldwide. Although bariatric surgery for the treatment of obesity associated with type 2 diabetes mellitus has been shown to be a safe and effective approach, its challenges have not been described systematically and comprehensively. This review is at the forefront of presenting and answering the challenges faced in the field of surgical management of obesity-associated diabetes mellitus today. It provides an up-to-date insight into the daily practice of clinicians.

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INTRODUCTION

Obesity and diabetes mellitus (DM) are now public health problems that threaten the health of people around the world. Obesity is associated with increased morbidity and mortality from various diseases. Historically, DM has long been recognized as a permanent disease that is difficult to cure. In recent years, with the rapid development of metabolic surgery, bariatric surgery has become an effective way to treat obesity and type 2 DM (T2DM)[1]. However, the surgical treatment of obesity and diabetes also faces significant challenges: For example, is bariatric surgery indicated for patients with type 1 DM (T1DM)? Is it suitable for people over 70 or for children under 16? Is it appropriate for people of normal weight or non-diabetics? How is new-onset diabetes managed after bariatric surgery? Here, our review will address these poignant questions and lead to more comprehensive studies in the future. We conducted a comprehensive search of PubMed, MEDLINE, CNKI, Wanfang, Embase, Google Scholar, Scopus, Wiley, Cochrane, and ScienceDirect online databases, as well as the medRxiv and bioRxiv gray literature, using the keywords "bariatric surgery", "diabetes", "obesity", and "metabolic surgery" either individually or in combination, and a final update of the latest cutting-edge researches in bariatric surgery was performed using Reference Citation Analysis (https://www.referencecitation-analysis.com).

Bariatric surgery for the treatment of obesity combined with DM not only results in significant and long-lasting weight loss[2], but also significantly reduces morbidity and mortality from a wide range of obesity-associated diseases, including T2DM[3], cardiovascular disease[4], obstructive sleep apnea[5], non-alcoholic fatty liver disease[6], and osteoarthritis[7]. Bariatric surgery has become an illuminating scientific model that has evolved from the treatment of obesity, to the treatment of T2DM, and further to the treatment of a range of metabolic syndromes centered on obesity and its accompanying metabolic disorders (including endocrine[1], cardiovascular[4], respiratory[5], and reproductive disorders [8]). More surprisingly, bariatric surgery has been reported to improve proteinuria, with a profound effect on chronic kidney disease[9]. The clinical use of laparoscopic bariatric surgery has grown exponentially over the past two decades, and different types of bariatric surgery may have different outcomes. Table 1 lists the major surgical types of bariatric surgery available today, their respective weight loss potential, advantages and disadvantages, and complications.

CHALLENGE ONE, FROM AGE: < 18 OR > 65 YEARS OLD

Childhood obesity is now a truly global health problem[10]. Data suggest that childhood obesity is strongly associated with the development of certain comorbidities, including cardiovascular disease[11,12], endocrine/metabolic disorders [13], respiratory disease[14], and musculoskeletal problems[15]. In addition, obese children often develop psychosocial problems such as mood disorders, anxiety, prejudice, and low self-esteem[16]. A study by Shah *et al*[17] suggests that prepubertal bariatric surgery is safe and effective and may not require age criteria. A review summarizing recent data on long-term outcomes following bariatric surgery in severely obese adolescents suggests that bariatric surgery has a beneficial impact on both weight loss and resolution of comorbidities in severely obese adolescents[18], which may help to remove barriers to the referral of adolescents for bariatric surgery[19]. With the increasing number of cases of metabolic surgery for the treatment of obesity in children and adolescents, the latest guideline, 2019 edition, recommends an age range of 2-18 years for surgery[20]. Even more encouraging is the fact that research has shown that bariatric surgery for severely obese adolescents is cost-effective[21]. Although it is more expensive than not having the surgery, it significantly improves quality of life, and overall costs, including medical care and medication, decrease after surgery. In other words, bariatric surgery increases short- to medium-term healthcare costs but can save money in the long term[22, 23]. Of course, some researchers have said that although bariatric surgery in adolescents can result in sustainable weight loss and reverse many of the complications associated with severe obesity, its safety and long-term effectiveness remain unclear, and therefore large, long-term prospective studies are still needed to determine the role of surgical treatment in childhood obesity[24].

Historically, bariatric surgery has been performed in patients < 65 years old because of the balance of risks (surgical safety, survival time, and availability of lifestyle changes) and benefits of surgery in older patients due to reduced cardiorespiratory fitness and frequent comorbidity with other underlying diseases. As quality of life improves and longevity increases, the need for bariatric surgery in older patients > 65 years is increasing, and studies have shown that bariatric surgery is safe and effective in older patients > 65[25,26] and even > 70 years[27-29] and that older patients appear to have better cardiovascular risk improvement[30]. In addition, elderly patients who undergo bariatric surgery also experience improvements in other areas, including physical and mental functioning, work capacity, self-confidence, sexual activity, and health-related quality of life[31]. Therefore, age per se should not prevent older patients from receiving optimal bariatric surgery for obesity and related complications.

Table 1 Types of bariatric surgery, their respective weight loss potential, advantages, disadvantages and complications

Procedure	Target weight loss	Advantages	Disadvantages	Complications
Laparoscopic adjustable gastric banding	20%-25%	No anatomic alteration, removable, adjustable	Erosion, slip, and prolapse	Gastric ptosis, outlet obstruction, erosion of the gastric wall by gastric banding
Sleeve gastrectomy	25%-30%	Easy to perform, no anastomosis, reproducible, few long-term complications	Leaks difficult to manage, 20%-30% risk of GERD	Bleeding of the cutting edge, leakage and stenosis
Roux-en-Y gastric bypass	30%-35%	Effective for GERD, can be used as second stage after sleeve gastrectomy	Internal hernias possible, long-term micronutrient deficiencies	Anastomotic leakage, bleeding, incisional infection, anastomotic stenosis and malnutrition
Biliopancreatic diversion with duodenal switch	35%-45%	Long-lasting weight loss, especially effective in patients with very high BMI	GERD, potential for hernias, technically challenging	Diarrhoea, nutrient deficiency
Single anastomosisduodeno-ileal bypass with sleeve gastrectomy	35%-45%	Single anastomosis with strong metabolic effect and low rate of early complications	Nutritional and micronutrient deficiencies possible, duodenal dissection	GERD, bile reflux
Intragastric balloon	10%-12%	Endoscopic or swallowed, good safety profile	Temporary (6 months) therapy, early removal rate of 10%-19%	Abdominal pain, nausea and vomiting
One-anastomosisgastric bypass	35%-40%	Simpler to perform, strong metabolic effects, no mesenteric defects	Potential for bile reflux, long biliopancreatic limb	Malnutrition, diarrhea
Transpyloric bulb	14%	Outpatient endoscopic procedures with long implantation times	Gastric mucosal erosion	Gastric ulcer
Aspiration therapy	12%-14%	Endoscopy, treatment is completely reversible	Tube-related problems/complications, 26% early removal	Abdominal pain, gastrostomy site infection
Vagal nerve blocking therapy	8%-9%	No anatomic changes, low complication rate	Explant required for conversion to another procedure	Pain at neuroregulatory site, indigestion, nausea
Gastric electric stimulation	20%-30%	No anatomical changes, minimal surgical trauma, high surgical safety	Difficulty in determining electrode implantation position and electrical stimulation parameters	Perforation, electrode dislodgement, electrode failure
Left gastric artery embolization	3%-14%	No anatomical changes, minimal surgical trauma	Difficulty in selecting embolic materials and target vessels	Ulcer, abdominal pain, and vomiting

GERD: Gastroesophageal reflux disease; BMI: Body mass index.

CHALLENGE TWO, FROM SPECIAL POPULATIONS

T1DM

Bariatric surgery can significantly improve glycaemic control and even alleviate diabetes in people with T2DM, so can it also benefit people with T1DM?

The International Federation for the Surgery of Obesity and Metabolic Disorders stated in 2016 that bariatric surgery not only treats obesity-related diseases but also improves insulin resistance and reduces insulin requirements, so obese T1DM can be used as a therapeutic indication for bariatric surgery[32]. Studies have shown that bariatric surgery leads to significant improvements in weight and cardiometabolic variables and modest improvements in blood glucose with few reported adverse events in cohorts of patients with obesity and T1DM[33]. The study by Vilarrasa *et al*[34] described the long-term outcomes of bariatric surgery in a cohort of patients with T1DM: Bariatric surgery provided some benefits in terms of weight loss, insulin requirements, obesity comorbidities and diabetic complications in patients with T1DM, but the long-term impact on glycaemic control was likely to be small. The study by Landau *et al*[35] demonstrated the same point; in people with obesity and T1DM, weight loss after bariatric surgery was successful without significant improvement in glycaemic control. A meta-analysis involving 9 studies with a total of 78 patients showed that glycated hemoglobin (HbA1c), insulin dose, and body mass index (BMI) improved after surgery, although the improvement in HbA1c did not reach statistical significance (P = 0.40)[36]. It is worth noting that the postoperative risks of diabetic ketoacidosis and severe hypoglycemic episodes should also be considered when performing bariatric surgery in the T1DM population[35].

In summary, bariatric surgery has brought new hope to the treatment of obese T1DM and, apart from its controversial effect on blood glucose, it has played a significant role in reducing BMI, improving obesity-related diseases, improving

insulin sensitivity, and alleviating or even reversing diabetic complications. As a result of these positive results, more and more patients with T1DM are willing to undergo bariatric surgery.

Normal weight diabetics

More than 10 years ago, some animal studies have successively confirmed that bariatric surgery has certain effects on non-obese DM[37,38]. Bariatric surgery was performed in 69 patients with T2DM and a BMI of 21-29 kg/m^2, with a mean follow-up of 21.7 months, resulting in satisfactory glycaemic control in 95.7% of patients[39]. The study of Zhang *et al*[40] showed that Billroth II gastrojejunostomy for the treatment of non-obese patients with T2DM had similar short- and medium-term glycaemic control effects as Roux-en-Y gastric bypass (RYGB) surgery for the treatment of obese patients with T2DM. Malapan *et al*[41] included 29 non-obese (BMI < 27 kg/m^2) T2DM patients who underwent laparoscopic RYGB and showed a significant reduction in mean body weight, mean BMI, and mean waist circumference, as well as mean systolic and diastolic blood pressure over a one-year prospective follow-up; and other biochemical variables, including blood glucose, HbA1c, C-peptide, insulin, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and homeostatic model assessment insulin resistance were significantly improved. More surprisingly, ten patients with T2DM and a BMI < 24.0 kg/m^2 (mean BMI 23.8 kg/m^2 ± 1.2 kg/m^2) were reported to have undergone duodenojejunal bypass, with resolution of T2DM in one patient and significant reductions in fasting glucose, 2-hour postprandial glucose and HbA1c at all postoperative time points, without significant weight loss[42]. However, the sample sizes of these clinical trials are small, and further large, multicentre, prospective, randomized controlled trials are needed to confirm the role of bariatric surgery in mildly obese or normal-weight patients with T2DM.

Non-diabetics

For obese and diabetic patients, bariatric surgery can provide relief from both conditions. But can bariatric surgery benefit non-diabetic patients? Bariatric surgery has been shown to restore visual cortical plasticity in non-diabetic obese patients [43]. Luo *et al*[44] reported on the effects of bariatric surgery in diabetic and non-diabetic patients who were followed for five years, and final subgroup analyses showed a linear increase in the percentage of non-diabetic patients from the worst clinical outcome to successful weight loss, and even more unexpectedly, non-diabetics lost a higher percentage of excess weight than diabetics over all five years. In addition, the Michigan Bariatric Surgery Collaborative reported that patients without DM were more likely to achieve a post-operative BMI of less than 30 kg/m^2 within one year[45]. It is well known that the obese population is at an increased risk of microvascular complications, and the risk is even greater in patients with T2DM. Bashir *et al*[46] evaluated the effects of bariatric surgery on microvascular complications in diabetic and non-diabetic patients, and they concluded that obese patients with or without DM benefit from the improvement in microvascular complications with bariatric surgery.

Immunodeficient patients

Although the risks of obesity in people with human immunodeficiency virus (HIV) are unclear, the incidence of DM and cardiovascular disease is increased in HIV-infected people, whether they are obese or not[47]. Many of those treating obese HIV patients are concerned that weight loss is unsafe for these patients. Flancbaum *et al*[48] reported on the first cohort of HIV-infected patients to undergo bariatric surgery, including six morbidly obese patients with obesity-related comorbidities who had asymptomatic, stable HIV infection. In their study, none of the patients showed a clinically significant deterioration in CD4 count or immune status, or progression of acquired immunodeficiency syndrome, and all of the patients' comorbidities improved or resolved. Fazylov *et al*[49] reported two cases of morbidly obese, asymptomatic HIV-infected patients who underwent laparoscopic RYGB, both patients successfully lost weight and maintained their weight loss at the last follow-up. Yang *et al*[50] evaluated the efficacy and safety of bariatric surgery in three HIV-infected patients. In their three cases, bariatric surgery resulted in stable CD4 counts and undetectable viral loads. A growing body of data suggests that bariatric surgery can be safely performed in HIV-infected patients without progression to acquired immunodeficiency syndrome.

Cancer patients

Obesity is a major risk factor for cancer morbidity and mortality[51,52]. Whether the anatomical, physiological, and microbiome changes induced by bariatric surgery in the gastrointestinal tract result in an increased risk of cancer in this area remains an open question. Numerous studies have shown that bariatric surgery is beneficial in reducing morbidity and mortality from all cancers[53-55], particularly breast[56-58] and endometrial cancers[59,60]. In addition to the effects on cancer development, some studies have suggested that bariatric surgery improves cancer prognosis in severely obese patients[61,62]. However, esophagogastric cancers have been reported to be induced after bariatric surgery[63], but epidemiological studies have not shown a higher incidence of these cancers in patients undergoing bariatric surgery compared with the general population[64]. Equally distressing is the conflicting evidence regarding the risk of colorectal cancer after bariatric surgery, with several studies showing a reduced risk of colorectal cancer in patients who have undergone bariatric surgery[65,66], while Aravani *et al*[67] showed that bariatric surgery was not associated with an increased risk of colorectal cancer, and others even suggest that patients who have undergone bariatric surgery are at an increased risk of colorectal cancer[68,69]. There are still some unanswered questions about the possible effects of bariatric surgery on cancer risk in patients with obesity, and there is a need for prospective, randomized controlled, and longer studies in populations undergoing bariatric surgery.

In addition to the special populations mentioned above, it is also important to consider what to do when pregnancy and bariatric surgery conflict, with the best evidence to date coming from expert opinion[70,71] that either pregnancy should be postponed during the bariatric surgery phase or bariatric surgery should be postponed during pregnancy.

More high-quality randomized controlled trials are needed to explore the relationship between bariatric surgery and pregnancy in more detail.

CHALLENGE THREE, FROM RELAPSE, INCLUDES WEIGHT REGAIN AND NEW-ONSET DM

Postoperative recurrence rate

Although a large proportion of patients with T2DM who have undergone bariatric surgery initially experience remission, some patients later relapse. It is estimated that around half of patients regain 5% of their body weight within two years of bariatric surgery[72]. An observational study of 300 patients undergoing RYGB showed that 37% of patients regained ≥ 25% of their maximum weight loss after a mean follow-up of 7 years[73]. The incidence of weight regain ranges from 9% to 91%, depending on the definition used[74]. Similarly, patients are at risk of developing a new onset DM after bariatric surgery[75]. In an analysis of data from a non-randomized, prospective, controlled trial, Carlsson *et al*[76] found that the incidence of new-onset T2DM after bariatric surgery was 6.8 cases per 1000 patient-years during a 15-year follow-up period. In a single-center retrospective study, Nor Hanipah *et al*[77] investigated the incidence of new-onset DM over 17 years after bariatric surgery, and they found that the incidence of new-onset DM was 0.4%; weight regain was common among patients with new-onset DM (> 50%).

Prediction of postoperative recurrence

Many studies have tried to explore the best biological and clinical predictors of T2DM recurrence after surgery[78-81]. Unfortunately, it is still difficult to predict which patients will experience postoperative weight regain or new-onset DM. A prospective study of 175 patients with RYGB T2DM and a 5-year follow-up found that several baseline factors (duration of DM, number of DM medications, HbA1c) and status at 1 year (fasting blood glucose, number of DM medications, remission status, percentage total weight loss) predicted the rate of DM remission and recurrence at 5 years [82]. A growing number of studies have shown that shorter duration of T2DM, better preoperative glycaemic control, lower baseline HbA1c and waist circumference, and greater postoperative weight loss are associated with higher rates of T2DM remission and lower risk of recurrence[83-85]. In addition, it has been suggested that gender is also an important factor in T2DM recurrence, with men having a lower risk of T2DM recurrence[86,87].

Management of postoperative recurrence

Despite the risk of weight regain or a new onset of DM, surgery still has the potential to have beneficial metabolic effects in the long term. Therefore, a higher risk of recurrence should not be a reason for exclusion from surgery. Careful preoperative patient selection and preoperative optimization appear to be important. Patients with postoperative recurrence, either weight regain or diabetic recurrence, are usually re-operated. Previous studies have shown that early surgery reduces the risk of recurrence[85,86]. Therefore, patients with obesity and T2DM should prioritize metabolic surgery at an early stage. Of course, it seems equally important to optimize lifestyle changes in patients after bariatric surgery[88]. In addition, the use of anti-obesity medications may provide more options for patients and healthcare providers[89].

CHALLENGE FOUR, FROM THE OPTIMAL TIMING OF SURGERY

Exactly when surgery is most effective in diabetes remains controversial. Recent studies have confirmed that the chances of achieving complete remission are negatively correlated with the duration of DM[86]. The available evidence strongly supports that patients benefit more from bariatric surgery when it is performed in the pre-diabetic or early stages of DM, or even during periods of abnormal glucose tolerance. Reserving surgery for more advanced and complex stages of the disease appears to be less beneficial for the clinical course of DM[90]. Based on these findings, we should offer bariatric surgery to patients with T2DM at an early stage of their disease. This idea seems revolutionary in the treatment of obesity, and it is exactly the same idea that underlies early cancer screening - offering bariatric surgery as a last resort, after years of obesity and T2DM have wreaked havoc on the body, is like waiting until the cancer has metastasized throughout the body.

CHALLENGE FIVE: WHO BENEFITS MOST FROM BARIATRIC SURGERY?

Obesity has been shown to be a heterogeneous disease. Despite the effectiveness of bariatric surgery, there are large individual differences in surgical outcomes. If we can predict who will benefit most, we may be able to target bariatric surgery more accurately. Previous studies have identified several predisposing factors for benefit from bariatric surgery, including patient factors (*e.g.*, age, BMI, gender, history and duration of DM, family history, glycemic control, and comorbidities), surgical factors (*e.g.*, surgeon experience, type of surgery), and social factors (*e.g.*, socioeconomics, medical conditions, and social discrimination)[91,92]. It has been shown that over 10 years, the number of treatments required to prevent one additional death was 8.4 in diabetic patients compared with 29.8 in non-diabetic patients[93], so diabetic patients benefit more from bariatric surgery than non-diabetic patients in terms of both the relative and absolute risk of

reducing morbidity and mortality.

Due to the high degree of patient heterogeneity, it is difficult to tailor surgical techniques to all patients, the decision to operate is empirical and there is no robust evidence-based approach, and predicting which patients will benefit most from bariatric surgery remains a challenge at present. There is evidence that there is less variation in outcomes when identical twins or first-degree relatives undergo bariatric surgery compared with unrelated individuals, but the variation in outcomes increases when bariatric surgery is performed on couples or people living together in the same environment [94-97]. This suggests that there may be biological factors that predict response to bariatric surgery. Studies have shown that a variety of omics technologies such as genomics, transcriptomics, proteomics, metabolomics, and lipidomics can provide a holistic molecular view of systems biology[98]. Unfortunately, currently available biomarkers cannot be used in clinical practice[99]. Prediction of the efficacy of bariatric surgery seems to improve when clinical variables are combined with genetic testing[100]. More research is needed to accurately predict who will benefit most from bariatric surgery.

CHALLENGE SIX, FROM REACTIVE HYPOGLYCEMIA

Reactive hypoglycemia, or post-bariatric surgery hypoglycemia (PBSH), was first described by Service *et al*[101] and most commonly occurs 1-3 years after RYGB[102], but has also been reported after sleeve gastrectomy[103]. Reactive hypoglycemia is a notoriously difficult-to-manage metabolic complication of bariatric surgery, the presentation of which can be non-specific and unrecognizable, and for which there are no clear diagnostic criteria or standardized tests, making it a challenging condition for both surgeons and endocrinologists.

Clinical features

PBSH typically presents with palpitations, sweating, weakness, and dizziness one to three hours after a meal, and some patients experience severe and potentially life-threatening symptoms of hypoglycemia, including seizures, coma, or loss of consciousness. Repeated hypoglycemic events lead to reduced quality of life for patients[104] and are associated with increased all-cause mortality[105], risk of dementia[106], and risk of motor vehicle accidents[107], and severe hypoglycemia increases morbidity and mortality in patients with T2DM[108].

Incidence

The true incidence of PBSH remains uncertain, with previous reports ranging from 0.1% to 50.0%[109-111]. However, as there are cases where hypoglycemia goes undiagnosed due to undetected hypoglycemia[103], it is clear that the true incidence is likely to be underestimated. The incidence of hypoglycemia based on continuous glucose monitoring data has been reported to be as high as 75% in postoperative RYGB patients[112].

Mechanisms

The pathogenesis of PBSH is controversial and involves rather complex mechanisms that can be divided into insulin-dependent and non-insulin-dependent mechanisms. Insulin-dependent mechanisms include excessive insulin secretion by pancreatic beta cells, decreased insulin clearance, and increased insulin sensitivity. Non-insulin-dependent mechanisms include functional and structural adaptations in the gut after bariatric surgery that affect gastric emptying rate, glucose absorption, glucagon-like peptide-1 (GLP-1) levels, bile acid levels, gut microbiota, and counter-regulatory mechanisms to prevent hypoglycemia[113-118].

Management

The management of PBSH is equally challenging and requires a multidisciplinary approach that includes dietary, pharmacological, and surgical interventions. The first line of treatment for PBSH is dietary modification, particularly restriction of carbohydrate intake and avoidance of simple carbohydrates[119,120]. If dietary changes do not adequately control the patient's symptoms, pharmacological treatment with GLP-1 agonists, acarbose, growth inhibitor analogues, and calcium channel blockers may be considered[121-123]. Avexitide and glucagon pumps are two newer therapeutic options that have recently been tested[124,125]. Recent studies have shown that the acute effects of exercise training on glycaemic homeostasis after bariatric surgery can be used as a non-pharmacological adjunctive therapy[126]. Surgery is the last option when all other treatments have failed [127,128].

Predictors

In a prospective controlled Swedish study of patients treated with bariatric surgery for up to 31 years, male gender, older age, and higher HbA1c levels were associated with hypoglycemia-related events[129]. Previous studies have found that preoperative HbA1c, lower BMI, and greater postoperative weight loss are predictors of PBSH[109,130]. A study by Nielsen *et al*[131] showed that younger age and lower postoperative BMI were strong predictors of PBSH, while a study by Belligoli *et al*[132] demonstrated that the incidence of hypoglycemia was higher in younger patients with lower fasting blood glucose levels and higher triglyceride levels before laparoscopic sleeve gastrectomy. It has also been shown that the longer the duration of surgery, the higher the risk of hypoglycemia[109,133].

Much remains to be learned about the causes, diagnosis, and treatment of PBSH, and it will be necessary in the future to reach a consensus on the definition and diagnostic criteria for PBSH and to adopt a more scientifically intelligent diagnostic paradigm for the long-term monitoring of bariatric surgery patients in order to detect and reduce the risk of PBSH promptly. In conclusion, despite the risk of serious adverse events from reactive hypoglycemia, it is not sufficient

to be a contraindication to bariatric surgery.

Future prospects

As the prevalence of obesity continues to rise, medical technology improves, and health awareness increases, more and more people with DM will benefit from bariatric surgery. With further basic and clinical research, we believe that the challenges we face today will become clearer in the future. Looking ahead, if one day we discover the mechanism for treating diabetes surgically, looking back, bariatric surgery may be just one step in the treatment of diabetes; we even look forward to the day when a genetic means of curing diabetes in the truest sense of the word will be found, by increasing or eliminating a particular segment of a gene, or by increasing or suppressing the expression of a particular factor.

Limitations

This review also has some limitations. Firstly, most of the studies had a short follow-up period, and the advantages and disadvantages of bariatric surgery for specific populations require longer follow-ups to obtain more stable and reliable results. Secondly, we did not adequately discuss the effects of bariatric surgery on pregnancy and gender.

CONCLUSION

This review summarizes the types of procedures and their advantages and disadvantages for the surgical treatment of DM, highlights the serious challenges faced today, stands at the forefront of perspectives, systematically answers these poignant questions, and boldly envisions the future, providing invaluable insights into the field of surgical treatment of DM.

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FOOTNOTES

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MINIREVIEWS

Mechanism underlying the effects of exercise against type 2 diabetes: A review on research progress

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Abstract

Exercise has emerged as one of the important and effective non-drug therapies used for management of type 2 diabetes (T2D) in certain nations. The present report summarizes the latest findings from the research on the beneficial effect of exercise on T2D. The objectives were to provide references for the theoretical study and the clinical practice of exercise-based management of T2D, in addition to identify the limitations of the existing literature, thereby provide direction for future research in this field.

Key Words: Type 2 diabetes; Diabetes; Exercise; Mechanism; Inflammation

Core Tip: Exercise significantly benefits type 2 diabetes (T2D) management by enhancing insulin sensitivity, regulating glucose and lipid metabolism, and reducing inflammation. This review reveals how different exercise types, including aerobic, resistance, and flexibility exercises, contribute to these effects. It also highlights the need for personalized exercise programs to optimize T2D treatment. The article underscores the importance of incorporating exercise into comprehensive care strategies for T2D, pointing towards future research to refine and personalize exercise recommendations for individuals with T2D.

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INTRODUCTION

Diabetes incidence is increasing every year, and the reason is improved living standards, altered lifestyles, and changes in the methods used for food processing. Diabetes has consequently emerged as a global health crisis, contributing enormously to the economic burden, mortality, and disability worldwide[1]. Type 2 diabetes (T2D) accounts for 90%-95% of all cases of diabetes[2], and the number of people with T2D is expected to increase to 556 million by the year 2030[3]. Unfortunately, T2D is incurable and might present with various complications, such as cardiovascular diseases, stroke, kidney failure, and other critical conditions. Moreover, T2D ranks among the diseases with the highest mortality rate worldwide. Patients with T2D have to consume medication for their entire lives, and treatment costs are huge, placing an immense economic burden on the families of these patients while leading to a serious decline in the quality of life of the patients. Therefore, it is imperative to search for effective interventions for this metabolic disease. The objectives of the treatment for T2D should be to facilitate an individualized treatment plan, achieve and maintain optimal blood glucose and metabolism, lipid profile, and blood pressure level, and prevent or delay the development of any chronic complications[4].

In 1935, Joslin, a famous scholar who worked on diabetes, proposed that "physical activity should be regarded as a treatment tool for diabetes". However, the research exploring exercise therapy for T2D was scarce initially. In 1969, the term "exercise prescription" was officially adopted by the World Health Organization, following which the topic of the therapeutic effects of exercise on T2D began attracting considerable attention. Exercise is currently recommended as an important non-pharmacological therapeutic strategy for the management of T2D by certain major national and international guidelines, and is, therefore, considered critical to managing the cases of T2D and achieving and maintaining the desired therapeutic goals and improving the quality of life of the affected patients[5-9]. According to the statement of the American College of Sports Medicine, various kinds of physical activity, inclusive of although not limited to planned exercise, could greatly enhance the health and glycemic management of individuals of all ages with T2D, such as flexibility and balance exercise in adults[5]. Research has demonstrated that different types of exercise allow for intervening in T2D and could even reduce the incidence of diabetic complications *via* different mechanisms. Glucose metabolism, immune inflammation, endothelial function, intestinal flora, and epigenetics have been reported to play significant roles in the beneficial effect of exercise on T2D.

Among the various strategies adopted to combat T2D, exercise has been recognized as one of the most powerful approaches. However, the mechanism through which exercise contributes to diabetes management and prevention remains to be elucidated. In this context, the present review explored the latest findings of the research exploring the mechanism underlying the effect of exercise against diabetes to offer insights that could revolutionize the therapeutic approach adopted to overcome this chronic metabolic condition.

EFFECT OF EXERCISE ON T2D AND THE ASSOCIATED COMPLICATIONS

Studies have demonstrated that exercise effectively prevents the development of T2D and its associated complications by improving insulin resistance, lipid metabolism, and inflammatory reaction[10,11]. In the context of the complications associated with T2D, exercise reportedly improves cardiovascular complications by enhancing the expression of extracellular superoxide dismutase (EcSOD) in skeletal muscles to attenuate oxidative stress, aberrant cell signaling, and inflammation[12]. In addition, progressive resistance training reportedly improved muscle strength in knee extensors and flexors and the motor function of individuals with T2D polyneuropathy[13]. The animal experiments conducted to explore diabetes-induced kidney injury in T2D revealed that treadmill exercise training significantly suppressed the levels of albuminuria, tubulointerstitial fibrosis, inflammation, and oxidative stress in the kidneys of Wistar fatty rats[14].

Multiple exercise training modalities, such as aerobic exercise, resistance exercise, combined exercise, and flexibility training, are recommended by the American Diabetes Association, American College of Sports Medicine, European Society of Cardiology, Belgian Physical Therapy Association, and Exercise and Sports Science Australia[15] (Figure 1).

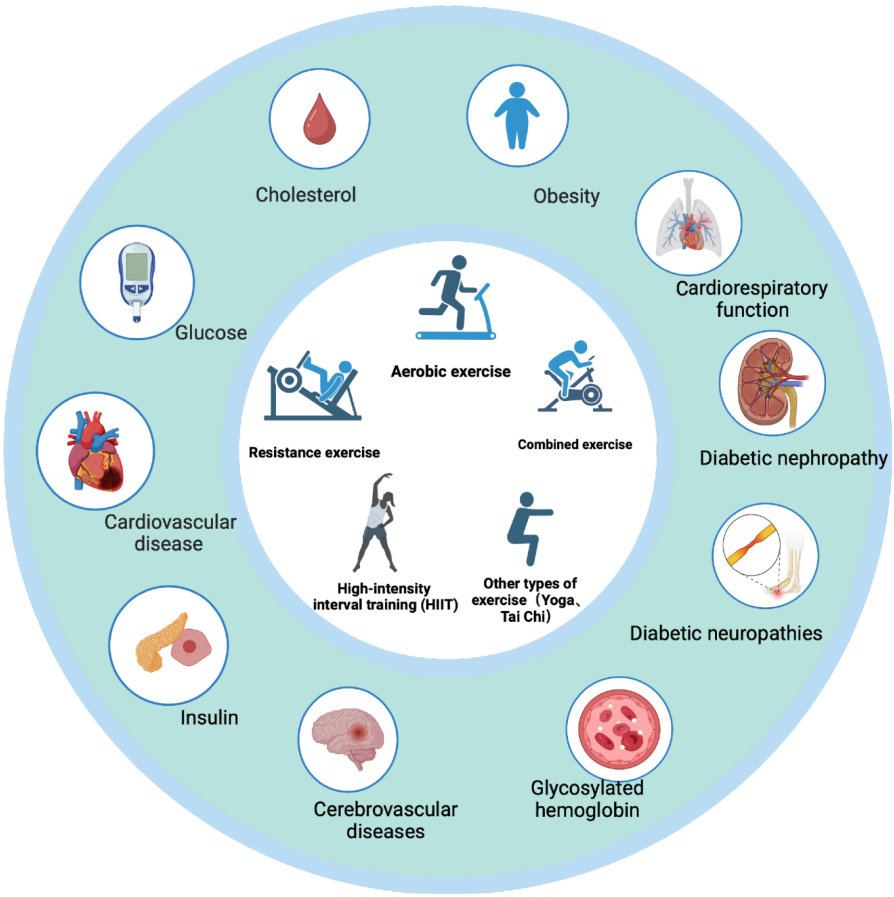

Figure 1 Effects of different types of exercise on type 2 diabetes and the associated complications. Citation: The authors have obtained the permission for figure using from the BioRender.com (Supplementary material)[41].

Aerobic exercise

Aerobic exercise training improves insulin sensitivity in adult T2D patients, while paralleling improved mitochondrial function[16]. An even-day training involving vigorous exercise reportedly improved glycemia in T2D, increased peripheral insulin sensitivity and responsiveness, and inhibited the production of hepatic glucose[17]. Aerobic exercise also improved the control of glycated hemoglobin (HbA1c) levels and increased cardiorespiratory fitness[6].

Resistance exercise

Resistance exercise training reportedly improved strength, bone mineral density, blood pressure, blood lipids, skeletal muscle mass, and insulin sensitivity in adult patients with T2D[5]. According to the guidelines of the American College of Sports Medicine and the American Diabetes Association, resistance exercise training provides optimal benefits for reducing the risk of cardiovascular diseases and minimizing injuries[8]. It has been demonstrated that resistance training exercises improve the control of blood glucose and HbA1c levels[18].

Combined exercise training

A combined aerobic and resistance exercise intervention might be superior to the implementation of either of these modalities separately and is effective in improving the levels of inflammatory, metabolic, and lipid markers in middle-aged and older adults with T2D[19]. The combined practice of aerobic and resistance exercises reportedly led to a greater reduction in the HbA1c levels than that achieved using any of the training modalities alone in adults with T2D[5].

High-intensity interval training

High-intensity interval training (HIIT) is a regimen comprising aerobic training conducted during the 65%-90% VO_2 peak or 75%-95% heart rate peak (HR peak) for a duration of 10 seconds to 4 minutes, followed by 12 seconds to 5 minutes of active or passive recovery.

As a potentially time-efficient modality, HIIT elicits significant physiological and metabolic adaptations. In adults with T2D, one session of HIIT (10 seconds × 60 seconds cycling at approximately 90% HRmax) reduces postprandial hyperglycemia while improving the cardiorespiratory fitness levels and reducing the HbA1c levels and the body mass index (BMI). HIIT also reduces the risk factors for developing cardiovascular diseases, enhances the diastolic function, increases the left ventricular wall mass, enhances the end-diastolic blood volume due to increased stroke volume and left ventricular ejection fraction, and improves the endothelial function[5,20].

Other types of exercise training

Yoga could lead to significant improvements in several indices significant for the management of T2D, including glycemic control, lipid levels, and BMI. A limited set of data suggests that yoga might also lower oxidative stress and blood pressure, enhance pulmonary and autonomic function, mood, sleep, and quality of life, and reduce the consumption of medication in adults with T2D[21]. Tai Chi, on the other hand, could improve glycemic management, balance, and neuropathic symptoms, in addition to enhancing certain dimensions of the quality of life[22].

MECHANISM UNDERLYING THE EFFECT OF EXERCISE AGAINST T2D

Mechanisms revealed through metabolomics

Glucose metabolism: T2D is a chronic metabolic disease characterized by the dysregulation of systemic glucose homeostasis. While the precise etiology of T2D remains to be comprehensively understood, studies have implicated impairments in key glucoregulatory functions in the pathogenesis of this disease. Exercise training, including both aerobic and resistance training, could ameliorate the hyperglycemia associated with T2D by stimulating alterations in skeletal muscle glucose transport and glucose metabolism[23]. Multiple studies have demonstrated that exercise training stimulates alterations in skeletal muscle glucose transport and glucose metabolism by improving the glycolytic capacity of skeletal muscles, decreasing the activity of the hexosamine pathway in skeletal muscles, increasing the glycogen content in skeletal muscles, and stimulating glucose flux *via* the pentose phosphate pathway in skeletal muscles. Owing to the duration of the exercise program, the exercise intensity, and the number of muscle groups stimulated, aerobic exercise may often lead to a pronounced effect on glucose transport and glucose metabolism compared to resistance exercise[24,25] (Figure 2).

Insulin sensitivity and insulin resistance: Insulin was one of the greatest scientific discoveries of the 20th century. Insulin plays a major role in the regulation of glucose in the body and also in the treatment of diabetes. The dominant control of glucose metabolism by insulin occurs under the regulation of complex and highly regulated hormonal and signaling cascades that may exert different and unique effects on skeletal muscles. As the primary tissue involved in insulin-stimulated glucose metabolism, skeletal muscles are a key driver of systemic glycemic control. Skeletal muscles also respond in a unique manner to muscle contraction or exercise with increased sensitivity to the subsequent insulin stimulation. Exercise training sensitizes the skeletal muscles to exhibit a glucose uptake response after insulin stimulation and might activate 5′ adenosine monophosphate-activated protein kinase (AMPK) in the muscles and promote enhanced translocation of insulin-stimulated glucose transporter 4 (GLUT4)[26]. Exercise training of muscle attenuates the subsequent insulin effects in the muscle, including enhanced expressions of muscle GLUT4 and hexokinase and increased mitochondrial capacity, capillarization, and insulin-dependent muscle blood flow[27].

Immune inflammation: Low-grade chronic inflammation *in vivo* is considered one of the pathogeneses of T2D, and pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 reportedly activate intracellular signaling molecules, which then increase the expressions of inflammatory mediators and impair insulin activity through the nuclear translocation of various nuclear factors[28]. A single strenuous exercise stimulates the local muscle tissue to release inflammatory factors such as TNF-α and IL-6 although without releasing these factors into the bloodstream, such that a single strenuous exercise does not exert much effect on the level of systemic pro-inflammatory factors. Long-term regular exercise, however, reduces the basal levels of the corresponding inflammatory factors and causes the body to produce physiological adaptation, such that the levels of inflammatory markers in the entire body are lowered, which then improves the function of pancreatic islets[19].

Lipid metabolism: Cell dysfunction caused by excessive intracellular lipid and ectopic accumulation which is referred to as lipotoxicity, and this phenomenon may subsequently inhibit the insulin signaling pathways, reduce insulin sensitivity, and impact the progression of T2D to a certain degree. It is currently believed that intramuscular lipids do not inherently exhibit lipotoxicity, and rather their metabolic intermediates exhibit lipotoxicity. Intramuscular fat (IMTG) dynamics are disrupted in patients with T2D and its precursor phase, causing the lipophilic intermediates such as diacylglycerol and ceramide to accumulate, and these intermediates then interfere with insulin production. Exercise accelerates the oxidation and conversion of IMTG, and although it increases the levels of intramuscular lipids as well, the reduced levels of lipid intermediates improve insulin function to a certain extent[29,30]. At the same time, patients with T2D have a higher unsaturated intramyocellular fat. Clinical studies have found that patients with T2D can increase contributions of the saturated intramyocellular fatty acid pool through endurance training, adapt to the lipid storage in muscle cells and improve insulin resistance[31].

Endothelial function: Endothelial cells maintain vascular homeostasis, and any imbalance in their physiological function leads to vasoconstriction, possible thrombosis, inflammation, *etc.*, which are the physiological and pathological signs of T2D and are considered important factors for vascular complications in patients with diabetes. Exercise, particularly aerobic exercise and a combination of aerobic and resistance exercises, enhance the vasodilation function[32]. The main mechanism through which exercise produces such changes is as follows: First, exercise increases the intravascular blood flow, enhances the endothelial cell shear force, and increases Nitrogen Oxide (NO) synthesis and bioavailability; next, exercise reduces factors that initiate endothelial dysfunction, such as oxidative stress and reduced expression of pro-inflammatory molecules; finally, exercise restores the function of endothelial progenitor cells, thereby promoting endothelial repair and angiogenesis. Since patients with T2D exhibit a continuous high glucose state in their bodies along with

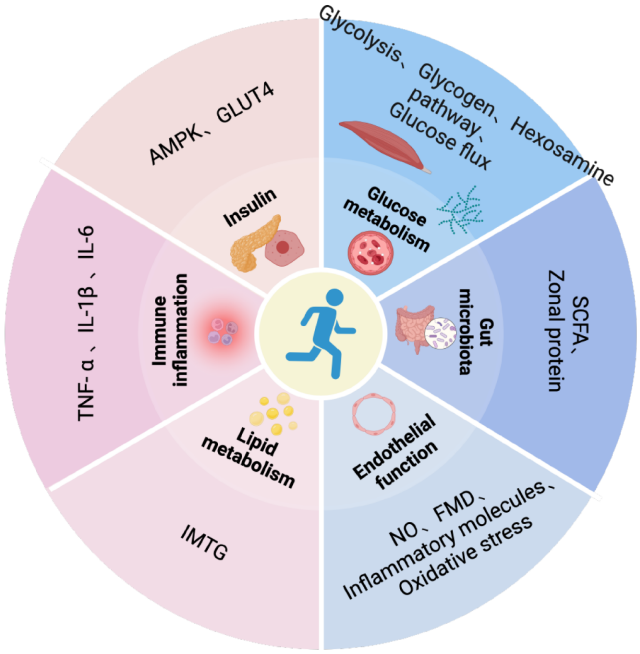

Figure 2 Effects of exercise against type 2 diabetes through reduced insulin resistance, improved insulin sensitivity, increased glucose transport and metabolism, reduced inflammatory response, and regulated lipid metabolism. AMPK: Activated protein kinase; IMTG: Intramuscular fat; FMD: Flow-mediated dilation; SCFA: Short-chain fatty acid; GLUT: Glucose transporter; TNF-α: Tumour necrosis factor alpha; IL: Interleukin. Citation: The authors have obtained the permission for figure using from the BioRender.com (Supplementary material)[41].

the accumulation of advanced glycosylation end products and reactive oxygen species in the circulation, the bioavailability of NO after exercise is lower than that observed in non-diabetic patients. Moreover, the capacity of mobilizing endothelial progenitor cells is weaker in patients with T2D compared to non-diabetic patients. These two factors lead to a lower improvement in flow-mediated dilation (FMD) after exercise in patients with T2D compared to non-diabetic patients. Nonetheless, the FMD of patients with T2D is improved significantly after exercise[33,34].

Gut microbiota composition and the intestinal barrier function: Currently, no definite conclusion has been reached on whether alterations in the intestinal microorganisms in patients with T2D are the cause or the consequence of diabetes. However, it is affirmative that changes in the gut microbiota play an important role in the progression of T2D. The diversity of gut bacteria is decreased in patients with T2D, mainly due to a decrease in the abundance of short-chain fatty acid (SCFA)-producing bacteria. In addition, increased permeability facilitates the entry of inflammatory factors present in the intestine into systemic circulation[35]. The interaction between SCFAs and G protein-coupled receptors increases the secretion of GLP-1, thereby regulating blood glucose levels. The mechanism through which exercise training induces changes in gut microbiota and the intestinal barrier function in patients with T2D remains to be understood, although the reasons for improvement in the internal environment of patients with T2D could include the following: On one hand, exercise training increases the abundance of SCFA-producing bacteria in the intestine, which increases the content of SCFA in the intestine and the blood, thereby partially improving insulin resistance; on the other hand, intestinal zonal protein disrupts the intestinal barrier and increases intestinal permeability, while exercise intervention reduces the concentration of zonal proteins[36,37].

Mechanisms revealed through transcriptomics

Epigenetic mechanisms, including DNA methylation, histone modifications, and RNA-mediated processes, are known to control gene activity and organism development. Disruption of the epigenetic balance could, therefore, lead to various pathologies and contribute to the development of diseases such as T2D (Figure 3). The differential gene expression analysis in the human islets of T2D revealed increased DNA methylation and decreased expressions of INS, PDX1, PPARGC1A, and GLP1R, which are associated with impaired insulin secretion. Meanwhile, high glucose and HbA1c levels could directly increase DNA methylation in these genes. Exercise is thought to partially regulate gene expression and phenotypic outcomes in epigenetics. It has been suggested that alterations in the DNA methylation of genes involved in the AMPK, insulin, and calcium signaling pathways in skeletal muscles after exercise, and these genes include MEF2A, RUNX1, NDUFC2, and THADA, which are reported to be important in T2D and muscles[38].

In addition to methylation, upregulation of the genes involved in insulin function and glucose metabolism, such as fatty acid synthase (FASN), was noted in the skeletal muscles of T2D mice, while the expressions of *LPIN1*, *TBC1D1*, *HK2*, *HMOX1*, *SORBS1*, *PPARGC1A*, and other genes were observed to be downregulated. After exercise intervention, most of the patients with T2D benefited from exercise to a certain extent[39]. The benefits manifested mainly as decreased levels of glycosylated hemoglobin, body fat percentage, and BMI. After exercise, FASN levels decreased in skeletal muscles,

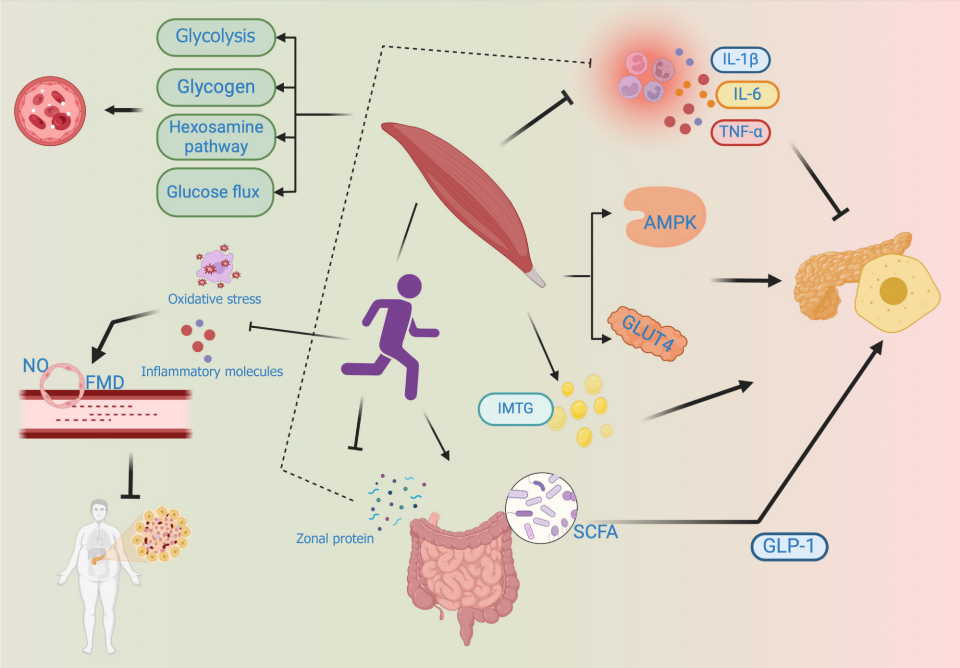

Figure 3 The molecular mechanisms underlying the effect of exercise against type 2 diabetes. AMPK: Activated protein kinase; IMTG: Intramuscular fat; FMD: Flow-mediated dilation; SCFA: Short-chain fatty acid; GLUT: Glucose transporter; TNF-α: Tumour necrosis factor alpha; IL: Interleukin. Citation: The authors have obtained the permission for figure using from the BioRender.com (Supplementary material)[41].

while the levels of LPIN1, TBC1D1, HK2, HMOX1, SORBS1, and PPARGC1A increased. However, certain patients presented no significant decrease in glycosylated hemoglobin and body fat percentage after exercise training. In these patients, the mRNA expressions of PPARα and ELOVL1, which are involved in lipid metabolism, and those of CHKB, CISD2, and FOXO1, which are involved in mitochondrial function, were lower compared to the corresponding levels in responsive patients[40].

CONCLUSION

The understanding of the multifaceted mechanisms through which exercise combats diabetes and its complications is essential for developing effective treatment strategies tailored to individual requirements, emphasizing lifestyle modifications, in addition to medication, as pillars of diabetic care. Numerous studies have demonstrated that exercise could prevent and treat T2D by reducing insulin resistance, improving insulin sensitivity, increasing glucose transport and metabolism, reducing the inflammatory response, and regulating lipid metabolism, among other mechanisms. Exercise might exhibit positive effects on T2D and the associated complications through epigenetic changes as well, although the specific mechanisms underlying such effects are yet to be entirely understood. Significant difficulties remain in issuing personalized exercise prescriptions for different T2D patients in clinical practice. Therefore, future research could include large-scale clinical trials conducted with T2D patients to provide further accurate and efficient guidance for the treatment of these patients.

The clinical evidence of the benefits of physical exercise as a treatment measure for patients with T2D is ample, well-recognized, and widely accepted, and, therefore, deserves to be incorporated into clinical treatment plans. However, several challenges are encountered when the exercise recommendations have to be implemented. The diverse set of exercise modalities available and the inconsistencies in the exercise prescription parameters render it difficult to perform a precise analysis of the dose-response relationship between physical activity and health outcomes. The lack of high-quality evidence regarding the dose-response relationship renders it challenging to recommend measurable and achievable exercise targets in physical activity guidelines. Therefore, exercise prescriptions have to be personalized for individuals according to their habits, preferences, motivations, and tolerance levels, rather than providing generic prescriptions, describing exercise duration, intensity, and frequency based on the clinical testing. Moreover, while exercise has been proven to manage T2D by reducing insulin resistance, improving insulin sensitivity, increasing glucose transport and metabolism, reducing the inflammatory response, and regulating lipid metabolism, it remains unclear whether exercise exerts sustained regulatory effects. These limitations highlight the importance of future research, which could overcome these obstacles by further investigating and elucidating the molecular biology-based mechanisms underlying the effects of exercise against T2D and further comprehensively evaluating the expected type and level-response relationship between exercise and T2D by using more standardized exercise prescription parameters, to realize the full clinical therapeutic effects of exercise. In addition, theoretical demonstration of the effects of exercise therapy against T2D requires further formal evaluation through prospective epidemiological studies.

FOOTNOTES

Author contributions: Peng CJ, Chen S, and Yan SY conducted the original search and wrote the first draft of the paper; Zhao JN and Luo ZW screened the selected articles and contributed to subsequent drafts of the manuscript; Luo ZW, Qian Y, and Zhao GL designed the outline of the manuscript; Qian Y and Chen S generated the original idea of this study and provided suggestions. Luo ZW and Zhao GL, as co-corresponding authors, contributed equally to this article (designed the study), while Peng CJ and Chen S were the co-first authors.

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MINIREVIEWS

Advances in the treatment of diabetic peripheral neuropathy by modulating gut microbiota with traditional Chinese medicine

Ye-Yao Li, Rui-Qian Guan, Zhi-Bo Hong, Yao-Lei Wang, Li-Min Pan

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Abstract

Diabetic peripheral neuropathy (DPN) is one of the strongest risk factors for diabetic foot ulcers (neuropathic ulcerations) and the existing ulcers may further deteriorate due to the damage to sensory neurons. Moreover, the resulting numbness in the limbs causes difficulty in discovering these ulcerations in a short time. DPN is associated with gut microbiota dysbiosis. Traditional Chinese medicine (TCM) compounds such as Shenqi Dihuang Decoction, Huangkui Capsules and Qidi Tangshen Granules can reduce the clinical symptoms of diabetic nephropathy by modulating gut microbiota. The current review discusses whether TCM compounds can reduce the risk of DPN by improving gut microbiota.

Key Words: Diabetes; Peripheral neuropathy; Traditional Chinese medicine; Gut microbiota; Diabetic foot ulcer; Treatment

Core Tip: Diabetic peripheral neuropathy (DPN) is associated with gut microbiota dysbiosis. Traditional Chinese medicine (TCM) compounds such as Shenqi Dihuang Decoction, Huangkui Capsules and Qidi Tangshen Granules can reduce the clinical symptoms of diabetic nephropathy by modulating gut microbiota. In this review, the authors discusse whether TCM compounds can reduce the risk of DPN by improving gut microbiota.

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INTRODUCTION

Diabetic peripheral neuropathy (DPN) is one of the commonest complications in patients with diabetes and the prevalence of DPN is as high as 50% and maybe even higher, which is also one of the main contributors of the high mortality in patients with diabetes. The duration of DPN is long with poor outcomes[1,2]. DPN is a heterogeneous disease and it has different symptoms which primarily includes neurological disorders such as limb numbness, usually symmetrical, stabbing pain and muscle weakness. Inappropriate treatment of DPN may lead to ulcers and gangrene, which probably results in limb loss and seriously affects quality of life in this population[3,4].

DPN is common in both type 1 and type 2 diabetes. The majority of preclinical and clinical studies focus on the pathogenic cause of hyperglycemia because prettily good control of blood glucose can delay the progression of DPN. Studies using diabetic animal models have confirmed several potential mechanisms to produce glucotoxicity and damage to the nervous system including post-translational modifications of proteins with glycans, aldose reductase, glycolysis and increased glucose metabolism through other pathways for the catabolism of glucose. However, it is becoming increasingly evident that factors except for hyperglycemia may also cause the occurrence, development and severity of neuropathy and neuropathic pain[5,6]. For example, peripheral nerves contain insulin receptors that transduce the neurotrophic and neurosupportive properties of insulin, independent of systemic glucose regulation, while it has becoming a focus of concern that the detection of neuropathy and neuropathic pain in patients with metabolic syndrome and poor glycemic control to protect against the pathogenic role of dyslipidemia in patients with type 2 diabetes and neuropathy[7-9].

DPN usually affects the sensory neuropathy and sensorimotor neuropathy. The sensory neuropathy is the most common form of DPN in patients with diabetes in clinical practice. It is characterized by gradual loss of nerve fibers of different sizes. In this progressive disease, the distal portions of the nerves in hands and feet are first affected with retraction of sensory axons at the end of the peripheral nervous system. This phenomenon is usually called “stocking & glove neuropathy”, which reflects damage to the longest sensory axons and thus is considered as a length-dependent neuropathy[10,11]. The reduction in the sensation and feeling is one of the commonest and earliest patterns of DPN and these symptoms occur gradually including tingling on the toes, pain and loss of feeling.

Clinical evidence for motor dysfunction is a little unusual in patients with diabetes and the symptoms only appear in 1% to 6% patients, usually in those confirmed with DPN. In the animal models of diabetes, early decreased motor nerve conduction velocities can be easily discovered. However, in both animal models and human studies, it has been proved that compound muscle action potential amplitude and muscular strength are reduced[12-15]. Although hyperglycemia is one of the factors that induce the development of nerve injurie, recently, evidence has demonstrated that damage to the small fibers of the peripheral nervous system may occur in individuals with impaired glucose tolerance independent of the diagnosis of hyperglycemia and diabetes[16]. In addition, keeping blood glucose under control can partly prevent and/or postpone the occurrence of DPN in patients with type 1 diabetes. However, it hardly benefits patients with type 2 diabetes. Hence, other diseases besides hyperglycemia may have an influence on the occurrence and development of DPN in type 2 diabetes. The multitude clinical manifestations of DPN including pain in 15% to 30% patients makes the identification of pathophysiology and treatment of DPN challenging[17-20].

ASSOCIATION BETWEEN GUT MICROBIOTA AND DPN

Risk factors for DPN include duration of diabetes, age, hemoglobin A1c (HbA1c), diabetic retinopathy, smoking and body mass index. A successful way of managing DPN involves exercise in combination with altered lifestyle and targeted diets as well as medication intervention. Studies showed that the occurrence of diabetes and its complications are associated with dysbiosis of the gut microbiota. The development of these disease are mainly related to the increased intestinal permeability, for which bacterial by-products can enhance inflammation by permeating the leaky intestinal barrier[21-23]. Species such as *Lactobacillus fermentans*, *Akkermansia muciniphila*, *Bacteroides fragilis*, and *Enterococcus rosenbergii* have been discovered to be associated with insulin sensitivity and glucose metabolism[24-26]. Gut bacteria *Ackermann* may impact the effects of metformin on glucose metabolism. *Bifidobacterium* and *Lactobacillus spp*. play a predominant role in the repair of the intestinal mucosal barrier. *Bifidobacterium spp*. that is discovered to be capable of producing bacteriocins is negatively associated with inflammation, hyperglycemia, and insulin resistance through preventing mucosal adhesion and maintaining intestinal barrier function. Probiotics and yogurt or milk containing *Bifidobacterium* and *Lactobacillus* can reduce fasting blood glucose and HbA1c in patients with diabetes. Based on this, we can come to a conclude that *Bifidobacterium* and *Lactobacillus* can inhibit the potential intestinal pathogens and enhance intestinal antioxidant capacity and digestive enzyme activities. Fecal microbiotas, kinds of Gram-negative bacteria, are negatively associated with HbA1c and can increase intestinal synthesis of glucagon like peptide-1, peptide YY, acetate and butyrate, and maintain blood glucose homeostasis[27].

MODULATING GUT MICROBIOTA WITH TRADITIONAL CHINESE MEDICINE FOR DPN

According to traditional Chinese medicine (TCM), DPN belongs to the field of "Xiaoke Disease". It is induced by the protracted course of not cured diabetes, poor glycemic control and erosion of the nerve cells. It is generally agreed that "internal heat" oriented pathogenesis of "deficiency of Yin and Jin and excess of dryness and heat" induced by congenital deficiency, long established inappropriate diet, long period of emotional disorder and overstrain associated disorder of internal organs are probably cause of diabetes. Lung, spleen and especially kidney are most affected locations. Gut microbiota dysbiosis will result in intestinal microbial imbalance and insulin resistance, which in further weaken one's physical defenses against infection providing a chance for the six evils to attack the human body. TCM have the potential to lessen the symptoms of DPN by modulating gut microbiota which is out of balance. TCM compounds follow the compatibility principle of "monarch, minister, assistant, and guide", which means herbs interact and restrict each other to achieve the optimally holistic effect through multiple targets, levels multiple pathways. For example, Shenqi Dihuang Decoction can regulate the spleen and stomach consequently building a healthy gut microbiome and constructing an integrated intestinal mucosal barrier[28].

Moreover, Huangkui Capsule's pharmacological effects, such as anti-inflammatory, antiviral, antibacterial, *etc*. can modulate the balance of gut microbiota from the perspective of inflammation treatment. Studies showed that adding Huangkui Capsule to the experimental group achieved decrease in 24 hours urine albumin-creatinine ratio and blood urea nitrogen content and increase in the number of Gram positive and negative bacteria in the intestine compared with the control group, indicating Huangkui Capsule can relieve the clinical symptoms of diabetic nephropathy[29]. Other studies demonstrated that Qidi Tangshen Granules can improve gut microbiota composition, reduce serum total bile acid level to alleviate the clinical symptoms of diabetic nephropathy[30]. In addition, TCM compounds such as Gegen Qinlian Decoction, Bupi Yishen formular, Baoshen formular, Qinshi Shenshu Capsules, Sanhuang Yishen Capsules and Fuzi Lizhong Pills also can improve the microorganism environment of intestines and stomach by modulating gut microbiota, suppressing the proliferation of harmful bacteria and increasing good bacteria in the gut. Studies[31,32] found those TCMs with effectiveness characterized by tonifying spleen, nourishing Qi, lifting Yang and Qi and harmonizing the stomach and intestines, and dredging Fu organs and sinking turbidity can actively alleviate nerve pain, repair damaged nerves and regulate the generation of inflammatory mediators through modulating gut microbiota. Meanwhile, the gut microbiota and its metabolic products can regulate glucose metabolism slowly, mediate immune response, regulate and promote the release of various signaling factors and facilitate recovery from DPN.

Diabetes is a multi-factor caused disease and its onset and development are thought to be profoundly influenced by the environment and hereditary factors. It is the main cause of Renal failure, cardiovascular disease, and retinopathy. A great microbial ecosystem lives in the human digestive tract and it co-evolutes with human beings to realize mutual benefits, maintain human health and prevent diseases. According to the "gut-kidney axis" hypothesis, gut microbiota dysbiosis is associated with diabetes and there is a mutual causality relationship between them. Gut microbiota can activate immune cells with its metabolic products and other constituents to trigger inflammatory reaction and accelerate the progression of complications in diabetes. Gut microbiota community derived antigens can induce conventional T cells differentiate into multiple effector cells such as Th2 cells, Th17 cells and T cells (Tregs) to regulate autoimmune response and immune homeostasis and have an impact on the onset and development of DPN. More and more evidence showed that changes in gut microbiota composition and function are associated with the increased risks for the onset and development of diabetes and its complications. Some studies investigating the influence of probiotic administration on blood glucose control and renal function in patients with diabetic nephropathy found that the use of probiotics (*Bifidobacterium*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*) can modulate gut microbiota in these patients, improve blood glucose control, and reduce fasting blood glucose, 2 hours postprandial serum glucose, HbA1c and microalbuminuria/creatinine (mAlb/Cr) levels with boosting therapeutic potential in the clinical practice[33-35]. The mechanism of its blood glucose lowering is probiotics can promote gut microbiota to yield insulinotropic polypeptide and glucagon-like peptide-1, which stimulate muscle glucose uptake and by this way reduce blood glucose.

Microbiome symbiosis plays a crucial role in regulating metabolism and reducing the risks for diabetes. However, the underlying mechanism has not been well understood[36,37] (Table 1). According to TCM, imbalance of spleen Qi's lifting and sinking function will further affect the intestine's digestion, absorption and elimination work. When Zhuo-Du (turbid-toxin) accumulates in the intestines, it is apt to cause gut microbiota dysbiosis with reduced number of beneficial microbe and overrun of harmful microbe. Gut microbiota dysbiosis and the imbalance of microecology will affect liver, spleen and kidney[38-40]. Studies showed that Huangkui Capsules can regulate the blood glucose level, elevate the distribution and subsets of gut microbiota and improve kidney function in patients with diabetic nephropathy[24].

CONCLUSION

Above all, DPN is associated with gut microbiota dysbiosis in diabetes. TCM compounds with the effectiveness of gut microbiota dysbiosis modulation such as Shenqi Dihuang Decoction, Huangkui Capsules and Qidi Tangshen Granules can reduce the relevant clinical symptoms of DPN. With diagnosis and treatment on the basis of an overall analysis of the illness and the patient's condition, TCM can modulate the species and amounts of gut microbiota to realize the balance and diversity of gut microbiota. In the further, TCM's role in modulating gut microbiota will gain increasing attention in the clinical practice. Relevant researches are warrant to profoundly investigate various TCMs' therapeutic efficacy for the complications of diabetes.

Table 1 Microbiome symbiosis plays a crucial role in regulating metabolism and reducing the risks for diabetes

Item	Role of microbiome symbiosis in regulating metabolism and reducing the risks for diabetes
1	Gut microbiota dysbiosis results in the production of short chain fatty acids such as butyric acid, propionic acid, and acetic acid, which will ruin the intestinal barrier integrity, activate inflammatory signaling cascade response and cause damage to multiple organs
2	Trimethylamine nitrogen oxide produced by the gut microbiota will increase the accumulation of cholesterol, which will lead to insulin resistance and raise the risk for diabetes
3	It is demonstrated that changes in the gut microbiota groups have an effect on intestinal permeability and inflammation in diabetes

FOOTNOTES

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ORIGINAL ARTICLE

Case Control Study

Autoantibodies against beta cells to predict early insulin requirements in pediatric patients with clinically diagnosed type 2 diabetes

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Abstract

BACKGROUND

Autoimmunity has emerged as a probable disease modifier in patients with clinically diagnosed type 2 diabetes mellitus (T2DM), that is, patients who have insulin resistance, obesity, and other cardiovascular risk factors, suggesting that the presence of glutamic acid decarboxylase (anti-GAD65), islet antigen 2 (anti-IA2), and zinc transporter 8 (anti-Zn8T) antibodies could have deleterious effects on beta cell function, causing failure and earlier requirement for insulin treatment.

AIM

To evaluate anti-GAD65, anti-IA2 and anti-Zn8T as predictors of early insulin requirement in adolescents with a clinical diagnosis of T2DM.

METHODS

This was a case–control study in patients with clinically diagnosed with T2DM (68 cases and 64 controls with and without early insulin dependence respectively),

male and female, aged 12–18 years. Somatometry, blood pressure, glucose, insulin, C-peptide, glycated hemoglobin A1c, and lipid profiles were assessed. ELISA was used to measure anti-GAD65, anti-IA2, and anti-Zn8T antibodies. Descriptive statistics, Pearson's χ^2 test, Student's t test, and logistic regression was performed. $P < 0.05$ was considered statistically significant.

RESULTS

There were 132 patients (53.8% female), with a mean age was 15.9 ± 1.3 years, and there was a disease evolution time of 4.49 ± 0.88 years. The presence of anti-GAD65, anti-IA2, and anti-Zn8T positivity was found in 29.5%, 18.2%, and 15.9%, respectively. Dividing the groups by early or no insulin dependence showed that the group with insulin had a higher frequency of antibody positivity: anti-GAD65 odds ratio (OR): 2.42 (1.112–5.303, $P = 0.026$); anti-IA2: OR: 1.55 (0.859–2.818, $P = 0.105$); and anti-Zn8T: OR: 7.32 (2.039–26.279, $P = 0.002$).

CONCLUSION

Anti-GAD65 positivity was high in our study. Anti-GAD65 and anti-Zn8T positivity showed a significantly depleted beta cell reserve phenotype, leading to an increased risk of early insulin dependence.

Key Words: Diabetes; Obesity; Adolescents; Autoimmunity; Beta cell; Insulin requirements

Core tip: We found that adolescent patients with type 2 diabetes mellitus (T2DM) showed a more aggressive phenotype of the disease, with a significant depletion of beta cell function, and where antibodies against pancreatic beta cells were associated with lower levels of insulin and C-peptide conferring a higher risk of early dependence on exogenous insulin. Therefore, in a pediatric patient with T2DM phenotype, the determination of pancreatic antibodies can be a clinical tool to predict early insulin requirements, leading to closer control of the disease to avoid chronic complications.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by a state of chronic hyperglycemia secondary to resistance to the action of insulin, with a progressive defect in beta cell function causing relative or absolute insulin deficiency[1,2]. The diagnosis of T2DM includes confirmation of the presence of DM according to the criteria of the American Diabetes Association, and the subsequent determination of the type of diabetes according to the clinical and biochemical phenotype[3]. Also, clinical data that may support the presence of T2DM are: Being overweight or obese, acanthosis nigricans, dyslipidemia, hypertension, and fatty liver. The rapid progression of T2DM is significantly influenced by genetic factors, with over 130 variants in different susceptibility and candidate genes identified as being linked to the disease[4]. However, due to the growing epidemic of obesity, these data can also be found in type 1 diabetes mellitus (T1DM), with the evolution of the diabetes being what can sometimes define the specific type in each individual[5].

The mechanisms involved in the pathophysiology of T2DM include 11 metabolic pathways that mediate hyperglycemia, which contribute to beta cell dysfunction; with immunological dysregulation and inflammation being recently described mechanisms that perpetuate elevated glucose levels[6,7]. Autoimmune destruction of the pancreatic beta cells is the main mechanism of damage in T1DM; however, these autoimmune markers may be also present in 15%–40% of patients with clinically diagnosed T2DM[8,9].

Various studies, primarily in the adult population, have connected the presence of autoantibodies with greater damage at the beta cell level, observing an earlier start of insulin treatment and representing a possible predictive marker of early insulin initiation[10-12].

Autoantibodies for glutamic acid decarboxylase (anti-GAD65), islet antigen 2 (anti-IA2), and zinc transporter 8 (anti-Zn8T) are used as markers for diagnosis and prognosis of T1DM by ELISA. The objective of the study was to evaluate the presence of anti-GAD65, anti-IA2, and anti-Zn8T antibodies as predictors of early insulin requirements in adolescents with T2DM.

MATERIALS AND METHODS

Study design and setting

A retrospective case–control study was performed in adolescents from the clinic for patients living with diabetes at the Federico Gómez Children´s Hospital in Mexico, from August 2018 to January 2023.

Participants

Inclusion criteria were as follows: (1) Adolescents aged 12–18 years; (2) Male or female sex; (3) Clinically diagnosed with T2DM according to their biochemical and clinical phenotype (overweight/obesity, acanthosis nigricans, and insulin resistance) with C-peptide at diagnosis of ≥ 0.5 ng/mL; (4) Early insulin dependence (cases): Patients requiring continuous insulin administration for at least 3 years after diabetes onset. The insulinization criterion was patients with poor metabolic control, defined by glycated hemoglobin A1c (HbA1c) ≥ 9.0%, and insulin treatment was with a basal-bolus scheme or basal insulin; (5) No insulin dependence (controls): Patients with treatment with biguanides (metformin), glucagon-like peptide 1 analogs (liraglutide), or only lifestyle modifications; and (6) Ketoacidosis and improved glycemic control: Patients who presented with ketoacidosis at the onset of diabetes and requiring insulin administration for < 4 mo due to improvement in glycemic control. Exclusion criteria were as follows: (1) Family history of early-onset diabetes: Patients with a history of diabetes before the age of 25 years in first and second-degree relatives, to rule out maturity-onset diabetes of the young; and (2) Diabetes duration: Number of years from diabetes onset until the measurement of antibodies was not specified as a criterion for inclusion or exclusion.

Anthropometric, biochemical, and immunological measurements

To perform the anthropometry, patients were evaluated with as little clothing as possible and without footwear. Weight assessment was performed with a digital scale (Seca 884, Hamburg, Germany) with an accuracy of 0.1 kg. Height was determined using a stadiometer (Seca 225) with an accuracy of 0.1 cm. Body mass index (BMI) was calculated using both of the aforementioned measurements. Waist circumference was measured at the end of expiration with a flexible nonelastic tape with an accuracy of 0.1cm (Seca 200), in a standing position, on the point mid-way between the lower edge of the last rib and the iliac crest. Blood pressure (BP) was measured with a mercury sphygmomanometer on the right arm held at the level of the heart, after a 5-min rest, with the subject in a sitting position, using a cuff appropriate for the age and size of the patient. Three BP measurements were made using the first and fifth Korotkoff sounds with the nearest reading every 2 mmHg, obtaining an average of the measurements.

After a 12-h fast, blood samples were obtained to measure: Glucose (Dimension RXL MAX; SiemensEuro, UK), insulin (chemiluminescence, IMULITE 1000; Siemens, Euro-DPC, Llanberis, UK), C-peptide (chemiluminescence, IMULITE 1000), HbA1c (Dimension RXL MAX), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (Hitachi 902, Tokyo, Japan). Low-density lipoprotein-cholesterol (LDL-C) measurement was calculated using the Friedewald formula (TC in mg/dL, HDL-C in mg/dL, triglycerides in mg/dL/5). To assess pancreatic reserve, the homeostatic model assessment of beta cell function (HOMA-β) index [(fasting insulin in mIU/mL × 20]/(fasting glucose in mmol/L 3.5)] was obtained.

Qualitative and quantitative determinations of anti-GAD65, anti-IA2, and anti-Zn8T antibodies were performed using ELISA. For anti-Zn8T the KRONUS kit (KR7730-96),Kronus, Star, ID, USA was used, and values ≥ 15 IU/mL were considered positive. The IBL International Kit (RE70391) (Tecan Trading, Switzerland) was used to measure anti-IA2Ab and anti-GAD65Ab, taking as positive the samples that had an optical density (OD) greater than that of the minimum control [calibrator cut-off (cc)], and as negative those less than that of the minimum control OD value. Absorbances were read at 450 nm using the Varioskas TM LUX multimode microplate reader from ThermoFisher Scientific (Waltham, MA, USA).

Statistical analysis

Descriptive statistical analysis (measures of dispersion and central tendency) of the demographic, clinical, biochemical, and immunological variables was performed. The normal distribution of variables was determined by the Kolmogorov–Smirnov test, expressing them in means and standard deviation, and variables without normal distribution were expressed in medians and minimum and maximum ranges. The Student *t* test or Mann–Whitney *U* test was used for independent samples depending on the distribution of the variables, and Pearson's χ^2 test was used for categorical variables. Logistic regression was performed to determine the odds ratio (OR) for early insulin dependence. SPSS version 23.0 was used, and $P < 0.05$ was considered statistically significant.

RESULTS

A total of 132 patients (53.8% female) were included in the study, with a mean age of 15.9 ± 1.3 years. The duration of diabetes was 4.49 ± 0.88 years. When dividing the groups by early insulin dependence, no significant differences were observed in age and sex, however, in terms of diabetes duration, a difference was shown (Table 1).

In the metabolic variables, no differences were observed in terms of TC, HDL-C, LDL-C, and triglycerides. Patients with insulin dependence show metabolic decontrol with higher HbA1c. C-peptide reserve was lower in the group with early insulin dependence with a decreased HOMA-β (Table 2).

Table 1 Demographic characteristics by insulin dependence, mean ± SD

Variable	Total (*n* = 132)	No insulin dependence (*n* = 64)	Insulin dependence (*n* = 68)	*P* value
Sex (M) (%)	71 (53.8)	34 (53.1)	37 (54.4)	0.51
Age (years)	15.9 ± 1.3	15.98 ± 1.3	15.9 ± 1.4	0.85
Weight (kg)	73.4 ± 10.1	72.2 ± 10.4	74.5 ± 9.8	0.06
Height (cm)	165.3 ± 6.4	164.7 ± 6.5	165.9 ± 6.3	0.11
BMI (kg/m^2)	25.2 ± 3.5	24.9 ± 3.8	25.5 ± 3.1	0.09
BMI z-score	1.39 ± 0.44	1.35 ± 0.46	1.43 ± 0.41	0.42
WC (cm)	87.4 ± 9.9	85.1 ± 10.6	89.1 ± 8.9	0.22
WHtR	0.52 ± 0.05	0.52 ± 0.06	0.53 ± 0.04	0.08
SBP (mm/Hg)	111.6 ± 11.2	110.3 ± 10.7	112.9 ± 11.6	0.05
DBP (mm/Hg)	72.0 ± 8.5	70.6 ± 7.8	73.3 ± 9.0	0.1
DD (years)	4.49 ± 0.88	4.73 ± 0.09	4.27 ± 0.70	0.03

BMI: Body mass index; WC: Waist circumference; WHtR: Waist-to-height ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DD: Diabetes duration in years.

Table 2 Metabolic characteristics by insulin dependence, mean ± SD

Variable	Total (*n* = 132)	No insulin dependence (*n* = 64)	Insulin dependence (*n* = 68)	*P* value
TC (mg/dL)	175.9 ± 48.3	164.2 ± 37.4	186.9 ± 54.8	0.54
HDL-C (mg/dL)	44.1 ± 9.3	44.6 ± 7.5	43.6 ± 10.8	0.11
LDL-C (mg/dL)	107.1 ± 41.1	93.3 ± 28.8	118.3 ± 47.5	0.1
TG (mg/dL)	158.5 ± 21.1	148.5 ± 14.0	187.9 ± 19.9	0.18
Fasting glucose (mmol/L)	10.95 ± 0.55	7.62 ± 0.37	14.07 ± 0.51	0.08
HbA1c (%)	8.8 ± 2.9	6.7 ± 1.6	10.8 ± 2.3	0.001
Insulin (mUI/mL)	11.4 ± 7.7	12.7 ± 9.6	10.3 ± 5.0	0.3
C- peptide (ng/dL)	1.4 ± 1.3	2.53 ± 1.2	0.41 ± 0.36	0.001
HOMA-β	85.38 ± 16.6	125.5 ± 15.6	50.9 ± 16.8	0.022

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein-cholesterol; TG: Triglycerides; HbA1c: Glycated hemoglobin A1c; HOMA-β: Homeostatic model assessment of beta cell function.

When dividing the groups by the presence of autoimmunity, significant differences were observed between the weight and height of the group positive for antibodies, with their values being lower compared to the group without autoimmunity (Table 3).

The percentage of obesity at the time of diagnosis in the total population was 60.5%; however, the percentage was reduced at the time of antibody measurement to 35.6%. There were no differences observed in metabolic variables in terms of TC, HDL-C cholesterol, LDL-C, and triglycerides. Despite having insulin therapy, patients had higher serum glucose, which was reflected in the levels of HbA1c; the latter of which was significant. Regarding the pancreatic reserve, C-peptide was lowest in the group with early insulin dependence, showing a decreased HOMA-β that indicated a lower pancreatic reserve (Table 4).

By analyzing the metabolic variables and dividing them into groups based on the presence or absence of antibodies, the group without early insulin dependence had lower insulin levels in the presence of autoimmunity as well as lower HOMA-β levels, which was significant (Table 4).

Control of glycemia in the total population studied was 40.2%; however, on stratifying the groups by early or no early insulin dependency, the group without insulin dependency had a higher percentage of glycemia control (81.3%), while in the other group, despite having insulin treatment, only 1.5% had glycemic control (P = 0.000).

The presence of anti-GAD65 was positive in 39 patients (29.5%), and, when dividing the groups by early or no early insulin dependence, anti-GAD65 was positive in 38.2% of the cases (Table 5).

Table 3 Demographic characteristics by autoimmunity

Variable	At the time of diagnosis NID	NID		*P* value	At the time of diagnosis ID	ID		*P* value
	NID (*n* = 64)	(Ab+) (*n* = 16)	(Ab-) (*n* = 48)		ID (*n* = 68)	(Ab+) (*n* = 27)	(A-) (*n* = 41)	
Sex (F) (%)		8 (50.0)	26 (54.1)	0.77		16 (59.2)	21 (51.2)	0.51
Age (years)	12.9 ± 0.7	15.9 ± 1.4	16.0 ± 1.3	0.87	13.1 ± 0.9	16.1 ± 1.4	15.7 ± 1.4	0.3
Weight (kg)	68.9 ± 13.9	67.2 ± 11.2	73.9 ± 9.6	0.04	74.6 ± 10.5	73.6 ± 8.4	75.0 ± 10.6	0.57
Height (cm)	159.2 ± 6.0	161.7 ± 7.6	165.7 ± 5.9	0.03	162.2 ± 5.96	165.9 ± 6.2	166.0 ± 6.5	0.95
BMI (kg/m^2)	26.0 ± 5.0	24.8 ± 3.3	25.0 ± 4.0	0.81	26.6 ± 4.2	25.6 ± 2.5	25.4 ± 3.5	0.85
BMI z-score	1.61 ± 0.6	1.25 ± 0.4	1.38 ± 0.4	0.31	1.68 ± 0.4	1.40 ± 0.3	1.46 ± 0.4	0.52
WC (cm)	92.6 ± 9.4	85.9 ± 9.5	85.1 ± 11.1	0.91	94.6 ± 10.8	89.0 ± 7.1	89.1 ± 10.0	0.95
WHtR (cm)	0.56 ± 0.06	0.53 ± 0.05	0.51 ± 0.07	0.47	0.56 ± 0.06	0.53 ± 0.03	0.53 ± 0.50	0.94
SBP (mmHg)	112 ± 12.2	109.8 ± 10.0	110.5 ± 11.0	0.82	18 ± 14.6	112.3 ± 13.2	113.2 ± 10.6	0.75
DBP (mmHg)	74.5 ± 8.4	70.7 ± 7.0	70.6 ± 8.1	0.96	78.8 ± 9.2	73.1 ± 10.2	73.5 ± 8.2	0.86
DD (years)		4.76 ± 0.95	4.72 ± 0.09	0.87		4.38 ± 0.82	4.19 ± 0.73	0.34

NID: No insulin dependence; ID: Insulin dependent; Ab: Antibodies; BMI: Body mass index; WC: Waist circumference; WHtR: Waist-to-height ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DD: Diabetes duration in years.

Table 4 Metabolic characteristics of the groups by autoimmunity

Variable	At the time of diagnosis NID	NID		*P* value	At the time of diagnosis ID	ID		*P* value
	NID (*n* = 64)	Ab+) (*n* = 16)	(Ab-) (*n* = 48)		ID (*n* = 68)	(Ab+) (*n* = 27)	(A-) (*n* = 41)	
TC (mg/dL)	164.8 ± 39	170 ± 32.9	162.1 ± 38.8	0.44	170 ± 37.5	189.2 ± 60.7	185.3 ± 51.3	0.77
HDL-C (mg/dL)	41.9 ± 11.4	45.8 ± 6.9	44.1 ± 7.7	0.42	43.6 ± 8.0	42.2 ± 8.0	44.5 ± 12.4	0.39
LDL-C (mg/dL)	97.9 ± 30	96.8 ± 23.2	94.8 ± 30.6	0.82	96.2 ± 29.3	124.8 ± 51.6	114.0 ± 44.8	0.38
TG (mg/dL)	136.4 ± 28	162.5 ± 21.1	143.8 ± 11.0	0.65	153.3 ± 22	172.6 ± 9.8	164.8 ± 10.9	0.75
Glucose (mmol/L)	14.77 ± 2.16	7.58 ± 2.93	7.64 ± 3.97	0.96	13.27 ± 2.55	13.66 ± 0.54	14.68 ± 0.25	0.43
HbA1c (%)	9.7 ± 2.7	6.8 ± 1.8	6.3 ± 0.9	0.91	9.2 ± 2.2	11.4 ± 2.6	10.4 ± 2.1	0.09
Insulin (mUI/mL)	16.7 ± 1.6	9.3 ± 4.8	13.8 ± 9.5	0.02	21.1 ± 2.8	9.5 ± 3.7	10.7 ± 5.7	0.04
C-peptide (ng/mL)	0.3 ± 0.09	2.3 ± 1.2	2.6 ± 1.2	0.42	1.1 ± 0.26	0.30 ± 0.25	0.40 ± 0.04	0.19
HOMA-β	72.3 ± 33.9	64.8 ± 45.3	125.7 ± 15.8	0.02	91.5 ± 30.6	25.8 ± 34.6	61.3 ± 20.2	0.02

NID: No insulin dependence; ID: Insulin dependent; TC: total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein-cholesterol; TG: Triglycerides; HbA1c: Glycated hemoglobin A1c; HOMA-β: Homeostatic model assessment of beta-cell function.

Regarding antibody titers, anti-Zn8T showed higher titers compared with the other antibodies in the total population; however, when dividing the groups by insulin dependence, lower levels of the three were found. Antibodies in the group without early insulin dependence were compared with those in the group with dependence; however, these differences were not significant (Table 6).

Multivariate analysis showed that the presence of anti-GAD65 antibody positivity conferred an OR = 2.42 (95% confidence interval: 1.04–5.60, *P* = 0.026), adjusted for sex, BMI, and diabetes duration. Anti-IA2 had an OR of 1.55 but this was not significant. As for anti-Zn8T, a higher OR of 7.32 was found compared with the other two antibodies (Table 7).

Table 5 Frequency of antibody positivity, *n* (%)

Variable	Total (*n* = 132)	No insulin dependence (*n* = 64)	Insulin dependent (*n* = 68)	*P* value
Anti-GAD 65	39 (29.5)	13 (20.3)	26 (38.2)	0.02
Anti-IA2	24 (18.2)	8 (12.5)	16 (23.5)	0.07
Anti-Zn8T	21 (15.9)	3 (4.7)	18 (26.5)	0
Anti-GAD65 + anti-IA2	20 (15.2)	5 (7.8)	15 (22.1)	0.02
Anti-GAD65 + anti-Zn8T	15 (11.4)	3 (4.7)	12 (17.6)	0.01
Anti-IA2 + anti-Zn8T	12 (9.1)	2 (3.1)	10 (14.7)	0.02
Three antibodies	12 (9.1)	2 (3.1)	10 (14.7)	0.02

GAD65: Glutamic acid decarboxylase; IA2: Islet antigen 2; Zn8T: Zinc transporter 8.

Table 6 Antibody titers according to insulin dependence

Variable	Total (*n* = 132)	No insulin dependence (*n* = 64)	Insulin dependent (*n* = 68)	*P* value
Anti-GAD65 (UI/mL)	11.0 (5-543)	6.8 (5-543)	12.0 (5-186)	0.69
Anti-IA2 (UI/mL)	14.6 (5-245)	8.45 (5-111)	21.6 (5-245)	0.6
Anti-Zn8T (UI/mL)	68.5 (15.9-178.2)	64.5 (47-74.5)	73.7 (15.9-178.2)	0.68

Median and minimum and maximum ranges. Mann–Witney *U* test. GAD65: Glutamic acid decarboxylase; IA2: Islet antigen 2; Zn8T: Zinc transporter 8.

Table 7 Estimation of risks according to antibodies

Variable	Odds ratio early insulin requirements (*n* = 132)	
	OR adj (95%CI)	*P* value
Anti-GAD65	2.42 (1.112- 5.303)	0.026
Anti-IA2	1.55 (0.859- 2.818)	0.101
Anti-Zn8T	7.32 (2.039-26.279)	0.002
Anti-GAD65 + Anti-IA2	3.34 (1.136- 9.814)	0.028
Anti-GAD65 + anti-Zn8T	4.35 (1.168-16.248)	0.028
Anti-IA2 + anti-Zn8T	5.34 (1.123-25.431)	0.035
Anti-GAD65 + Anti-IA2 + anti-Zn8T	5.34 (1.123-25.431)	0.035

Adjusted for age, body mass index, and time of evolution of diabetes. GAD65: Glutamic acid decarboxylase; IA2: Islet antigen 2; Zn8T: Zinc transporter 8.

DISCUSSION

Although T2DM is traditionally considered a nonautoimmune disease, it is currently known that it is multifactorial, involving 11 well-established mechanisms, with immune dysregulation being one of them[6]. In international guidelines, such as that of the German Diabetes Association in its clinical practice guideline for the diagnosis, therapy and monitoring of diabetes in children and adolescents from 2019, it only suggests taking laboratory tests to provide additional information and power to differentiate between T1DM and T2DM[13]. However, the 2018 Canadian clinical practice guidelines on T2DM in pediatric age state that diagnosis can be difficult, because 10%–20% may find one or more positive antibodies, so other factors need to be considered[14].

In that sense, there are confounding factors, since there may be T1DM with an accelerating phenotype, that is, overweight/obesity and insulin resistance that will end with faster beta cell exhaustion. However, the onset of diabetes in puberty with an insidious and more prolonged clinical picture may be factors strongly associated with T2DM in addition to those already known[15]. In our study, the patients had a phenotype of insulin resistance, dyslipidemia, overweight/obesity initially, which even persisted years later, allowing a prolonged time without exogenous insulin of > 3 years, which is not compatible with a "honeymoon" period in a context of T1DM, therefore, their clinical behavior was more

towards T2DM.

According to ISPAD 2022, it is important to evaluate autoimmunity preferably with the four antibodies as long as exogenous insulin has not been started, since the anti-insulin antibody may not be assessable after treatment. This guideline recognizes the presence of autoimmunity in a subgroup of T2DM, which, given the positivity of the antibodies, predicts early insulin requirements as we reported in this study[16]. However, a key point is the follow-up of these patients, since the evolution and behavior of the disease can also be a factor to consider to reclassify diabetes as type 2, and prolonged suspension of exogenous insulin with good glycemic control for months or years will give rise to considering it as type 2, as was the case in our study.

In the present study, 51.5% of adolescent patients clinically diagnosed with T2DM presented with an earlier dependence on insulin treatment, which is a shorter time compared to the adult population when periods of > 6 years are observed, as referred to in the UKPDS group in the UK[11].

When dividing the groups by early insulin dependence and by positive antibodies, it was observed that the group without insulin therapy and positive antibodies showed lower weight and shorter height compared to the insulin-dependent group and positive antibodies, but without being significant, as well as in BMI, which differed from that reported by Turner *et al*[11], who reported that patients with early insulin dependence had a lower BMI.

Regarding the metabolic variables, the glycemic control exceeded the percentage reported in the literature of 20%–25% [9,17]. The lipid profile did not show significant differences between the groups. However, lower insulin levels were observed in antibody-positive patients without early insulin dependency, as well as lower C-peptide levels. This correlates with lower endogenous production of insulin; similar to the determination of HOMA-β where a significantly lower pancreatic reserve of insulin occurred in the presence of autoimmunity, similar to that reported by Bottazzo *et al* [12]. However, these patients with antibody positivity without insulin dependence have a HOMA-β similar to the group without antibody but with insulin dependence, which suggests they experienced continuous beta cell failure to rule out probable evolution of T1DM. We are aware that the participants in the present study who had positive antibodies, especially two or more, could be representative of a group of patients with undetected autoimmune diabetes.

The anti-GAD65 positivity was higher than that reported in the UKPDS study (6%) and the TODAY study (5.9%)[9,11]. Regarding anti-IA2, it was more frequent than the 2.2% reported by Bottazzo *et al*[12]; however, these percentages varied when the groups were stratified by age ranges, being higher in the younger groups[12]. The positivity of both antibodies was also higher than the 3.9% reported in the TODAY study[9].

Anti-GAD65 was the most frequent antibody in the group with early insulin dependence and was similar to the 38% reported by Turner *et al*[11]. Anti IA2 was the second antibody that predominated in the group with early insulin dependence, but it was lower than that reported by Buzzetti *et al*[10], who, when stratifying by age, found a positivity of 93% in the youngest group (< 45 years). The positivity of combined antibodies showed than anti-GAD65 and anti-IA2 was the most prevalent combination in the insulin-dependence group. However, this was lower than the UKPDS data, with 100% in the under 45 years, but in the over 45 years the percentage dropped to 40%.

The OR for anti-GAD65 was significant, but this was lower than that reported by Turner *et al*[11] with an OR of 13.0, possibly due to the sample size. Regarding anti-AI2, a lower OR was also reported by Bottazzo *et al*[12]. Lastly, despite the fact that anti-Zn8T positivity was lower, its presence conferred a higher risk compared to the other two antibodies. When two antibodies were positive, the combination of positive anti-IA2 and anti-Zn8T conferred an OR of 5.34, which was sustained when there was positivity for all three antibodies.

Our study is supported by Lampasona *et al* in 2010, who reported that ZnT8As can be detected in some patients with adult-onset autoimmune diabetes and appear to be a useful marker for distinguishing different clinical phenotypes[18].

Determination of the four antibodies against beta cells should be integrated into the current clinical practice guidelines. Their positivity can complement the diagnosis in a more comprehensive way and predict more rapid depletion of the pancreatic reserve, which leads to closer treatment and monitoring of the patient, avoiding lack of glycemic control and the presence of complications in the medium or long term.

While our study provides valuable insights into the role of antibody positivity in characterizing T2DM phenotypes and predicting early insulin dependence, there were several limitations that should be considered. First, our sample size may not be large enough to generalize the findings to all patients with T2DM, and larger studies are needed to confirm these results. Second, the cross-sectional design does not allow for the establishment of a causal relationship between antibody positivity and beta cell depletion. Longitudinal studies would be necessary to track changes over time and to establish causality. Third, the study was conducted at a single tertiary hospital, which may limit the generalizability of the findings to other populations or healthcare settings.

Finally, we did not account for potential confounding factors such as genetic predisposition, lifestyle factors, and concurrent medical conditions, which could influence the development of antibody positivity and insulin dependence. Despite these limitations, our findings suggest that antibodies such as anti-GAD65, anti-Zn8T, and anti-IA2 are valuable markers in the management of T2DM, highlighting the need for more comprehensive studies in this area.

Interactions with drugs and antigens: There is a need to explore potential interactions between the autoimmune antibody panel and various drugs, natural antigens, or acquired antigens present in the body. It remains unclear whether medications or external antigens could influence the expression or levels of these autoantibodies, thereby affecting their diagnostic and prognostic value.

Pharmacological interactions: Some drugs might modulate immune responses or interfere with antibody production, potentially affecting the accuracy of the autoimmune panel.

Natural and acquired antigens: The presence of other antigens, whether natural or acquired through infections, could affect the immune response, leading to false positives or negatives in antibody testing.

Despite these limitations, our findings suggest that antibodies such as anti-GAD65, anti-Zn8T, and anti-IA2 are valuable markers in the management of T2DM. These antibodies highlight the need for more comprehensive studies to understand their interactions and to improve diabetes management strategies effectively.

CONCLUSION

We can conclude that patients clinically diagnosed with T2DM demonstrate an early depletion of endogenous insulin reserves since > 50% required the application of exogenous insulin as a cornerstone in their treatment. Antibody positivity was associated with a phenotype of depleted beta cell reserves, thus lower insulin levels, conferring a higher risk of early insulin dependence. Anti-GAD65 was the most prevalent. However, positivity for anti-Zn8T conferred a greater risk of early insulin dependence, and its combination with positive anti-IA2 conferred an even greater risk. Therefore, these antibodies can be useful specific immunological markers in the approach to T2DM to characterize the patient more specifically. When they are positive it could be possible to further intensify diabetes education and tighten glycemia control to prevent further depletion of the beta cells, and delay chronic complications that accompany the disease.

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FOOTNOTES

Author contributions: Molina JM recruited, compiled, wrote, and edited the manuscript; Gómez RA and Herrera JR contributed to the writing of the protocol and the manuscript; Medina PG supervised the development of the study; Martínez NL and Hernández B participated in the processing of the antibodies; García Y participated in the recruitment of patients. All authors have read and approved the final manuscript.

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Institutional review board statement: The study aligned with the General Health Law, article 17 in research, respecting universal principles of ethics, and complied with the Official Mexican Standard NOM-012-SSA2-2012 that establishes criteria for the execution of research projects in humans. The study was approved by the research, ethics, and biosafety committee of the Federico Gómez Children's Hospital in Mexico, registered under the HIM 2018/068 protocol.

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ORIGINAL ARTICLE

Retrospective Study

Clinically significant changes in anal sphincter hiatal area in patients with gestational diabetes mellitus and pelvic organ prolapse

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Abstract

BACKGROUND

The prevalence of pelvic organ prolapse (POP) increases with age and parity. Specifically, the prevalence of POP among women aged 20 to 39 is 9.7%, while it rises to 49% among women over 80 years old. Additionally, as the number of deliveries increases, the prevalence of POP also rises accordingly, with a rate of 12.8% for women with one delivery history, 18.7% for those with two deliveries, and 24.6% for women with three or more deliveries. It causes immense suffering for pregnant women.

AIM

To evaluate the relationship between the levator ani muscle's hiatus (LH) area and POP in patients with gestational diabetes mellitus (GDM) using perineal ultrasound.

METHODS

The study cohort comprised 104 patients aged 29.8 ± 3.7 years who sought medical care at our institution between January 2021 and June 2023. All were singleton pregnancies consisting of 75 primiparas and 29 multiparas, with an average parity of 1.7 ± 0.5. According to the POP diagnostic criteria, the 104 subjects were divided into two groups with 52 members each: POP group (patients with GDM combined with POP) and non-POP group (patients with GDM without POP). Perineal ultrasound was used to measure differences in the anteroposterior diameter, transverse diameter, and LH area. Receiver operating characteristic curves were drawn to determine the optimal cutoff values for the LH anteroposterior diameter, transverse diameter, and area for diagnosing POP.

RESULTS

Statistically significant increase in the LH area, anteroposterior diameter, and lateral diameter were observed in the POP group compared with the non-POP group ($P < 0.05$). Both groups exhibited markedly elevated incidence rates of macrosomia and stress urinary incontinence. For the POP group, the area under the curve (AUC) for the LH area was 0.906 with a 95% confidence interval (CI): 0.824-0.988. The optimal cutoff was 13.54cm², demonstrating a sensitivity of 83.2% and a specificity of 64.4%. The AUC for the anteroposterior diameter reached 0.836 with a 95%CI: 0.729-0.943. The optimal cutoff was 5.53 cm with a sensitivity of 64.2% and a specificity of 73.4%. For the lateral diameter, its AUC was 0.568 with a 95%CI: 0.407-0.729. The optimal cutoff was 4.67 cm, displaying a sensitivity of 65.9% and a specificity of 69.3%. Logistic regression analysis unveiled that age, body weight, number of childbirths, total number of pregnancies, and gestational weight gain constituted the independent risk factors for the cooccurrence of GDM and POP.

CONCLUSION

Three-dimensional perineal ultrasonography of LH size and shape changes can effectively diagnose POP. Age, weight, number of births, number of pregnancies, and weight gain during pregnancy are independent risk factors affecting the cooccurrence of GDM and POP. GDM can increase the LH area in patients, and an enlarged LH leads to an increased incidence of POP.

Key Words: Ultrasound; Levator ani muscle hiatus; Gestational diabetes mellitus; Pelvic organ prolapse

Core Tip: This study aimed to evaluate the relationship between the levator ani muscle's hiatus (LH) area and pelvic organ prolapse (POP) in patients with gestational diabetes mellitus using perineal ultrasound. Conclusion: Three-dimensional perineal ultrasonography of LH size and shape changes can effectively diagnose POP.

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INTRODUCTION

The levator ani muscle is the most robust layer of the female pelvic floor and plays the most important supportive role [1]. Each side of the levator ani muscle is composed of three parts: Pubococcygeus, iliococcygeus, and ischiococcygeus. The fibers of the pubococcygeus on both sides extend posteriorly, downward, and inward to form an elliptical gap known as the "levator ani muscle's hiatus" (LH). The LH is the herniation pathway for organ prolapse, with the urethra, vagina, and rectum sequentially passing through it from front to back. To a certain extent, the size of the LH can reflect the support provided by the pelvic floor structure to the pelvic organs[2,3].

Gestational diabetes mellitus (GDM) is a disease unique to female pregnancy and its incidence shows an annually increasing trend[4,5]. GDM increases the incidence of complications in mothers and fetuses and the risk of pelvic floor functional disorders in patients. It is one of the high-risk factors affecting female pelvic floor function[6,7]. Inadequate glycemic control escalates the likelihood of substantial weight gain among expectant mothers and the probability of giving birth to infants with a birth weight above average for their gestational age, thereby imposing pronounced stress on the integrity of the pelvic floor structure. Furthermore, suboptimal glycemic management may result in significant diminishments in the levator ani muscle's thickness, constrictions in ligamentous width, and a diminution in collagen concentration within both the pelvic floor and the rectus abdominis muscular fibers, resulting in varying degrees of muscle weakness[8,9]. The incidence of pelvic organ prolapse (POP) is steadily rising, making it a significant health burden for women worldwide[10]. In patients with POP, the pelvic support structures are damaged and their supporting strength is weakened, leading to the displacement of the uterus, bladder, vagina, and surrounding tissues from their normal anatomical positions and causing damage to the functional state of these pelvic organs. This condition poses a considerable threat to the female reproductive system and affects the patients' psychological health and quality of life. In this study, a comparative analysis was conducted to investigate the correlation between the LH area and POP in individuals diagnosed with GDM. By utilizing transperineal ultrasound as the primary diagnostic tool, 52 patients with GDM and concurrent POP were compared with another group of 52 patients diagnosed with GDM but without POP. The findings are presented below.

MATERIALS AND METHODS

General information

This study was approved by the Ethics Committee of People's Hospital Affiliated to Shandong First Medical University, who agreed to waive the informed consent. A total of 104 patients who sought medical care at our institution between January 2021 and June 2023 were meticulously chosen as the study cohort. Their ages spanned 21-42 years with a mean age of 29.8 ± 3.7 years. All the participants experienced singleton pregnancies. Among them, 75 were primiparous and 29 had previously given birth, resulting in an average parity of 1.7 ± 0.5. According to the diagnostic criteria for POP, the participants were categorically divided into two distinct groups with 52 individuals each: POP group and non-POP group. The inclusion criteria were as follows: (1) Met the diagnostic criteria for GDM; (2) Met the diagnostic criteria for POP according to "Practical Pelvic Floor Ultrasonography"; (3) No history of POP before pregnancy; (4) No history of chronic cough or urinary incontinence before pregnancy; and (5) Was informed and voluntarily joined the study. An oral glucose tolerance test was conducted between 23 and 25 weeks of pregnancy and involved the intake of 250-300 mL of sugar water containing 75 g of glucose powder. The normal blood glucose levels at fasting and 1 and 2 hours post consumption were < 5.1, 10.0, and 8.5 mmol/L, respectively. A diagnosis of GDM was made if any blood glucose value reached or exceeded these standards. The exclusion criteria were as follows: (1) Not meeting the diagnostic criteria for GDM; (2) Preexisting diabetes before pregnancy; and (3) History of chronic cough or urinary incontinence before pregnancy. No statistically significant distinctions in the demographic characteristics were found between the two cohorts of patients ($P > 0.05$), affirming their comparability. For detailed data, please refer to Tables 1 and 2.

Methods

Detection method: Following enrollment into the study, the patients in the POP group underwent POP Quantification (POP-Q) staging[11]. All patients were subjected to fasting ultrasonography. The GE Voluson E8 color Doppler ultrasound diagnostic system with a RAB4-8-D three-dimensional (3D) volume probe was utilized at a frequency of 2-8 MHz. Prior to the examination, the patients were instructed to empty their rectum and bladder (with appropriate bladder filling, residual urine < 50 mL) and then assisted into the lithotomy position. A disposable condom was used to cover the probe, which was then placed at the perineum for two-dimensional ultrasound imaging. This imaging captured the mid-sagittal plane of the patient's pelvic floor, measuring the position and movement of pelvic organs, checking for funnel formation at the internal urethral orifice, and assessing the openness of the posterior angle of the bladder and urethra. Afterward, the 3D imaging acquisition mode was activated to capture standard 3D volume images for the LH. After image collection, a four-dimensional volume offline analysis software was used for postprocessing and data analysis. All ultrasound examinations were conducted by the same physician with extensive clinical experience in the relevant department.

Observational indicators: The anteroposterior and transverse dimensions of the LH in the patients were quantified by utilizing the two-point distance method, and the LH area was determined by employing the area tracing method.

POP assessment: According to the POP-Q staging criteria[12], prolapse was categorized into stage 0, I-II (mild prolapse), and III-IV (severe prolapse). The stage of prolapse for each parturient was recorded based on the organ with the maximum prolapse.

Statistical analysis

All data were subjected to rigorous statistical analysis using SPSS software version 26.0. Quantitative data were presented as mean ± SD. Prior to intergroup comparisons, assessments for normality and homogeneity of variances were executed. For group comparisons, independent samples *t*-tests were employed, and statistical significance was denoted by $P < 0.05$. Receiver operating characteristic (ROC) curves were meticulously constructed to delineate the incidence rates of POP within both cohorts and scrutinize its correlation with the anteroposterior and lateral dimensions of the LH. The area under the curve (AUC) was also computed to quantify these associations.

RESULTS

Comparison was performed between the two study groups. The following notable findings were observed in the POP group: 47 cases of stress urinary incontinence, 36 cases of bladder prolapse, 12 cases of uterine prolapse, 4 cases of rectal prolapse, 23 cases of macrosomia, and 35 cases of diminished pelvic floor muscle strength. Meanwhile, the non-POP group exhibited 45 instances of stress urinary incontinence, 27 cases of macrosomia, and 35 cases of reduced pelvic floor muscle strength. The LH area, anteroposterior diameter, and lateral diameter in the POP group were significantly enlarged compared with those in the non-POP group ($P < 0.05$). In particular, the AUC for the LH area within the POP group was 0.906 with a 95%CI: 0.824-0.988. The optimal cutoff was 13.54 cm², showcasing a sensitivity of 83.2% and a specificity of 64.4%. The AUC for the anteroposterior diameter of the LH reached 0.836 with a 95%CI: 0.729-0.943. The optimal cutoff was 5.53 cm, displaying a sensitivity of 64.2% and a specificity of 73.4%. For the lateral diameter of the LH, the AUC was 0.568 with a 95%CI: 0.407-0.729. The optimal cutoff was at 4.67 cm, exhibiting a sensitivity of 65.9% and a specificity of 69.3%. During examinations, attention should be paid to discerning the occurrence of POP. Logistic regression analysis indicated that age, weight, number of births, number of pregnancies, and weight gain during pregnancy are independent risk factors for the cooccurrence of GDM and POP. The incidence rates of macrosomia and

Table 1 Comparison of general information between the two groups of patients (mean ± SD)

Group	Height (cm)	Age (years)	Weight (kg)	Primiparas (cases)	Multiparas (cases)	Pregnant once (cases)	Pregnant ≥ 2 times (cases)	Weight gain during pregnancy (kg)
POP group (n = 52)	163.74 ± 6.11	28.28 ± 3.15	59.88 ± 8.41	39	13	35	17	20.05 ± 2.78
Non-POP group (n = 52)	165.39 ± 6.53	28.36 ± 2.64	58.44 ± 9.59	36	16	37	15	19.98 ± 2.59

In the comparison of two groups, $P > 0.05$. POP: Pelvic organ prolapse.

Table 2 Comparison of levator ani muscle's hiatus area, anteroposterior diameter, and lateral diameter between two groups of pelvic organ prolapse patients, mean ± SD

Group	Number	LH area (cm²)	LH anteroposterior diameter (cm)	LH lateral diameter (cm)
POP group	52	14.94 ± 1.58	6.69 ± 0.72	4.18 ± 0.56
Non-POP group	52	13.65 ± 0.97	5.15 ± 0.45	4.04 ± 0.17

LH: Levator ani muscle's hiatus; POP: Pelvic organ prolapse.

stress urinary incontinence were relatively high in both groups, indicating that GDM can increase the LH area. An enlarged LH increases the incidence of POP and the risk of pelvic organ dysfunction and fetal complications.

Sonographic characteristics of LH

In the non-POP group, the LH appeared diamond-shaped with a compact and orderly internal structure. In the POP group, the LH morphology was primarily diamond shape, "V"-shape, or "U"-shape with a loosely arranged and disordered internal structure. The anteroposterior diameter, transverse diameter, and area of the LH in the POP group were significantly larger than those in the control group.

Area and anteroposterior and lateral diameters of LH

The LH in the POP group had an area of 14.94 ± 1.58 cm², anteroposterior diameter of 6.69 ± 0.72 cm, and lateral diameter of 4.18 ± 0.56 cm, all of which were significantly greater than those in the non-POP group (13.65 ± 0.97, 5.15 ± 0.45, and 4.04 ± 0.17 cm, respectively; $P < 0.05$) as indicated in Table 2.

POP-Q staging

Among the 52 patients in the POP group, 35 (34.61%) were in POP-Q stage I, 8 (15.38%) in stage II, 7 (13.46%) in stage III, and 2 (3.84%) in stage IV. The LH area, anteroposterior diameter, and lateral diameter for each stage are presented in Table 3.

Incidence of macrosomia and stress urinary incontinence in both groups

In the POP group of 52 patients, 23 (44.23%) had macrosomia and 47 (90.38%) showed stress urinary incontinence. In the non-POP group of 52 patients, 27 (52.93%) had macrosomia and 45 (86.54%) showed stress urinary incontinence. However, the disparity was not significant ($P > 0.05$) as illustrated in Table 4.

ROC curves for LH area and anteroposterior and lateral diameters

In the POP group, the LH area yielded an AUC of 0.906 with a 95%CI: 0.824-0.988. The optimal cutoff was 13.54 cm², with an associated sensitivity of 83.2% and specificity of 64.4%. For the anteroposterior diameter of the LH in the same group, the AUC was 0.836 with a 95%CI: 0.729-0.943. The optimal cutoff was 5.53 cm, demonstrating a sensitivity of 64.2% and a specificity of 73.4%. For the lateral diameter of the LH within the POP group, the AUC was 0.568 with a 95%CI: 0.407-0.729. The optimal cutoff was 4.67 cm, with a sensitivity of 65.9% and a specificity of 69.3%. These significant findings are visually represented in Figure 1.

Analysis of risk factors for GDM

Logistic regression analysis revealed that age, weight, number of births, number of pregnancies, and weight gain during pregnancy are independent risk factors influencing the cooccurrence of GDM and POP as shown in Table 5.

Table 3 Pelvic organ prolapse quantification staging, anteroposterior diameter, and lateral diameter, mean ± SD

POP-Q stage	Number	LH area (cm²)	LH anteroposterior diameter (cm)	LH lateral diameter (cm)
I	35	14.58 ± 0.54	5.86 ± 0.41	3.57 ± 0.55
II	8	15.73 ± 0.50	6.86 ± 0.62	4.28 ± 0.23
III	7	16.23 ± 0.29	7.23 ± 0.18	4.75 ± 0.11
IV	0	0	0	0

LH: Levator ani muscle's hiatus; POP-Q: Pelvic organ prolapse quantification.

Table 4 Incidence of macrosomia and stress urinary incontinence in two groups of pelvic organ prolapse patients, *n* (%)

Group	Number	Macrosomia	Stress urinary incontinence	Pelvic floor muscle weakness
POP group	52	23 (44.23)	47 (90.38)	35 (67.31)
Non-POP Group	52	27 (52.93)	45 (86.54)	26 (50.00)

Comparison between the two groups, $P > 0.05$. POP: Pelvic organ prolapse.

Table 5 Multifactorial logistic regression analysis

Factor	β	SE	Wald statistic	95%CI
Age	3.005	0.570	4.698	1.542-14.399
Weight	2.819	0.799	6.112	1.778-40.774
Parity (number of births)	1.052	0.501	3.301	1.737-4.721
Gravidity (number of pregnancies)	2.318	0.452	3.686	2.467-3.741
Gestational weight gain	1.401	0.672	3.500	3.418-3.895

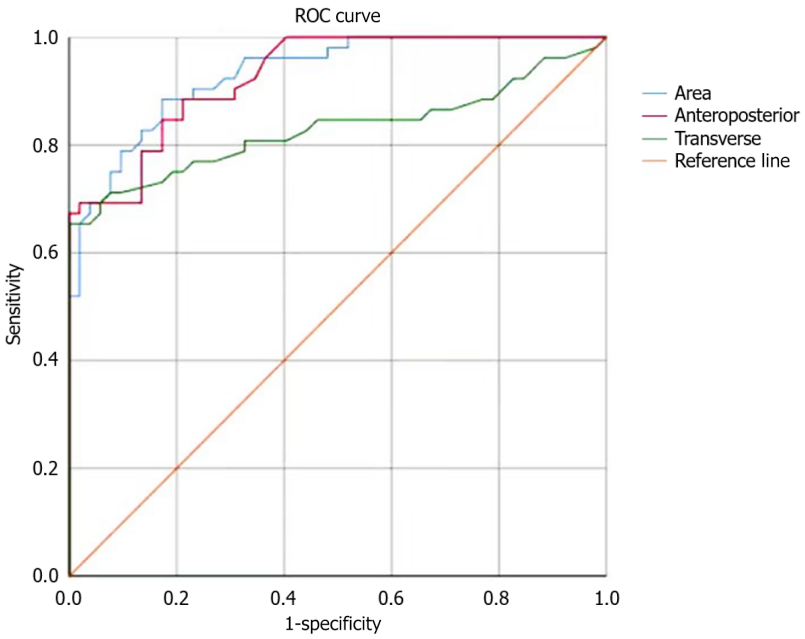

Figure 1 Levator ani muscle's hiatus area, anteroposterior diameter, and lateral diameter in the pelvic organ prolapse group. ROC: Receiver operating characteristic.

DISCUSSION

GDM is a distinct condition among pregnant women, occurring exclusively during pregnancy. Its incidence has gradually increased in recent years. According to literature, the incidence of GDM in the Asian population is approximately 11.5% [13]. Poor glycemic control during pregnancy heightens the risk of complications for the mother and fetus and the likelihood of macrosomia. The majority of women with GDM experience weight gain. Gomes *et al*[14] showed that maternal overweight or obesity can lead to an increased risk of the same condition in children. Skrypnik *et al*[15] argued that persistent hyperglycemia in utero raises the risk of macrosomia, resulting in severe strain on the maternal pelvic floor. Pinheiro *et al*[16] found that women with GDM experience varying degrees of muscle weakness in the pelvic floor and rectus abdominis during pregnancy, along with a reduction in type I/II collagen in fast and slow muscle fibers. Marini *et al*[17] noted a significant increase in the incidence of urinary incontinence 2 years post-cesarean section in women with GDM; this phenomenon can be attributed to long-term hyperglycemia damaging the extracellular matrix and striated muscles of the urethra. A retrospective study by Sangsawang[18] suggested that the most common type of urinary incontinence during pregnancy is stress urinary incontinence. Physiological changes during pregnancy, such as the increased weight of the uterus and fetus, exert pressure on the pelvic floor muscles. GDM exacerbates this risk by increasing the weight of the uterus and fetus, thus raising the incidence of POP in women.

POP has a high incidence rate. In our study involving 104 patients, the POP group had significantly larger LH area and anteroposterior and transverse diameters than the non-POP group. In addition, the number of women with stress urinary incontinence in both groups was higher than the general incidence rate[19]. The levator ani muscle, a major muscle of the pelvic floor, attaches bilaterally to the inner side of the pelvic wall, is symmetrically arranged, and forms a downward funnel shape. It is divided into two parts based on the arrangement of its muscle fibers: Iliococcygeus and pubococcygeus. Both are integral to the levator ani and play a supportive role in maintaining the normal position of pelvic organs. The LH, formed by the bilateral levator ani and the pubic bone in front, is the largest portal in the peritoneum and a primary pathway for the descent of pelvic organs. The integrity of the levator ani and the morphology of the LH reflect the position and structural changes of the pelvic organs. An increased LH size is a significant factor in the increased risk of POP. This study compared the size of the LH area in women with GDM with or without POP using perineal ultrasonography. The findings revealed that the anteroposterior diameter, transverse diameter, and area of LH were significantly enlarged in the POP group ($P < 0.05$). A possible reason is the prolonged high glucose state during pregnancy that affects the uterine environment and increases the fetal weight, consequently stretching the pelvic floor muscles and enlarging the LH. As a result, the incidence of POP increases.

This study established cutoff values for the LH area and anteroposterior and lateral diameters for the early screening and treatment of POP in GDM using ROC curve analysis. In the POP group, the LH area demonstrated an AUC of 0.906 with a 95%CI: 0.824-0.988. The optimal cutoff was 13.54 cm², showcasing a sensitivity of 83.2% and a specificity of 64.4%. For the anteroposterior diameter, the AUC was 0.836 with a 95%CI: 0.729-0.943. The optimal cutoff was 5.53 cm, revealing a sensitivity of 64.2% and a specificity of 73.4%. For the lateral diameter, the calculated AUC was 0.568 with a 95%CI: 0.407-0.729. The optimal cutoff was 4.67 cm, displaying a sensitivity of 65.9% and a specificity of 69.3%. Dietz[20] suggested that an LH area exceeding 25 cm² serves as a diagnostic criterion for abnormal expansion, which is contradictory with our study findings. This discrepancy may be attributed to potential variations in race and geographical region. Logistic regression analysis showed that age, weight, number of births, number of pregnancies, and weight gained during pregnancy are independent risk factors for the cooccurrence of GDM and POP.

CONCLUSION

Women diagnosed with GDM face an elevated probability of experiencing diminished pelvic floor muscle strength, POP, postpartum stress urinary incontinence, and macrosomia subsequent to childbirth. GDM stands as a substantial risk factor for the development of postpartum pelvic floor dysfunction.

FOOTNOTES

Author contributions: Wang QH and Liu LH initiated the project; Ying H and Chen MX designed the experiment and conducted clinical data collection; Liu LH, Zhou CJ and Li H performed postoperative follow-up and recorded data; Wang QH and Liu LH conducted a number of collation and statistical analysis, and wrote the original manuscript; all authors have read and approved the final manuscript.

Institutional review board statement: This study was approved by the Ethics Committee of People's Hospital Affiliated to Shandong First Medical University.

Informed consent statement: The ethics committee agrees to waive informed consent.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Efficacy comparison of multipoint and single point scanning panretinal laser photocoagulation in non-proliferative diabetic retinopathy treatment

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Abstract

BACKGROUND

Non-proliferative diabetic retinopathy (NPDR) poses a significant challenge in diabetes management due to its microvascular changes in the retina. Laser photocoagulation, a conventional therapy, aims to mitigate the risk of progressing to proliferative diabetic retinopathy (PDR).

AIM

To compare the efficacy and safety of multi-spot *vs* single-spot scanning panretinal laser photocoagulation in NPDR patients.

METHODS

Forty-nine NPDR patients (86 eyes) treated between September 2020 and July 2022 were included. They were randomly allocated into single-spot (n = 23, 40 eyes) and multi-spot (n = 26, 46 eyes) groups. Treatment outcomes, including best-corrected visual acuity (BCVA), central macular thickness (CMT), and mean threshold sensitivity, were assessed at predetermined intervals over 12 months. Adverse reactions were also recorded.

RESULTS
Energy levels did not significantly differ between groups ($P > 0.05$), but the multi-spot group exhibited lower energy density ($P < 0.05$). BCVA and CMT improvements were noted in the multi-spot group at one-month post-treatment ($P < 0.05$). Adverse reaction incidence was similar between groups ($P > 0.05$).

CONCLUSION
While energy intensity and safety were comparable between modalities, multi-spot scanning demonstrated lower energy density and showed superior short-term improvements in BCVA and CMT for NPDR patients, with reduced laser-induced damage.

Key Words: Panretinal laser photocoagulation; Non-proliferative diabetic retinopathy; Efficacy comparison; Multipoint; Single point; Treatment assessment

Core Tip: This study compares the therapeutic effectiveness of multipoint and single-point scanning panretinal laser photocoagulation in non-proliferative diabetic retinopathy (NPDR) patients. The results showed that both treatment modalities had similar energy intensity and safety profiles, but the multipoint scanning mode had a lower energy density. In the short term, the multipoint scanning mode demonstrated better improvement in best-corrected visual acuity and central macular thickness compared to the single-point mode. Additionally, the multipoint mode resulted in less laser damage. These findings suggest that multipoint scanning may be a preferred treatment approach for NPDR patients.

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INTRODUCTION

Diabetes is a comprehensive disease caused by absolute or relative insulin deficiency, leading to reduced sensitivity of target tissue cells to insulin, resulting in metabolic disturbances of proteins, fats, water, electrolytes, *etc*[1,2]. Data show that in recent years, with the improvement of residents' living standards, the prevalence of diabetes has been increasing year by year. In 2013, the prevalence of diabetes in adults in China was about 10.9%, with a total number of cases of approximately 110 million, ranking first in the world[3,4]. The most typical clinical manifestation of diabetes is elevated blood sugar, and long-term hyperglycemia can cause varying degrees of damage to multiple organs. Diabetic retinopathy (DR), caused by long-term hyperglycemia stimulation leading to retinal microvascular damage, is one of the common complications in the late stage of diabetes and the main cause of visual impairment and blindness in diabetic patients[5, 6]. Although global awareness of diabetes prevention and treatment has increased in recent years, the incidence of DR has not decreased, especially in low-income and middle-income countries.

Non-proliferative DR (NPDR) is a type of DR, accounting for approximately 19.1% of the total cases, which is significantly higher than the 2.8% of proliferative DR (PDR)[7]. The central macular thickness (CMT) refers to the thickness of the macula, which is the central part of the retina responsible for sharp, central vision. It's a crucial measure in assessing retinal health, especially in conditions like DR and age-related macular degeneration, where thickening of the macula can indicate disease progression. Clinical practice has indicated that early clinical symptoms of NPDR include bleeding and exudation, and it may progress to late stages with vascular proliferation and even blindness. Therefore, early treatment of NPDR is of great significance in improving the quality of life of diabetic patients[8]. Currently, treatment options for NPDR include medication intervention, laser photocoagulation, and vitrectomy. Among them, laser photocoagulation is widely used in clinical practice and has been proven in multiple studies to effectively improve clinical symptoms in NPDR patients[9,10]. However, there is still some controversy regarding the selection of laser treatment techniques for NPDR. Traditional laser photocoagulation for NPDR often uses single-spot or continuous single-spot emission, which, although effective, carries the risk of inducing visual field narrowing and decreased visual acuity [11]. In recent years, the clinical value of multi-spot scanning laser in the treatment of NPDR has been discovered. This study, through the establishment of a control group, found that compared to single-spot laser, the multi-spot scanning laser mode helps in the short-term visual recovery of NPDR patients after surgery, and the energy density is lower than that of single-spot scanning laser.

MATERIALS AND METHODS

General information

A total of 49 patients (86 eyes) diagnosed with NPDR and treated in our hospital from September 2020 to July 2022 were selected as the research subjects. They were randomly separated into the Single Spot Group (n = 23, 40 eyes) and the Multiple Spot Group (n = 26, 46 eyes). The approval of the Hospital Ethics Committee has been obtained for this study. The patients had given consent to participate in the study or treatment.

Inclusion criteria: (1) Age ≥ 18 years; (2) Type II diabetes patients; (3) Diagnosed with NPDR through fundus examination; and (4) Complete clinical data.

Exclusion criteria: (1) Patients with malignant tumors; (2) Patients with a history of laser photocoagulation therapy of the fundus; (3) Patients with poor dilation of the pupil or posterior synechiae that make laser photocoagulation therapy difficult; (4) Patients with coagulation disorders; (5) Patients already enrolled in other ongoing clinical studies; and (6) Pregnant or lactating women.

Intervention methods

The Single Spot Group received single-spot scanning laser photocoagulation therapy. The exposure time was set at 100-300 ms, and the total retinal photocoagulation was divided into four sessions, with each session targeting one quadrant starting from the inferior temporal quadrant. If the patient had concurrent macular edema, macular photocoagulation was performed first. The interval between the two photocoagulation sessions was 7 days, and the number of photocoagulated spots per session was approximately 300-500. The Multiple Spot Group received multiple-spot scanning laser photocoagulation therapy, using an approach from the periphery to the center. A total of six sessions were performed to complete the total retinal photocoagulation, with approximately 230 spots per session and a total of approximately 1500-2000 spots. The retinal photocoagulation procedures for both groups were performed by the same physician.

Observation indicators and evaluation criteria

Clinical data, including sex, age, body mass index, smoking, drinking, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, *etc*, were collected for both groups. The laser energy used during the therapies was recorded for both groups, and the energy density was calculated. Follow-up evaluations were conducted 12 months after therapy to assess the effectiveness rate, based on improvements of ≥ 2 Lines in best-corrected visual acuity (BCVA) indicating improvement, improvements of ≥ 2 Lines indicating worsening, and no change. BCVA and CMT were measured before therapy, 1 month, 6 months, and 12 months after therapy for inter-group comparisons. The incidence of adverse reactions such as iritis and vitreous hemorrhage within 12 months of follow-up after therapy was also analyzed.

Statistical analysis

Normality was assessed for continuous variables using Shapiro-Wilk test. The t-test utilized in this study was one-tailed. Continuous data are presented as mean ± SD, and comparisons between groups were made using one-tailed t-tests. Data collection was performed using Excel 2021. Data processing and analysis were conducted using SPSS 19.0. Categorical data are presented as percentages (%), and comparisons between groups were made using χ^2 tests. A significance level of $P < 0.05$ was used to determine statistical significance.

RESULTS

Comparison of baseline clinical data

A total of 46 patients were included in this study, including 26 patients in the multipoint group and 23 patients in the single point group. The baseline clinical data of the enrolled patients, such as sex, age, body mass index, ALT, AST, serum creatinine, and creatine kinase levels, were included in the study, and intergroup distinctions were compared. The comparison showed no obvious distinctions in baseline clinical data of patients ($P > 0.05$), indicating good comparability (Table 1). There was no obvious distinction in laser energy ($P > 0.05$), but the energy density of the multipoint group was lower than that of the single point group, and the distinction was obvious ($P < 0.05$) (Table 2 and Figure 1).

The effective rate was different

In the multipoint group, the visual acuity improved in 17 cases, remained unchanged in 26 cases, and decreased in 3 cases. There was no obvious distinction in the change of visual acuity ($P > 0.05$) (Table 3 and Figure 2).

Comparison of LogMAR BCVA before and after therapy

Before therapy, there was no obvious distinction in LogMAR BCVA ($P > 0.05$). After 1 month of therapy, the LogMAR BCVA in the multipoint group was lower than the single point group, and the distinction was obvious ($P < 0.05$). However, there was no obvious distinction in LogMAR BCVA after 6 months and 12 months of therapy ($P > 0.05$) (Table 4, Figure 3A).

Table 1 Comparison of baseline clinical data, *n* (%)

Clinical parameters	Multi-point mode, *n* = 26	Single point mode, *n* = 23	t/χ^2	*P* value
Male sex	16 (61.64)	12 (52.17)	0.437	0.509
Average age in years	44.92 ± 6.35	44.22 ± 6.40	0.384	0.703
Average body mass index in kg/m^2	22.80 ± 2.87	23.95 ± 2.50	1.486	0.144
Smoking	3 (11.54)	4 (17.39)	0.341	0.559
Alcohol drinker	3 (11.54)	3 (13.04)	0.026	0.873
ALT in U/L	29.11 ± 14.54	29.83 ± 14.13	0.175	0.862
AST in U/L	34.22 ± 16.64	36.20 ± 17.02	0.411	0.683
FBG in mmol/L	5.20 ± 0.85	5.30 ± 1.01	0.376	0.708
Creatinine in μmol/L	72.48 ± 14.73	63.26 ± 17.67	1.992	0.052
Creatine kinase in U/L	181.87 ± 135.88	177.37 ± 114.54	0.124	0.902
Uric acid in μmol/L	373.43 ± 95.80	389.90 ± 96.51	0.599	0.552
Concurrent diseases				
Hypertension	2 (7.69)	4 (17.39)	1.068	0.301
Nonalcoholic fatty liver disease	2 (7.69)	1 (4.35)	0.238	0.626
Liver cirrhosis	1 (3.85)	0 (0.00)	0.903	0.342

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FBG: Fasting blood glucose.

Table 2 Comparison of surgical indicators

Groups	Eyes	Laser energy in mW	Energy density in mW·ms/μm^2
Multipoint group	46	551.93 ± 64.25	0.51 ± 0.08
Single point group	40	540.64 ± 73.77	2.02 ± 0.92
t	-	0.759	11.094
P value	-	0.450	0.000

Table 3 Distinctions in therapy response rates, *n* (%)

Groups	Eyes	Changes in vision		
		Improve	Unchanged	Decline
Multipoint group	46	17 (36.96)	26 (56.52)	3 (6.52)
Single point group	40	8 (20.00)	25 (62.50)	7 (17.50)
χ^2	-	2.983	0.574	2.509
P value	-	0.084	0.317	0.113

Comparison of CMT before and after therapy

Before therapy, there was no obvious distinction in CMT ($P > 0.05$). After 1 month of therapy, the CMT in the multipoint group was lower than the single point group, and the distinction was obvious ($P < 0.05$). However, there was no obvious distinction in CMT after 6 months and 12 months of therapy ($P > 0.05$) (Table 5, Figure 3B).

Comparison of the incidence rates of adverse reactions

In the multipoint group, there was 1 case of iritis and 1 case of vitreous hemorrhage. The overall occurrence rate of adverse reactions is 7.69% (2/26). There was no obvious distinction in comparison to the single point group, which had an incidence rate of 17.39% (4/23) (Table 6, Figure 4).

Table 4 Comparison of LogMAR best-corrected visual acuity before and after therapy

Groups	Eyes	Pre-therapy	1 month after surgery	6 months after surgery	12 months after surgery
Multipoint group	46	0.65 ± 0.08	0.54 ± 0.10	0.49 ± 0.11	0.51 ± 0.10
Single point group	40	0.68 ± 0.09	0.60 ± 0.10	0.48 ± 0.09	0.53 ± 0.06
t	-	1.637	2.775	0.457	1.103
P value	-	0.105	0.007	0.649	0.273

Table 5 Comparison of central macular thickness before and after therapy

Groups	Eyes	Pre-therapy	1 month after surgery	6 months after surgery	12 months after surgery
Multipoint group	46	446.36 ± 175.99	366.74 ± 102.17	346.41 ± 85.47	345.72 ± 82.03
Single point group	40	455.08 ± 118.94	423.35 ± 94.54	359.14 ± 76.18	349.35 ± 86.54
t	-	0.265	2.653	0.724	0.200
P value	-	0.792	0.010	0.471	0.842

Table 6 Comparison of the incidence of adverse reactions, *n* (%)

Groups	Cases	Iritis	Vitreous hemorrhage	Tractional retinal detachment	Overall incidence
Multipoint group	26	1 (3.85)	1 (3.85)	0 (0.00)	2 (7.69)
Single point group	23	2 (8.70)	1 (4.35)	1 (4.35)	4 (17.39)
Fisher	-	-	-	-	1.068
P value	-	-	-	-	0.301

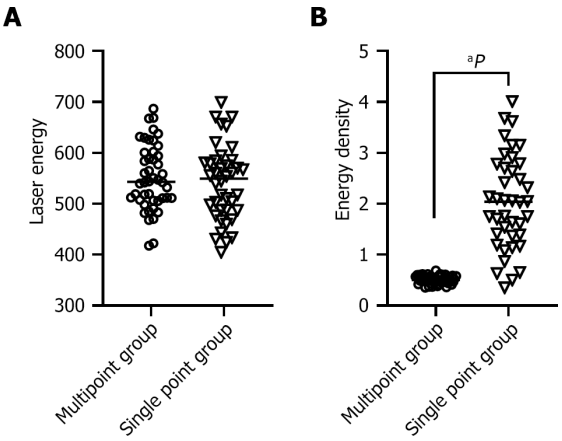

Figure 1 The laser energy in the multipoint group and single point group. A: The difference of laser energy between multi-point group and single-point group was not statistically significant ($P > 0.05$); B: The energy density of multi-point group was lower than that of single-point group, and the difference was statistically significant ($P < 0.05$). $^aP < 0.05$.

DISCUSSION

Diabetes is a metabolic-related disease characterized by high blood sugar levels. DR is one of the common complications of diabetes, and with the progression of the disease, almost every diabetic patient will develop different manifestations of complications. Among them, DR is the most common[12-15]. From a clinical perspective, DR can be categorized into two forms based on the development of new blood vessels: PDR and NPDR. NPDR is an initial indication, while PDR is a more advanced stage[13]. The early clinical symptoms of NPDR are not obvious, and patients' vision does not obviously change. However, as the condition worsens, patients may experience an obvious reduction in vision, along with manifestations such as retinal capillary dilation, and the presence of hemorrhages, exudates, and cotton wool spots[14]. Early intervention is crucial in NPDR, and proactive surgery can help to seal retinal vascular leaks, prevent new bleeding, and

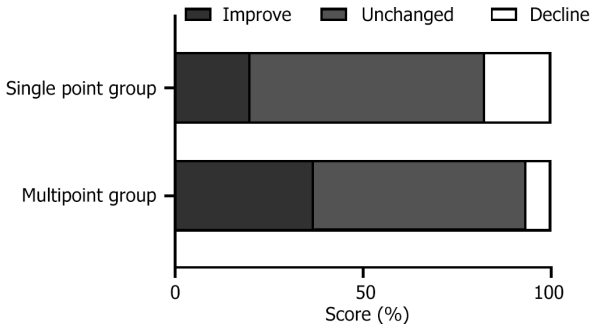

Figure 2 Distinction in therapy effectiveness.

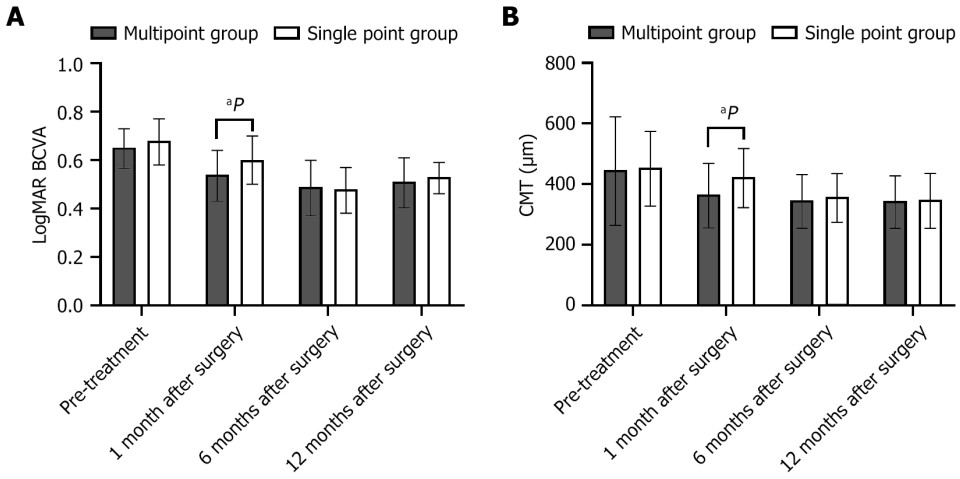

Figure 3 Comparison of LogMAR best-corrected visual acuity between the two groups before and after treatment. A: LogMAR best-corrected visual acuity; B: Central macular thickness. There was no significant difference between the multi-point group and the single-point group before treatment ($P > 0.05$), and the multi-point group was lower than the single-point group 1 month after treatment ($P < 0.05$), and there was no significant difference between the multi-point group and the single-point group 6 months and 12 months after treatment ($P > 0.05$). $^aP < 0.05$. CMT: Central macular thickness.

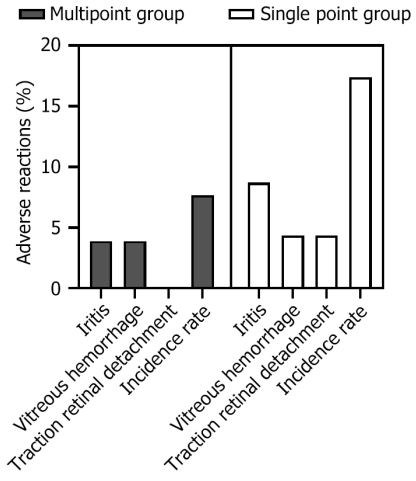

Figure 4 Comparison of adverse reaction rates.

suppress the development of neovascularization, thereby achieving therapy goals[15]. In this study, the clinical value of multi-point scanning laser photocoagulation and single-point scanning laser photocoagulation in NPDR patients was analyzed using a control group. The results showed no obvious distinction in the therapy efficacy between multi-point scanning laser photocoagulation and single-point scanning laser photocoagulation, suggesting similar effectiveness of both procedures. The mechanism of laser photocoagulation in the therapy of NPDR can be summarized as follows: (1) The thermal coagulation effect of laser can transform high oxygen-consuming photoreceptors into low oxygen-consuming glial components, thereby redistributing oxygen to other retinal tissues and improving retinal blood circulation[16]; (2)

Laser can destroy the ischemic areas of the retina, preventing neovascularization within those regions[17]; (3) By disrupting the outer barrier of the retina, laser allows oxygen previously trapped in the outer retina to reach the inner retina, thus improving oxygen supply to the inner retina[18]; and (4) Photocoagulation helps to inhibit the overexpression of cyclooxygenase-2 and vascular endothelial growth factor in NPDR patients, preventing the occurrence of neovascularization[19]. The manner in which laser photocoagulation is applied does not obviously affect its effectiveness, and therefore, there is no obvious distinction in the clinical efficacy.

Furthermore, follow-up was conducted on patients in the single-point and multipoint groups. The results showed that at 1 month after therapy, the LogMAR BCVA and CMT were obviously lower in the multipoint group in comparison to the single point group. The authors of this study analyzed the reasons as follows: Although single-point scanning laser has less powerful laser energy, it has higher energy density. After absorption by the target tissue, it produces a high-temperature effect in a short period of time, causing greater damage to the choroid and retina, resulting in a higher risk of reactive macular edema[20]. On the other hand, multi-point scanning laser utilizes a pre-designed short pulse sequence to rapidly complete pan-retinal photocoagulation, keeping the exposure time below 50 ms. The reduction in energy density and shorter laser irradiation time effectively limit the range of thermal conduction, minimizing damage to the retinal pigment epithelium and photoreceptor layer, and obviously reducing CMT in patients after surgery[21,22]. At 6 months and 12 months of follow-up, there was no obvious distinction in LogMAR BCVA and CMT. The reason for this is that both single-point and multi-point scanning laser photocoagulation can effectively control the exacerbation of diabetic macular edema, which is consistent with the similar therapy efficacy observed. Finally, the study compared the occurrence of complications during follow-up, and found no obvious distinction in the incidence of adverse reactions, indicating that both single-point scanning laser photocoagulation and multi-point scanning laser photocoagulation are safe in the therapy of NPDR.

CONCLUSION

Multi-point and single-point scanning laser photocoagulation have similar therapeutic effects on NPDR, with similar laser energy intensity and safety. However, multi-point scanning has lower energy density in comparison to single-point scanning, and in the short term after therapy, the multi-point approach shows better improvement in BCVA and CMT for NPDR patients, with less laser-induced damage. Although this study compared the distinctions in the effectiveness of multi-point scanning laser photocoagulation and single-point scanning laser photocoagulation in the therapy of NPDR, it has limitations such as a small sample size and relatively short follow-up time. Adding laboratory examination results to the study would help further compare the advantages and disadvantages of the two.

FOOTNOTES

Author contributions: Zhang YZ, Gong H, Yang J, Bu JP, and Yang HL designed the research study; Zhang YZ, Gong H, and Bu JP performed the research; Zhang YZ, Gong H, and Yang J contributed new reagents and analytic tools; Zhang YZ, Bu JP, and Yang HL analyzed the data and wrote the manuscript; All authors have read and approved the final manuscript.

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Clinical trial registration statement: This study has not yet been registered with clinical trials.

Informed consent statement: All research participants or their legal guardians provided written informed consent prior to study registration.

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ORIGINAL ARTICLE

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Association between composite dietary antioxidant index and stroke among individuals with diabetes

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Abstract

BACKGROUND

Recent research has underscored the potentially protective role of dietary antioxidants against chronic conditions, such as cardiovascular diseases and stroke. The composite dietary antioxidant index (CDAI), which reflects the overall intake of key dietary antioxidants, has been identified as a crucial metric for exploring this relationship. Although previous research has shown a negative correlation between CDAI levels and stroke risk in prediabetic individuals, there remains a substantial gap in understanding this association among individuals with diabetes, who are at an inherently greater risk for cerebrovascular events.

AIM
To investigate the association between CDAI and stroke risk in individuals with diabetes.

METHODS
Using a cross-sectional study design, this investigation analyzed data from the National Health and Nutrition Examination Survey spanning from 2003 to 2018 that included 6735 participants aged over 20 years with diabetes. The CDAI was calculated from 24-h dietary recalls to assess intake of key antioxidants: Vitamins A, C, and E; carotenoids; selenium; and zinc. Multivariate logistic regression and restricted cubic spline analysis were used to rigorously examine the relationship between CDAI and stroke risk.

RESULTS
The participant cohort, with an average age of 59.5 years and a slight male majority, reflected the broader demographic characteristics of individuals with diabetes. The analysis revealed a strong inverse relationship between CDAI levels and stroke risk. Remarkably, those in the highest quintile of CDAI demonstrated a 43% lower prevalence of stroke compared to those in the lowest quintile, even after adjustments for various confounders. This finding not only highlights the negative association between CDAI and stroke risk but also underscores the significant potential of antioxidant-rich diets in reducing stroke prevalence among patients with diabetes.

CONCLUSION
Our findings suggested that CDAI was inversely associated with stroke prevalence among patients with diabetes. These results suggest incorporating antioxidant-rich foods into dietary regimens as a potential strategy for stroke prevention.

Key Words: Stroke; Diabetes; Composite dietary antioxidant index; National Health and Nutrition Examination Survey; Cross-sectional study

Core Tip: Previous research on the composite dietary antioxidant index (CDAI) and its impact on stroke risk among individuals with diabetes is limited. Our study addressed this gap by examining the association between higher CDAI scores and stroke prevalence. Our findings revealed that higher CDAI scores correlated with reduced stroke risk in this population, indicating that a diet rich in diverse antioxidants may play a crucial role in mitigating stroke risk among individuals with diabetes.

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INTRODUCTION

Stroke is the second largest cause of death worldwide and a major contributor to long-term disability[1]. Additionally, the chronic condition of diabetes, which is prevalent globally, has been identified as a significant stroke risk factor[2,3]. Importantly, individuals with diabetes face a 1.5 to 2-fold greater risk of stroke than individuals without diabetes, which intensifies with a longer duration of diabetes[4]. Additionally, patients with diabetes tend to experience worse post-stroke outcomes and a heightened risk of recurrent strokes[5-10]. The 2024 ADA Standards of Care underscore the critical role of dietary management in diabetes to mitigate the risk of associated complications, including stroke[11].

Hyperglycemia, a hallmark of diabetes, is known to induce mitochondrial dysfunction and endoplasmic reticulum stress, leading to increased accumulation of reactive oxygen species (ROS). This buildup is crucial in causing cellular damage, hastening the development of diabetes-related complications like stroke[12-15]. Furthermore, hyperglycemia-induced oxidative stress is implicated in the upregulation of proinflammatory factors, triggering cellular apoptosis and impairing nitric oxide release[16]. This interplay between oxidative stress and inflammation forms a vicious cycle, exacerbating the progression of atherosclerosis, a critical process in stroke pathogenesis[17,18]. Emerging research suggests that increasing daily dietary antioxidant intake can increase plasma antioxidant levels, effectively mitigating oxidative stress-related damage[19,20]. Therefore, dietary modifications aimed at reducing oxidative stress present a promising approach for decreasing stroke risk in individuals with diabetes.

The efficacy of individual antioxidants, such as vitamin E and carotenoids, in reducing cardiovascular disease (CVD) risk remains controversial[21-26]. Measurements based on individual antioxidants, including vitamins A, C, E, zinc, selenium, and total carotenoids, may not accurately capture overall antioxidant intake. In contrast to individual antioxidants, the composite dietary antioxidant index (CDAI) provides a more extensive evaluation of total antioxidant

consumption, which correlates with specific inflammatory biomarkers, including tumor necrosis factor-α and interleukin-1β[27,28]. Although elevated CDAI scores have been inversely associated with the risks of coronary heart disease, stroke, depression, and cancer[17,29-31], its specific impact on stroke risk among individuals with diabetes is yet to be studied. To the best of our knowledge, this study represents the initial investigation into the correlation between the CDAI and stroke risk within a diabetic population. Our cross-sectional analysis examines how CDAI relates to stroke risk among this group, which may inform future preventive and therapeutic approaches.

MATERIALS AND METHODS

Study population

The National Health and Nutrition Examination Survey (NHANES) supplies essential information related to the health and diet specifics of the United States population. Employing a stratified, multistage probability sampling approach, NHANES ensures a demographically representative sample. Data collection encompasses structured personal interviews in participants' homes, comprehensive health assessments at mobile examination centers, and laboratory analysis of collected specimens. Every participant consented to the study by signing an informed consent form.

Our study focused on adults aged 20 years or older with hyperglycemia, participating in NHANES from 2003 to 2018. Diagnosis of diabetes mellitus in participants was established in accordance with the Standards of Medical Care in Diabetes. Criteria included a fasting plasma glucose level of at least 126 mg/dL (7.0 mmol/L), a hemoglobin A1c level of 6.5% or higher (48 mmol/mol), a self-reported diagnosis, or prior use of antidiabetic drugs. Exclusion criteria included being under 20 years of age, pregnancy at study onset, lack of dietary information, or absence of stroke condition data. After rigorous screening, 6735 individuals diagnosed with diabetes from the 2003-2018 NHANES cohorts were selected for analysis. Figure 1 illustrates the detailed screening process and the participant breakdown.

Dietary assessment

Dietary data were collected through structured interviews at the NHANES. Dietary intake data from the subjects was gathered *via* recall interviews conducted by experienced technicians, with participants asked to specify the food and beverages they consumed in the 24 h prior to the interview. This information facilitated the estimation of energy consumption, various nutrients, and other dietary components. The assessment of dietary antioxidants focused on six key antioxidants: Vitamin A; vitamin C; vitamin E; zinc; selenium; and total carotenoids. Total carotenoids were quantified as the sum of five distinct carotenoids: α-carotene; β-carotene; β-cryptoxanthin; lutein/zeaxanthin; and lycopene. To evaluate the cumulative impact of dietary antioxidants on stroke risk in patients with diabetes, we utilized a modified CDAI[32]. This index was derived from the six mentioned antioxidants and is calculated through the following steps. First, we stratify each antioxidant intake by sex, calculating the mean and standard deviation for males and females separately. Next, we standardize each individual's antioxidant intake. For each individual, we subtract the sex-specific mean from their intake and then divide by the sex-specific standard deviation. In this way, we obtain the standardized intake of dietary antioxidants. Finally, we aggregate the standardized intakes of the six antioxidants to calculate each individual's overall standardized intake of dietary antioxidants. The following formula is used for the calculation:

$$CDAI = \sum_{i=1}^{6} \frac{xi - \mu i}{Si}$$

In this formula, xi represents the daily intake of antioxidant i; μi represents the sex-specific mean value of xi for the antioxidants i; Si represents the standard deviation for μi.

Diagnosis of stroke

Stroke diagnosis in this research was determined based on participants' self-reported medical history of stroke[33]. During the NHANES interview process, participants were directly asked, "Have you ever been informed by a doctor or other health professional that you experienced a stroke?" A positive response to this question was considered indicative of a prior stroke occurrence. Conversely, a negative response was interpreted as an indication that the participant had no history of stroke. This method aligns with standard practices in epidemiological research, where self-reported medical histories are commonly used to identify previous health events.

Covariates

Informed by clinical expertise and prior research, this study carefully considered various potential confounders that might influence the association between the CDAI and stroke risk, integrating them as covariates in our analysis. These included demographic data such as age, sex, and race/ethnicity, educational levels spanning from below high school to college education and above, marital status categories, economic indicators like the family poverty-income ratio (PIR), lifestyle aspects like smoking habits and alcohol consumption, and health-related metrics like body mass index (BMI), daily energy intake, and histories of hypertension and hypercholesterolemia. This thorough selection of covariates ensured a robust analysis by accounting for potential confounders in the association under investigation.

Statistical analysis

This study utilized data from the NHANES, a complex, multistage, cluster research survey conducted by the National Center for Health Statistics in the United States. To ensure representativeness of the United States population, the analysis

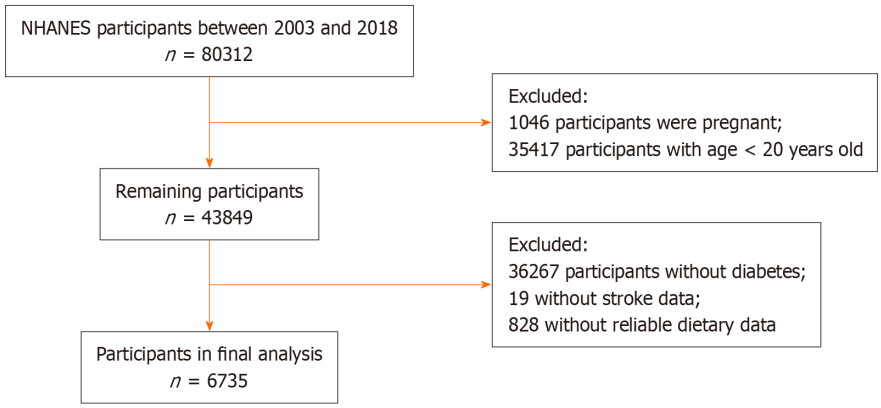

Figure 1 Overview of the study design.

was weighted according to NHANES-specific sample weights. The study population was divided into two groups for analysis: Those with a history of stroke and those without. Continuous variables with a normal distribution were presented as means ± standard deviation, while those with skewed distributions were expressed as medians with interquartile range and subjected to log transformation to approximate normality prior to analysis. Categorical variables were shown as percentages (%).

To assess differences between groups, independent sample *t*-tests or non-parametric Mann-Whitney *U* tests were employed for continuous variables, and χ^2 tests were utilized for categorical variables. Multivariate logistic regression analyses were performed to explore the relationship between CDAI and stroke. Three distinct models were employed: An unadjusted model 1; model 2 adjusted for demographic variables such as age, sex, race, education level, marital status, and family PIR; and model 3 that included additional adjustments for lifestyle and comorbidity like alcohol consumption, smoking status, BMI, energy intake, hypertension, and hypercholesterolemia.

Results were presented as odds ratios (ORs) and 95% confidence intervals (CI). The dose-response relationship between CDAI and stroke risk was analyzed using restricted cubic spline functions. Additionally, subgroup analyses were conducted to further validate the stability of the results. All statistical analyses were performed with R software, version 4.3.2 (http://www.R-project.org). A two-tailed *P* value of less than 0.05 was considered statistically significant.

RESULTS

In this study, 6735 participants from the NHANES dataset were rigorously screened and included in the analysis. The mean age of the subjects was 59.5 ± 13.6 years, and 51.5% of the participants were males. Among these, 611 individuals were identified as having a history of stroke. The baseline characteristics of the study population, encompassing age, sex, race, education, marital status, family PIR, alcohol consumption status (drinker), smoking status, BMI, and histories of hypertension and hypercholesterolemia, are detailed in Table 1. A comparative analysis revealed significant distinctions in several clinical characteristics between the stroke and non-stroke groups. Notably, the stroke group comprised older participants ($P < 0.001$), and significant racial composition differences were observed ($P < 0.001$). Additionally, the stroke group demonstrated lower levels of educational and economic status compared to the non-stroke group. Prevalence rates of hypertension and hypercholesterolemia were also found to be significantly higher in the stroke group, underscoring the potential impact of these conditions on stroke occurrence. The non-stroke group exhibited significantly higher levels of vitamin A, vitamin E, zinc, selenium, and carotenoids compared to their counterparts in the stroke group (Table 2). However, no significant difference in vitamin C was observed between the two groups ($P = 0.093$).

As a continuous variable, CDAI exhibited a negative correlation with stroke prevalence, with an OR of 0.91 (95%CI: 0.89-0.94) in unadjusted logistic regression analysis (Table 3). After adjusting for potential confounders, the negative association between CDAI and stroke remained statistically significant (OR = 0.96, 95%CI: 0.92-0.99). Of note, individuals with the highest quintile of CDAI (Q5) displayed a 43% lower stroke risk compared to those in the lowest quintile (Q1) (OR = 0.57, 95%CI: 0.40-0.83). Moreover, a dose-response relationship was observed, indicating a gradual increase in stroke risk as CDAI levels decreased (Figure 2).

Additionally, a comprehensive subgroup analysis was conducted to further elucidate the association between CDAI and stroke risk across various demographic and clinical subgroups. These subgroups were delineated based on age, sex, BMI, race, smoking status, alcohol consumption, hypertension, and hypercholesterolemia (Table 4). However, the subgroup analysis did not reveal any significant statistical interactions, suggesting a consistent relationship between CDAI and stroke risk across these diverse groups.

Table 1 Characteristics of subjects with or without stroke

Characteristics	Total, *n* = 6735	Non-stroke, *n* = 6124	Stroke, *n* = 611	*P* value
Age in years	59.5 ± 13.6	58.8 ± 13.6	67.4 ± 10.7	< 0.001
Sex				0.156
Male	51.5	51.8	48.7	
Female	48.5	48.2	51.3	
Race				< 0.001
Non-Hispanic White	60.7	60.3	65.0	
Non-Hispanic Black	15.4	15.2	17.2	
Mexican American	9.8	10.1	6.4	
Other Hispanic	5.7	6.0	2.5	
Other race	8.4	8.4	8.8	
Education				< 0.001
Lower than high school	10.6	10.4	13.2	
High school	39.4	38.6	48.1	
Higher than high school	50.0	51.0	38.6	
Marital status				0.053
Married/living with partner	62.5	63.3	54.7	
Widowed/divorced/separated	27.9	26.8	40.1	
Never married	9.6	10.0	5.2	
Poverty income ratio				< 0.001
≤ 1.30	26.2	25.5	33.1	
1.3 to ≤ 3.5	39.7	39.1	47.3	
> 3.5	34.1	35.4	19.6	
Smoking status				0.007
Nonsmoker	48.6	49.2	42.7	
Former smoker	34.7	34.4	37.1	
Current smoker	16.7	16.4	20.2	
Alcohol use				0.002
No	33.3	32.7	40.1	
Yes	66.7	67.3	59.9	
Energy intake, kcal	1940.82 ± 900.52	1967.09 ± 909.80	1655.64 ± 734.62	< 0.001
BMI in kg/m^2				0.429
< 25.0	11.77	11.77	11.86	
25 to < 30	25.68	25.88	23.36	
≥ 30	62.55	62.35	64.78	
Hypertension				< 0.001
No	34.3	36.0	16.4	
Yes	65.7	64.1	83.6	
Hypercholesterolemia				0.002
No	38.6	39.1	32.5	
Yes	61.4	60.9	67.5	
CDAI	0.28 ± 3.97	0.38 ± 4.01	-0.77 ± 3.29	< 0.001

Data are presented as mean (SD) or *n* (%). BMI: Body mass index; CDAI: Composite dietary antioxidant index.

Table 2 Comparison of each component of the composite dietary antioxidant index among the non-stroke group and stroke group

Variables	Total, *n* = 6735	Non-stroke, *n* = 6124	Stroke, *n* = 611	*P* value
Vitamin A in mcg	449 (252-736)	453 (255-743)	400 (220-676)	0.026
Vitamin C in mg	50.6 (21.4-105.2)	51.3 (22.0-105.4)	44.2 (18.0-101.3)	0.093
Vitamin E in mg	5.99 (3.77-9.23)	6.10 (3.81-9.38)	5.13 (3.28-7.46)	< 0.001
Zinc in mg	8.99 (6.10-13.06)	9.09 (6.20-13.18)	7.81 (5.04-11.66)	< 0.001
Selenium in mcg	94.3 (65.1-131.2)	95.9 (66.3-133.4)	79.1 (55.8-111.5)	< 0.001
Carotenoid in mcg	4740.0 (1675.0-10809.0)	4851.0 (1759.2-10902.8)	3655.0 (1124.0-9827.0)	0.001

Data are presented as the median (interquartile range).

Table 3 Association of composite dietary antioxidant index and stroke

Factor	Model 1		Model 2		Model 3	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
CDAI (continuous)	0.91 (0.89-0.94)	< 0.001	0.93 (0.90-0.96)	< 0.001	0.96 (0.92-0.99)	0.019
CDAI-Q1	Ref.	-	Ref.	-	Ref.	-
CDAI-Q2	0.70 (0.55-0.88)	0.003	0.70 (0.55-0.89)	0.004	0.74 (0.57-0.96)	0.024
CDAI-Q3	0.59 (0.46-0.75)	< 0.001	0.60 (0.46-0.77)	< 0.001	0.68 (0.51-0.90)	0.007
CDAI-Q4	0.56 (0.43-0.72)	< 0.001	0.62 (0.48-0.80)	< 0.001	0.72 (0.53- 0.98)	0.037
CDAI-Q5	0.38 (0.29-0.51)	< 0.001	0.46 (0.34-0.61)	< 0.001	0.57 (0.40-0.83)	0.003
P for trend	< 0.0001		< 0.0001		0.007	

Model 1 was unadjusted; Model 2 was adjusted for age, sex, race, education, marital status and poverty income ratio; Model 3 was further adjusted for drinker, smoker, body mass index, energy intake, hypertension and hypercholesterolemia. Subjects with a composite dietary antioxidant index in the lowest quintile group served as the reference group. CDAI: Composite dietary antioxidant index; CI: Confidence interval; OR: Odds ratio; Q: Quintile; Ref.: Reference.

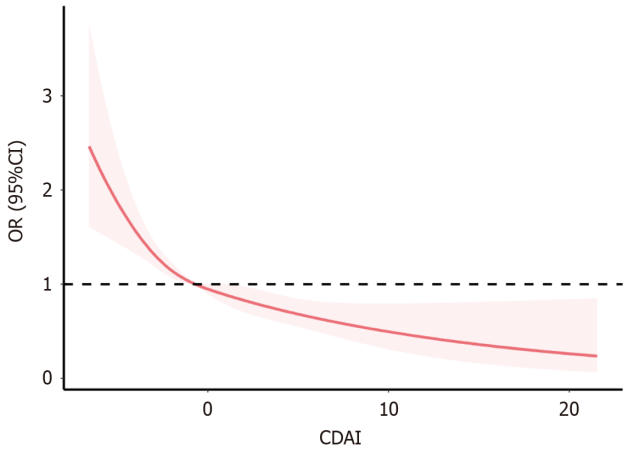

Figure 2 Restricted cubic spline plot of the association between composite dietary antioxidant index and stroke. The shaded part represented the 95% confidence interval (CI). CDAI: Composite dietary antioxidant index; OR: Odds ratio.

DISCUSSION

In our study, higher CDAI scores were associated with a decreased prevalence of stroke specifically in individuals with diabetes, a novel focus on this high-risk group. The observed dose-response relationship indicated that the risk of stroke inversely correlated with CDAI levels, gradually increasing as CDAI decreased. Importantly, this association remained significant even after accounting for conventional risk factors such as BMI, hypertension, and hypercholesterolemia. These findings suggested that a higher intake of antioxidants, as quantified by the CDAI, may confer a protective effect against stroke in individuals with diabetes, highlighting the unique impact of antioxidants on this population's heightened oxidative stress, a recognized risk factor for stroke.

Previous studies have not specifically focused on patients with diabetes when examining the relationship between dietary antioxidants and stroke. Our research is the first to highlight the significant role of CDAI in reducing stroke risk among individuals with diabetes, and through restricted cubic splines, we observed that as CDAI increased, the risk of stroke gradually decreased. Our study's finding contributes to a growing understanding of the impact of diet on chronic inflammation and oxidative stress, key factors in metabolic diseases and cardiovascular health[34,35]. The CDAI, which is designed to reflect the anti-inflammatory potential of dietary components, underscores the role of antioxidants in neutralizing oxidative stress and is implicated in the pathogenesis of atherosclerosis and vascular diseases[36]. Diets rich in antioxidants, targeting ROS, may not only protect against CVD but also are inversely associated with all-cause and CVD-related mortality in adults with diabetes, providing unique insights into the dietary management of this high-risk group[17,37].

This aligns with research indicating that dietary antioxidants can act as neuroprotective agents, safeguarding brain tissues and potentially ameliorating conditions leading to stroke[18-20]. A deficiency in dietary antioxidants is postulated to elevate stroke risk, likely through mechanisms linked to oxidative stress. Excessive ROS, in conjunction with lipid peroxidation, neuroinflammatory responses, and blood-brain barrier disruption, contribute to brain tissue injury[2-4]. Moreover, post-ischemic ROS activity can stimulate nuclear transcription factors and trigger the release of proinflammatory factors, leading to a localized neuroinflammatory response[25].

Oxidative stress also plays a role in the destruction of tissue surrounding hematomas following cerebral hemorrhage [26], and the capacity of the central nervous system for maintaining redox homeostasis is vital for post-stroke brain tissue recovery[5]. The efficacy of antioxidants involves electron donation to free radicals, thus diminishing cellular damage and curbing inflammatory responses[14]. The protective potential of antioxidants extends to the prevention and management of atherosclerosis, a key stroke risk factor, by reducing ROS generation and preventing oxidative damage to lipoproteins [21,22].

The components of CDAI, especially vitamins A, C, and E, are crucial in this context. These non-enzymatic antioxidants help neutralize oxidative stress, a key mechanism in stroke pathogenesis. Vitamin E, for example, effectively scavenges free radicals and protects cell structures, including lipoproteins, from oxidative damage[38,39]. In adult stroke-prone spontaneously hypertensive rats, vitamins C and E mitigate oxidative stress, enhance vascular function and structure, and inhibit hypertension progression potentially through the modulation of enzyme systems responsible for free radical generation[40].

In a 22-year prospective population-based study, it was found that higher dietary intakes of antioxidant vitamins A, C, and E significantly reduced the risk of adverse cardiovascular outcomes among Chinese individuals[41]. Carotenoids offer neuroprotection through the inhibition of neuroinflammation, microglial activation, and the excitotoxic pathway, as well as by modulating autophagy, attenuating oxidative damage, and activating defensive antioxidant enzymes[42]. This protective effect was shown in a 13-year cohort study in which higher plasma levels of carotenoids, indicative of fruit and vegetable intake, were associated with a reduced risk of ischemic stroke[43]. In addition to vitamins, micronutrients in the CDAI, such as zinc and selenium, also contribute significantly to mitigating oxidative stress and inflammation. Zinc acts by suppressing oxidative stress through inhibition of NADPH oxidase[44,45], while selenium, along with selenoproteins, prevents cellular damage due to lipid peroxidation[46].

The CDAI is a widely utilized metric in nutritional research to explore the correlation between dietary antioxidant intake and diverse diseases, particularly those associated with oxidative stress. Previous investigations have indicated a negative correlation between the CDAI and diabetes prevalence[47]. Additionally, research has shown that higher CDAI levels might mitigate the risk of diabetic kidney disease and mortality among individuals with diabetes[48]. Moreover, higher CDAI levels exhibit a significant association with reduced CVD mortality in individuals with diabetes, with those in the highest quartile experiencing a 53% decrease in risk compared to those in the lowest quartile[37]. Collectively, these findings suggest that higher CDAI levels confer protective benefits against multiple health risks, particularly cardiovascular risks, for individuals with diabetes.

Aligning with our findings in individuals with diabetes, the study in the general population has observed an inverse relationship between CDAI and stroke[29]. In the general population, the highest tertile of CDAI was associated with a 23% reduction in stroke prevalence compared to the lowest tertile (OR = 0.77; 95%CI: 0.64-0.92). Remarkably, in our cohort of individuals with diabetes, those in the highest quintile of CDAI exhibited a 43% lower prevalence of stroke compared to those in the lowest quintile, even after adjusting for various confounders. However, it is noteworthy that our study population had a higher prevalence of hypertension, indicating a higher baseline risk for stroke.

However, there exists a degree of heterogeneity in the findings across various studies. While our research supports the notion that increased antioxidant intake is correlated with reduced stroke risk, some studies have reported no significant associations or conflicting results[39,49]. This discrepancy could stem from variations in study designs, population demographics, and the specific types of antioxidants analyzed. For instance, our study's focus on a composite index rather than individual antioxidants might explain some of these differences. Moreover, the relationship between dietary antioxidants and stroke risk is intricate, as demonstrated by studies showing varied impacts among different sex and age

Table 4 Stratified analyses of the associations between the composite dietary antioxidant index and stroke

Characteristics	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for interaction
Sex						0.135
Male	Ref.	0.88 (0.62-1.24)	0.58 (0.39-0.88)	0.63 (0.40-0.99)	0.58 (0.34-0.98)	
Female	Ref.	0.60 (0.40-0.88)	0.77 (0.52-1.14)	0.82 (0.53-1.26)	0.56 (0.33-0.95)	
Age in years						0.088
≤ 60	Ref.	0.44 (0.25-0.78)	0.46 (0.25-0.85)	0.74 (0.41-1.34)	0.41 (0.19-0.88)	
> 60	Ref.	0.88 (0.65-1.17)	0.80 (0.58-1.11)	0.74 (0.52-1.07)	0.68 (0.45-1.03)	
BMI						0.300
< 30	Ref.	0.65 (0.45-0.95)	0.48 (0.31-0.74)	0.57 (0.36-0.91)	0.45 (0.26-0.79)	
≥ 30	Ref.	0.81 (0.58-1.14)	0.85 (0.60-1.21)	0.86 (0.58-1.26)	0.67 (0.42-1.07)	
Race						0.424
White	Ref.	0.75 (0.50-1.13)	0.86 (0.55-1.33)	0.94 (0.58-1.51)	0.74 (0.42-1.31)	
Others	Ref.	0.73 (0.52-1.02)	0.56 (0.38-0.81)	0.59 (0.39-0.88)	0.48 (0.29-0.78)	
Smoker						0.611
Yes	Ref.	0.68 (0.49-0.95)	0.54 (0.37-0.79)	0.67 (0.45-1.00)	0.48 (0.29-0.78)	
No	Ref.	0.77 (0.51-1.16)	0.83 (0.54-1.27)	0.74 (0.46-1.19)	0.67 (0.39-1.17)	
Drinker						0.225
Yes	Ref.	0.61 (0.42-0.88)	0.44 (0.29-0.68)	0.52 (0.33-0.81)	0.40 (0.24-0.68)	
No	Ref.	0.94 (0.59-1.48)	0.89 (0.54-1.47)	1.04 (0.61-1.78)	0.84 (0.44-1.59)	
Hypertension						0.843
Yes	Ref.	0.75 (0.57-1.00)	0.66 (0.48-0.90)	0.71 (0.51-1.00)	0.55 (0.36-0.82)	
No	Ref.	0.69 (0.36-1.30)	0.87 (0.44-1.71)	0.84 (0.40-1.75)	0.79 (0.32-1.91)	
Hypercholesterolemia						0.375
Yes	Ref.	0.68 (0.49-0.95)	0.66 (0.46-0.95)	0.57 (0.38-0.85)	0.47 (0.29-0.76)	
No	Ref.	0.87 (0.56-1.34)	0.61 (0.37-1.00)	1.01 (0.61-1.70)	0.72 (0.38-1.34)	

The odds ratio and 95% confidence interval were obtained from multivariable logistic regression models after adjusting for age, sex, race, education, marital status, poverty income ratio, drinker, smoker, body mass index, energy intake, hypertension and hypercholesterolemia. BMI: Body mass index; Ref.: Reference.

groups. For example, a Swedish cohort study observed a link between dietary antioxidants and ischemic stroke risk in females but not in males. In light of these findings, our study underscored the potential benefit of a holistic dietary approach, emphasizing a high antioxidant intake in managing stroke risk among individuals with diabetes. Nevertheless, the specific mechanisms underlying the CDAI-stroke relationship warrant further investigation to elucidate the complex interplay between diet, oxidative stress, and vascular health in patients with diabetes.

Our study's strength lies in its use of a comprehensive, multicomponent index to assess dietary antioxidant intake and its large, representative sample. Despite the strengths of our study, it is important to acknowledge certain limitations. First, the cross-sectional study was unable to establish a causal relationship between the CDAI and stroke. Future research should aim to elucidate this relationship through long-term follow-up and cohort studies. Second, the assessment of dietary intake, which forms the basis for calculating CDAI, is not without potential errors and inaccuracies. Dietary data collected through self-report can be subject to recall bias and may not accurately capture day-to-day variations in individual dietary patterns. Third, stroke assessment relied on questionnaire responses, lacking a more detailed classification of stroke types. Fourth, although numerous relevant covariates were included in the analysis, it is possible that not all confounding factors were accounted for. Unidentified confounders might skew the results. Future studies should aim for more comprehensive control of these factors to validate and refine our findings. Moreover, our study's findings are most applicable to the American population, given that dietary habits can vary significantly across different racial and ethnic groups. This geographic and cultural specificity limits the generalizability of our conclusions. Therefore, further research in diverse populations is needed to broaden our understanding of the association between CDAI and stroke risk globally.

CONCLUSION

In summary, this cross-sectional study found that adults diagnosed with diabetes in the United States with high levels of the CDAI, which measures the overall antioxidant quality of the diet, tend to have a lower risk of stroke. Furthermore, our smooth curve fitting analysis revealed a negative relationship between CDAI and stroke. Based on these findings, it is recommended that patients with diabetes maintain an appropriate intake of dietary antioxidants to increase their CDAI, thereby reducing stroke-related risk factors. In the future, rigorous prospective studies are imperative to validate our findings and provide deeper insights.

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FOOTNOTES

Author contributions: Wang CF and Ding Y designed the research; Zhang HQ and Shi J performed data processing and statistical analysis, drafted the manuscript, and revised the manuscript; Zheng XY, Luo SH, and Yue T provided valuable insights and guidance throughout the research process; Weng JH, Wang XL, Wang H, and Su XY participated in the revision of the manuscript; Wang CF and Ding Y contributed to data interpretation and manuscript discussion; All authors contributed to the article and approved the final manuscript. Zhang HQ and Shi J contributed equally to this work as co-first authors. The reasons for designating Zhang HQ and Shi J as co-first authors are as follows. First, Zhang HQ and Shi J provided their respective specialized skills and knowledge, playing a critical role in data analysis and interpretation. Second, Zhang HQ and Shi J conducted extensive literature reviews together, providing a solid theoretical foundation for the study. Third, they played a significant role in writing and revising the manuscript, ensuring its high quality and rigor. In summary, Zhang HQ and Shi J were actively involved in every stage of the project, from initial design to data collection and final analysis, demonstrating their comprehensive involvement and substantial contributions. There are several reasons for designating Wang CF and Ding Y as co-corresponding authors. First, they provided important guidance and supervision throughout the study, ensuring scientific rigor and accuracy. Second, they combined expertise and skills from different fields, offering a comprehensive perspective that enriched the diversity of the research. Third, Wang CF and Ding Y played key roles in resource coordination and project management, and both provided funding support, facilitating the smooth conduct of the study. Additionally, they will continue to support and guide the research in the post-submission stages, ensuring the ongoing quality and impact of the research findings. This dual leadership also enhanced the credibility and reliability of the manuscript, reflecting the team's collaborative spirit and interdisciplinary approach. In conclusion, designating Zhang HQ and Shi J as co-first authors and Wang CF and Ding Y as co-corresponding authors not only reflects the highly collaborative spirit of our team during the research process but also highlights the equal contributions and diverse expertise of each member in their respective fields. This team collaboration model ensures the comprehensiveness, rigor, and innovation of the research, making our findings more reliable and impactful.

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ORIGINAL ARTICLE

Basic Study

Functional analysis of the novel mitochondrial tRNA^{Trp} and tRNA$^{Ser(AGY)}$ variants associated with type 2 diabetes mellitus

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Abstract

BACKGROUND

Mutations in mitochondrial tRNA (*mt-tRNA*) genes that result in mitochondrial dysfunction play important roles in type 2 diabetes mellitus (T2DM). We previously reported a large Chinese pedigree with maternally inherited T2DM that harbors novel *mt-tRNA*^{Trp} *A5514G* and *tRNA*$^{Ser(AGY)}$ *C12237T* variants, however, the effects of these *mt-tRNA* variants on T2DM progression are largely unknown.

AIM

To assess the potential pathogenicity of T2DM-associated *m.A5514G* and *m.C12237T* variants at genetic, molecular, and biochemical levels.

METHODS

Cytoplasmic hybrid (cybrid) cells carrying both *m.A5514G* and *m.C12237T* variants, and healthy control cells without these mitochondrial DNA (mtDNA) variants were generated using trans-mitochondrial technology. Mitochondrial features, including *mt-tRNA* steady-state level, levels of adenosine triphosphate (ATP), mitochondrial membrane potential (MMP), reactive oxygen species (ROS), mtDNA copy number, nicotinamide adenine dinucleotide (NAD^+)/NADH ratio, enzymatic activities of respiratory chain complexes (RCCs), 8-hydroxy-deoxyguanine (8-OhdG), malondialdehyde (MDA), and superoxide dismutase (SOD) were examined in cell lines with and without these *mt-tRNA* variants.

RESULTS

Compared with control cells, the *m.A5514G* variant caused an approximately 35% reduction in the steady-state level of *mt-tRNA*^{Trp} ($P < 0.0001$); however, the

m.C12237T variant did not affect the *mt-tRNA*$^{Ser(AGY)}$ steady-state level (P = 0.5849). Biochemical analysis revealed that cells with both *m.A5514G* and *m.C12237T* variants exhibited more severe mitochondrial dysfunctions and elevated oxidative stress than control cells: ATP, MMP, NAD^+/NADH ratio, enzyme activities of RCCs and SOD levels were markedly decreased in mutant cells (P < 0.05 for all measures). By contrast, the levels of ROS, 8-OhdG and MDA were significantly increased (P < 0.05 for all measures), but mtDNA copy number was not affected by *m.A5514G* and *m.C12237T* variants (P = 0.5942).

CONCLUSION

The *m.A5514G* variant impaired *mt-tRNA*^{Trp} metabolism, which subsequently caused mitochondrial dysfunction. The *m.C12237T* variant did not alter the steady-state level of *mt-tRNA*$^{Ser(AGY)}$, indicating that it may be a modifier of the *m.A5514G* variant. The *m.A5514G* variant may exacerbate the pathogenesis and progression of T2DM in this Chinese pedigree.

Key Words: Type 2 diabetes mellitus; Mitochondrial tRNA genes; Novel variants; Oxidative stress; Mitochondrial dysfunctions

Core Tip: We established cytoplasmic hybrid (cybrid) cells with *m.A5514G* and *m.C12237T* variants, and control cells without these variants. The *m.A5514G* variant decreased *mt-tRNA*^{Trp} stability, whereas the *m.C12237T* variant did not alter the stability of *mt-tRNA*$^{Ser(AGY)}$. More severe mitochondrial dysfunction was observed in mutant cybrids than in control cells, indicating that the *m.A5514G* variant impaired *mt-tRNA*^{Trp} metabolism and mitochondrial functions and increased cellular oxidative stress, which play central roles in type 2 diabetes mellitus (T2DM) progression. By contrast, the *m.C12237T* variant acted as a modifier of the *m.A5514G* variant. Our study provides novel insight into the pathophysiology of maternally transmitted T2DM caused by novel *mt-tRNA* variants.

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INTRODUCTION

Diabetes is a common endocrine disease caused by the presence of chronic hyperglycemia. This complex disorder can be divided into type 1 and type 2. Type 2 diabetes mellitus (T2DM) is a major public health problem that affects approximately 10% of the Chinese adult population[1]. T2DM is characterized by high blood glucose in the context of insulin resistance (IR) and relative insulin deficiency. Furthermore, T2DM-associated complications, such as cardiovascular and peripheral vascular diseases, diabetic retinopathy, foot problems and nephropathy, remain very big challenge for clinicians[2]. Tremendous progression has been made in understanding the pathophysiology of T2DM over the past decades[3-5], with both genetic variations and environmental factors being involved in T2DM pathogenesis[6,7]. Nevertheless, the early prevention, diagnosis, and treatment of T2DM remain far from satisfactory.

Deregulation of mitochondrial oxidative phosphorylation (OXPHOS) is widely accepted as a major cause of T2DM and IR[8], diminished OXPHOS contributes to IR through elevated reactive oxygen species (ROS) production, and disrupted insulin receptor signaling[9]. Mitochondria are present in most eukaryotic cells and their primary role is to provide energy in the form of adenosine triphosphate (ATP) *via* OXPHOS[10]. They have their own DNA, mitochondrial DNA (mtDNA), which is 16569-bp in length and encodes 13 polypeptides, two rRNAs and 22 tRNAs[11]. Although *mt-tRNA* genes account for only approximately 10% mitochondrial genome, more than two third of mitochondrial disease-related variations are localized in this region[12]. An early landmark discovery in T2DM research was the identification of a 10.4-kb large deletion in mtDNA[13] and the m.A3243G mutation in *mt-tRNA*$^{Leu(UUR)}$[14]. A growing number of mtDNA variants has since been reported, for example, *mt-tRNA*^{Glu} A14692G[15], *mt-tRNA*^{Thr} G15897A[16] and *mt-tRNA*^{Gly} T10003C[17] are potential pathogenic variants affecting T2DM predisposition. Exactly how these *mt-tRNA* variants contribute to disease onset, however, remains poorly understood, and, there is an urgent need for additional studies to determine how mitochondrial dysfunction mediates the onset or progression of T2DM.

To understand the molecular basis of mitochondrial diabetes and to provide valuable information for its diagnosis, prevention, and treatment, we previously performed a systematic and extended screen for *mt-tRNA* gene variants in a cohort of 370 Chinese patients with T2DM and 631 healthy controls who were recruited from Hangzhou First People's Hospital and Quzhou People's Hospital in Zhejiang Province of China. We showed that *mt-tRNA*$^{Leu(UUR)}$ A3243G, *ND6* T14502C[18], *ND4* G11696A[19], *ND5* T12338C and *mt-tRNA*^{Ala} T5587C variants[20] are involved in the pathogenesis of maternally inherited T2DM. More recently, we reported a large Chinese pedigree with maternally transmitted T2DM, intriguingly, among 18 matrilineal relatives of this pedigree, six individuals suffered from diabetes. The age at T2DM onset ranged from 40 to 70 years, with an average of 52 years. Mutational analysis of nuclear genes (*GJB2*, *GJB3*, *GJB6*, and

TRMU) indicated no functional variants. Sequence analysis of entire mitochondrial genomes in matrilineal relatives revealed the presence of novel *mt-tRNA*^{Trp} *A5514G* and *mt-tRNA*$^{Ser(AGY)}$ *C12237T* variants[21]. The *m.A5514G* variant disrupted a highly conserved base-pairing (3A-70T) in the Acceptor arm of *mt-tRNA*^{Trp}, whereas the *m.C12237T* variant created a novel Watson-Crick base-pairing (11A-31T) in the Variable region of *mt-tRNA*$^{Ser(AGY)}$ (Figure 1). We therefore hypothesized that change in the *mt-tRNA*^{Trp} and *mt-tRNA*$^{Ser(AGY)}$ secondary structures caused by the *m.A5514G* and *m.C12237T* variants caused aberrant *mt-tRNA* metabolism which led to mitochondrial dysfunction and T2DM progression [22]. However, the pathophysiology of T2DM-related *m.A5514G* and *m.C12237T* variants remains largely undetermined.

To elucidate the pathogenic mechanism of the novel *mt-tRNA*^{Trp} and *mt-tRNA*$^{Ser(AGY)}$ variants, we generated cybrid cell lines derived from four T2DM patients carrying both *m.A5514G* and *m.C12237T* variants, together with four cybrids derived from control subjects without these mtDNA variants but belonging to the same mtDNA haplogroup. We examined whether the *m.A5514G* and *m.C12237T* variants affected *mt-tRNA* steady-state levels by northern blotting, and evaluated mitochondrial characteristics including levels of mtDNA copy number, malondialdehyde (MDA), superoxide dismutase (SOD), 8-hydroxy-deoxyguanine (8-OhdG), ATP, mitochondrial membrane potential (MMP), ROS, nicotinamide adenine dinucleotide (NAD^+)/NADH ratio and respiratory chain complexes (RCCs) activities in the mutant and control cybrids.

MATERIALS AND METHODS

Subjects

Members of a Han Chinese family with T2DM were enrolled *via* Hangzhou First People's Hospital as a part of genetic screening program for T2DM-associated mtDNA novel variants, as described previously[21]. The protocol used in this investigation was in accordance with the principles expressed in the 1975 Declaration of Helsinki, revised in 2008. The Ethics Committee of Hangzhou First People's Hospital approved this study (No. KY-20240327-0100-01). All participants, including four affected matrilineal relatives with T2DM (III-5, III-10, III-18, and III-22) bearing the *m.A5514G* and *m.C12237T* variants, belonged to mtDNA haplogroup G2a according to East Asian phylogeny[23]. Four genetically unrelated healthy subjects (C1, C2, C3, and C4) lacking these *mt-tRNA* variants and also belonging to human mtDNA haplogroup G2a were selected as controls. Written informed consent for participating in this study and for publication of case details was obtained from all subjects enrolled in the study.

Generation of cybrid cell lines

Cybrid cells can incorporate human mitochondria and perpetuate the mtDNA-encoded components of the incorporated mitochondria, which keeping the nuclear background constant[24]. To generate cybrids, the platelets of four patients with both *m.A5514G* and *m.C12237T* variants (III-5, III-10, III-18, and III-22), together with four controls (C1, C2, C3, and C4) were fused with 143B-ρ^0 206 cells, as described previously[25]. The 143B-ρ0 206 cells were grown in DMEM (Thermo Fisher Scientific, Waltham, MA, United States) supplemented with 10% FBS (Sigma, Aldrich, St. Louis, MO, United States) and 50 μg/mL uridine. The cybrid transformants were cultured in high-glucose DMEM containing 10% FBS at 37 °C in a humidified CO2 incubator.

To determine successful construction of cybrids, PCR-Sanger sequencing was performed to examine the presence of the *m.A5514G* and *m.C12237T* variants. The primer for amplification of the *mt-tRNA*^{Trp} gene were: Forward, 5′-CTA ACC GGC TTT TTG CCC-3′; reverse: 5′-ACC TAG AAG GTT GCC TGG CT-3′. The primers for amplification of the *mt-tRNA*$^{Ser(AGY)}$ gene were: forward, 5′-TAT CAC TCT CCT ACT TAC AG-3′; reverse: 5′-AGA AGG TTA TAA TTC CTA CG-3′. The PCR products were purified, sequenced and compared with an updated version of the human mitochondrial genome sequence to detect the variants (GenBank Accessible No: NC_012920.1)[26].

Northern blot analysis

Total mitochondrial RNA was obtained from mitochondria isolated from mutant and control cybrid cell lines (approximately 2 × 10^8 cells) using the TOTALLY RNATM kit (Ambion, Thermo Fisher Scientific), as described previously[27]. For northern blotting analysis of *mt-tRNA*, 2 μg of total mitochondrial RNA was electrophoresed through a 10% polyacrylamide/8M urea gel in Tris borate-EDTA buffer. The sequences for digoxigenin (DIG)-labeled probes specific to *mt-tRNA*^{Trp}; *mt-tRNA*$^{Ser(AGY)}$ and 5S rRNA were: 5′-AGA AAT TAA GTA TTG CAA CTT ACT GAG GGC-3′; 5′-GAG AAA GCC ATG TTG TTA GAC ATG GGG GCA-3′ and 5′-GGG TGG TAT GGC GGT AGA C-3′, respectively. Hybridization and quantification of band density were performed as previously described[28].

Analysis of ATP levels

The CellTiter-Glo® luminescent cell viability assay (Promega, Madison, WI, United States) was used to measure ATP levels in mutant and control cybrids according to the manufacturer's instructions[29]. Cells were seeded into white 96-well plates at 1 × 10^5 cells per well and cultured for 20 hours to reach approximately 70% confluence. Cells were then lysed by the addition of 100 μL CellTiter-Glo® working solution to each well and incubation for 2 minutes in the dark. Cell lysate solution (200 μL) was then transferred to an opaque 96-well plate. A 200 μL solution of culture medium and working solution at a ratio of 1:1 was used as a control. ATP concentration was determined using a 96-well fluorescence detector.

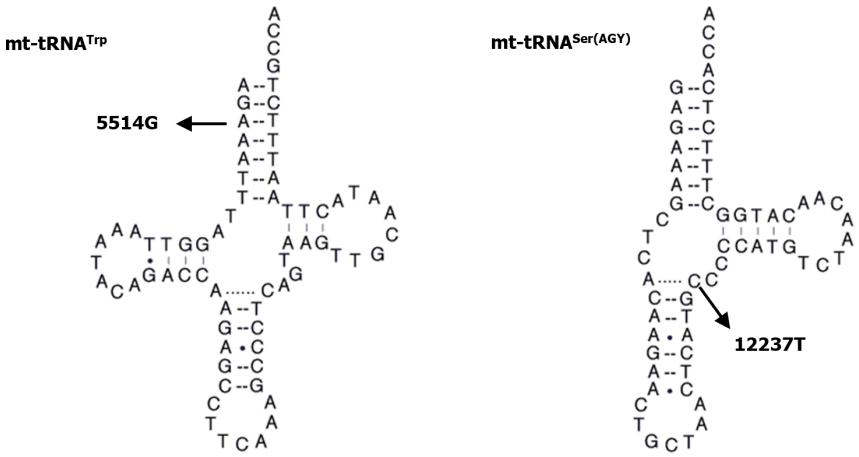

Figure 1 The secondary structures of *mitochondrial tRNA*^{Trp} and *mitochondrial tRNA*$^{Ser(AGY)}$, arrows indicated the positions of the *m.A5514G* and *m.C12237T* variants. mt-tRNA: Mitochondrial tRNA.

MMP analysis

The JC-1 MMP Assay Kit (Abcam, United States) was used to detect MMP in mutant and wild type cell lines. The JC-1 dye fluoresces in healthy cells with high levels of MMP, and green in cells with low levels of MMP[30]. JC-1 probe (1 μg/mL) was added to approximately 1 × 10^5 cells and incubated for 30 minutes in the dark. Cells were then washed twice with PBS and immediately analyzed by FACSCanto II flow cytometry (BD Biosciences, United States).

Analysis of mitochondrial ROS production

Mutant and control cybrid cells were seeded in 6-well plates (1 × 10^5 cells/well) and loaded with 10 μmol/L 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Beyotime, China) at 37 °C for 20 minutes. Cells were then washed with PBS three times and subsequently evaluated by FACSCanto II flow cytometry (BD Biosciences, United States).

Determination of NAD$^+$/NADH ratio

The NAD$^+$/NADH ratio is a measure of global energy capacity because the activities of rate-limiting enzymes involved in the tricarboxylic acid cycle (TCA), ketone production and glycolysis are regulated by this ratio[31,32]. The NAD$^+$/NADH ratio was determined for 1 × 10^5 cells using the NAD$^+$/NADH Assay Kit (Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions. Standard curves were generated for quantification.

Determination of mtDNA copy number

mtDNA copy number was determined using a quantitative real-time PCR (qRT-PCR) method, as described elsewhere [33]. Genomic DNA was extracted from 3 mL of blood from each participant using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For qRT-PCR analysis, 100 ng genomic DNA was used as template. The primers specific for the mtDNA-ND1 gene were: Forward, 5′-CCTAGC CGT TTA CTC AAT CCT-3′; reverse: 5′-TGA TGG CTA GGG TGA CTTCAT-3′; the primer sequences for nuclear (hemoglobin) gene were: forward, 5′-GCT TCT GAC ACA ACT GTG TTC ACT AGC-3′; reverse: 5′-CAC CAA CTT CAT CCA CGT TCA CC-3′. The qRT-PCR reaction was performed on an ABI 7900 instrument (Thermo Fisher Scientific) using SYBR Green Realtime PCR Master Mix (Bioteke, China). Relative expression levels were normalized by the $2^{-\Delta\Delta CT}$ method[34]. Samples were assayed in triplicate.

Analysis of oxidative stress-related biomarkers

To determine the effects of the *m.A5514G* and *m.C12237T* variants on oxidative stress[35], the concentrations of MDA and SOD in four cybrids with the *m.A5514G* and *m.C12237T* variants (III-5, III-10, III-18, and III-22), and in four control cell lines (C1, C2, C3, and C4) were analyzed using colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The serum levels of 8-OhdG were quantified by a competitive enzyme linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Nikken Foods, St. Louis, MO, United States). Samples were assayed in triplicate.

Analysis of enzymatic activities of mitochondrial RCCs

RCCs comprise four enzyme complexes (I-IV), which are embedded in the inner mitochondrial membrane and catalyze the transfer of reducing equivalents from high energy compounds. To analysis the activities of respiratory chain enzymes, an enriched mitochondrial fraction was isolated from mutant and control cybrid cells by centrifugation, as described previously[36]. The protein concentration was determined using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, United States). The enzymatic activities of RCCs were assayed spectrophotometrically, and the results were normalized to the activity of citrate synthase, a mitochondrial matrix enzyme[37].

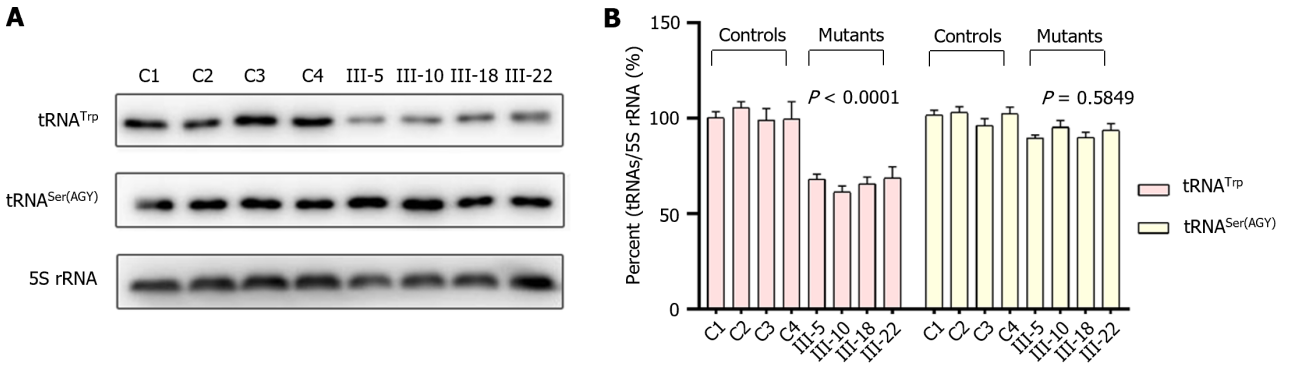

Figure 2 Analysis of steady-state levels of mitochondrial tRNA^{Trp} and mitochondrial tRNA$^{Ser(AGY)}$ by Northern blotting. A: 2 μg mitochondrial tRNA (mt-RNA) were electrophoresed through a denaturing polyacrylamide gel and hybridized with DIG-labeled oligonucleotide probes for *mt-tRNA*^{Trp}, *mt-tRNA*$^{Ser(AGY)}$, and 5S rRNA; B: Qualification of *mt-tRNA* levels.

Redefining the pathogenic roles of the m.A5514G and m.C12237T variants

We used the revised pathogenicity scoring system for *mt-tRNA* variants proposed by Yarham *et al*[38]. This scoring system gives special weight to functional data, which are considered the gold standard methods for assigning pathogenicity. The scoring system was as follows: ≤ 6 points, neutral polymorphism; 7-10 points, possibly pathogenic; 11-13 points (not including evidence from single-fiber, steady-state level, or trans-mitochondrial cybrid studies), probably pathogenic; ≥ 11 points (including evidence from single fiber, steady-state level or trans-mitochondrial cybrid studies), definitely pathogenic.

Statistical analysis

Data are presented as the mean ± SD. Statistical analysis of results was performed using GraphPad Prism 9.0 software (GraphPad. Software Inc., La Jolla, CA, United States). Student's *t*-test or the Mann-Whitney test were used to assess the difference between two groups. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Pedigree information and establishment of cybrid cell lines

We previously described a large Han Chinese pedigree with maternally transmitted T2DM that harbors the *mt-tRNA*^{Trp} A5514G and *mt-tRNA*$^{Ser(AGY)}$ C12237T variants[21]. Six individuals (one man and five women) of 18 matrilineal relatives suffered from T2DM. The age at onset varied among these six individuals from 40 to 70 years, with an average of 52 years.

To analyze mitochondrial functions, cybrid cell lines were derived from four subjects (III-5, III-10, III-18, and III-22) aged < 50 and from four genetically unrelated healthy subjects (C1, C2, C3, and C4) belonging to the same mtDNA haplogroup for use as controls. The platelets of these subjects were fused with the mtDNA-less human ρo206 cell line and cybrid clones were isolated by growing in selective DMEM, according to a previously described protocol[25]. The cybrid cell lines were analyzed by direct sequencing for the presence of the *m.A5514G* and *m.C12237T* variants and for homoplasmy.

mt-tRNA analysis

To investigate whether the *m.A5514G* and *m.C12237T* variants perturbed *mt-tRNA* metabolism, we subjected mt-RNAs from mutant and control cell lines to northern blotting and hybridized them with DIG-labeled oligodeoxynucleotide probes for *mt-tRNA*^{Trp} and *mt-tRNA*$^{Ser(AGY)}$. As shown in Figure 2A, the steady-state level of *mt-tRNA*^{Trp} in mutant cell lines was significantly decreased, whereas the steady-state level of *mt-tRNA*$^{Ser(AGY)}$ was not affected in either mutant or wild type cells. For comparison, the *m.A5514G* variant caused approximately 35% reduction in the level of *mt-tRNA*^{Trp} (P < 0.0001) after normalization of 5S rRNA, however, the *m.C12237T* variant had little impact on the *mt-tRNA*$^{Ser(AGY)}$ steady-state level (P = 0.5849) (Figure 2B).

ATP levels are decreased in m.A5514G and m.C12237T cybrid cells

We next assessed whether the variants influenced ATP generation by using a luciferin/Luciferase assay. As shown in Figure 3A, the average levels of ATP production in mutant cells decreased approximately 22.1% compared with control cells (P = 0.0016).

The m.A5514G and m.C12237T variants reduce the levels of MMP

The effects of *m.A5514G* and *m.C12237T* variants on MMP was measured using the fluorescence probe, JC-1, in control and mutant cells. The relative ratios of the FL_{590}/FL_{529} geometric means between control and mutant cells were assessed to

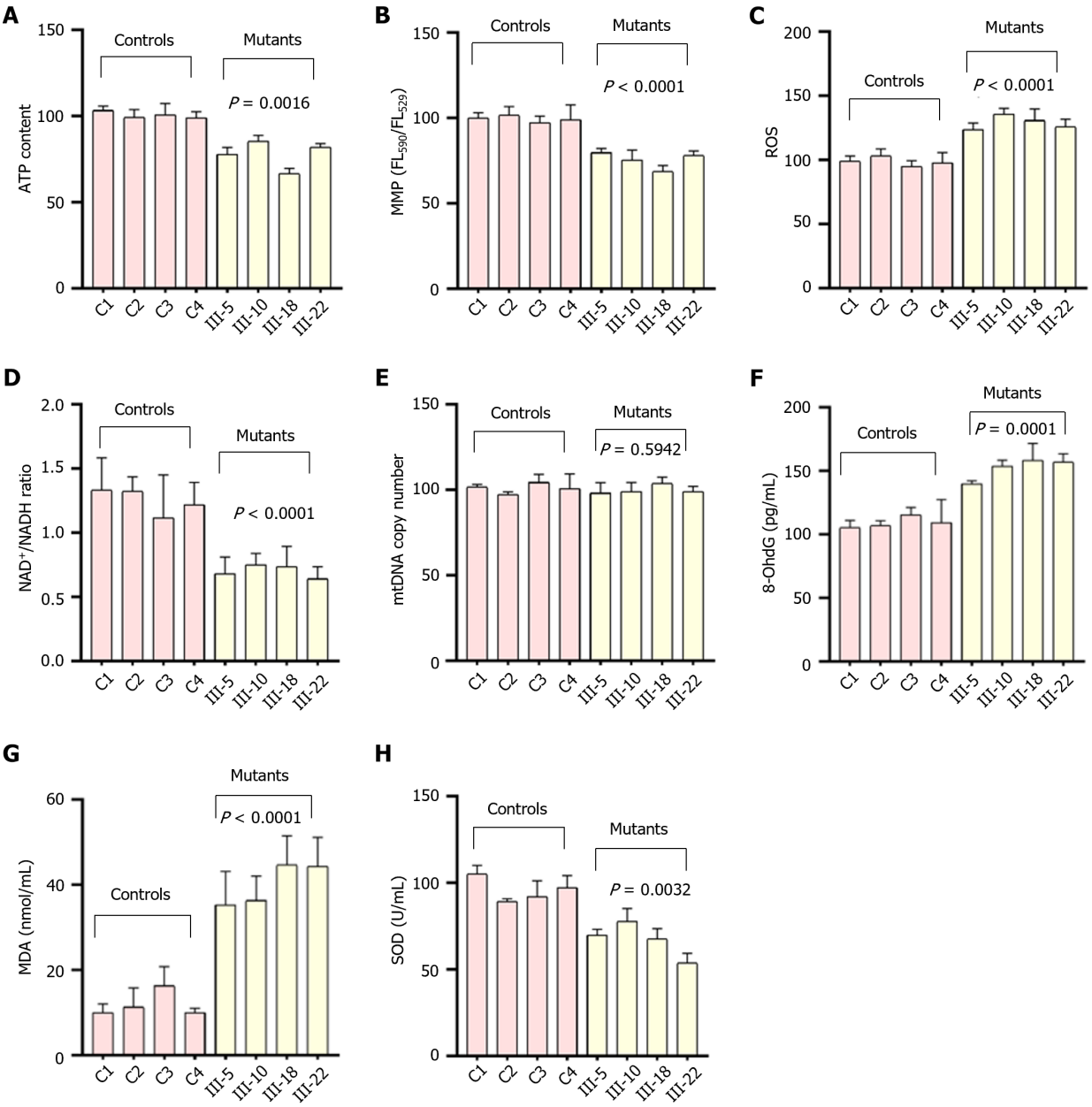

Figure 3 Analysis of mitochondrial functions in mutant and control cybrids. A: Adenosine triphosphate analysis; B: Mitochondrial membrane potential levels; C: Reactive oxygen species analysis; D: Nicotinamide adenine dinucleotide/NADH ratio; E: Mitochondrial DNA copy number; F: 8-hydroxy-deoxyguanine levels; G: Malondialdehyde levels; H: Superoxide dismutase levels. ATP: Adenosine triphosphate; MMP: Mitochondrial membrane potential; ROS: Reactive oxygen species; NAD: Nicotinamide adenine dinucleotide; mtDNA: Mitochondrial DNA; 8-OhdG: 8-hydroxy-deoxyguanine; MDA: Malondialdehyde; SOD: Superoxide dismutase.

depict the MMP level. As shown in Figure 3B, cells carrying *mt-tRNA* variants had 25.6% less MMP compared with control cells ($P < 0.0001$).

ROS production is enhanced in m.A5514G and m.C12237T cybrid cells

We used flow cytometry and DCFH-DA probe loading to assess ROS production in mutant and control cell lines. As shown in Figure 3C, mutant cells bearing the *m.A5514G* and *m.C12237T* variants exhibited increased ROS production, with an average increase of 127.5% relative to control cells ($P < 0.0001$).

The NAD⁺/NADH ratio is decreased in m.A5514G and m.C12237T cybrid cells

The NAD⁺/NADH ratio is involved in central carbon metabolism, nucleotide synthesis and lipid metabolism[39]. We measured the NAD⁺/NADH ratio in mutant and control cell lines and demonstrated that the *mt-tRNA* variants significantly reduced the NAD⁺/NADH ratio (Figure 3D).

mtDNA copy number is not affected by m.A5514G and m.C12237T variants

mtDNA copy number is a biomarker for both mitochondrial quality and function[40]. We therefore analyzed the mtDNA

Table 1 Redefining the pathogenic roles of the *m.A5514G* and *m.C12237T* variants

Scoring criteria	*m.A5514G* variant	Score/20	*m.C12237T* variant	Score/20	Classification
More than one independent report	Yes	2	Yes	2	≤ 6 points: Neutral polymorphisms; 7-10 points: Possibly pathogenic; 11-13 points (not including evidence from single fiber, steady-state level, or trans-mitochondrial cybrid studies): Probably pathogenic; ≥ 11 points (including evidence from single fiber, steady-state level or trans-mitochondrial cybrid studies): Definitely pathogenic
Evolutionary conservation of the base pair	No changes	2	No changes	2	
Variant heteroplasmy	No	0	No	0	
Segregation of the mutation with disease	Yes	2	Yes	2	
Biochemical defect in complex I, III, or IV	Yes	2	No	0	
Evidence of mutation segregation with biochemical defect from single fiber studies	No evidence	0	No evidence	0	
Mutant *mt-tRNA* steady-state level or evidence of pathogenicity in trans-mitochondrial cybrid studies	Strong evidence	5	No evidence	0	
Maximum score	Definitely pathogenic	13	Neutral polymorphism	6	

content in peripheral blood. As shown in Figure 3E, there were no differences in mtDNA copy number among patients with the *m.A5514G* and *m.C12237T* variants and controls ($P = 0.5942$).

Serum 8-OhdG levels are increased in T2DM individuals

8-OhdG, a critical biomarker of DNA damage, plays an important role in T2DM progression[41]. The concentrations of serum 8-OhdG in T2DM and control subjects were measured by ELISA. As shown in Figure 3F, patients with *mt-tRNA* variants had much higher concentrations of 8-OhdG compared with controls ($P < 0.0001$).

The m.A5514G and m.C12237T variants increase cellular oxidative stress

We next explored whether the *mt-tRNA* variants affected cellular oxidative stress. Individuals carrying the *mt-tRNA* variants showed much higher levels of MDA ($P < 0.0001$; Figure 3G) but significantly decreased levels of SOD ($P = 0.0032$) (Figure 3H) compared with controls.

The m.A5514G and m.C12237T variants inhibit the activities of Complex I and IV in cybrids

Mitochondrial Complex I is the major site for catalyzing oxidation of NADH. We therefore analyzed the enzyme activities of Complex I-IV in cybrids. As shown in Figure 4, the activities of Complex I and IV were dramatically inhibited ($P < 0.0001$, both), while Complex II and III enzyme activities remained unaffected ($P = 0.5019$ and 0.5523, respectively) in the *m.A5514G* and *m.C12237T* variants cybrids.

Pathogenicity of the m.A5514G and m.C12237T variants

Total scores of the *m.A5514G* and *m.C12237T* variants were 13 and 6 points, respectively, (Table 1), indicating that they were "definitely pathogenic" and "neutral polymorphism", respectively.

DISCUSSION

In the present study, we investigated the causal roles of T2DM-related *m.A5514G* and *m.C12237T* variants at genetic, molecular, and biochemical levels. The *m.A5514G* variant was initially described in a neonatal patient with multiple RCC defects[42], and the *m.C12237T* variant was first reported in patients with Leber's Hereditary Optic Neuropathy (LHON) [43]. Subsequently, we showed these variants to be associated with T2DM[21]. At the molecular level, the *m.A5514G* variant occurs at conventional position 3 in the Acceptor arm of *mt-tRNA*^{Trp}, which is highly conserved among species. Importantly, the *m.A5514G* variant abolishes the 3A-70T Watson-Crick base-pairing, which is important in recognizing its cognate tryptophanyl-tRNA synthetase during translation[44]. This causes misreading or misrecognition by RNase P[45]. Interestingly, the *m.T7512C* variant, which occurs at the same position as *mt-tRNA*$^{Ser(UCN)}$, was suggested to be associated with mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and with myoclonic epilepsy with ragged red fibers (MERRF) overlap syndrome[46]. We therefore speculate that the *m.A5514G* variant, which is similar to the *m.T7512C* variant, may alter the stability of mutant *mt-tRNA*^{Trp}, as indicated by our northern blotting

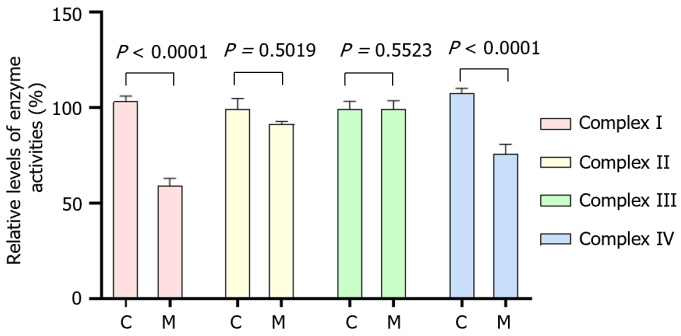

Figure 4 Analysis of enzymatic activities of mitochondrial respiratory chain complexes in four cybrids with mitochondrial tRNA variants and four control cell lines without these variants. C: Control group; M: Mutant group.

results. Indeed, the average steady-state level of *mt-tRNA*^{Trp} was significantly reduced to 35% of control levels, which is below the proposed threshold to produce a clinical phenotype associated with an *mt-tRNA* mutation[47]. These data lead us to believe that the *m.A5514G* variant impairs *mt-tRNA*^{Trp} metabolism.

Genetically, unlike other canonical *mt-tRNA*s, *mt-tRNA*$^{Ser(AGY)}$ is a highly unique tRNA in humans, lacking the entire D-loop structure[22]. In fact, the homoplasmic *m.C12237T* variant is located at position 31 in the Variable region of *mt-tRNA*$^{Ser(AGY)}$, which creates a new 11A-31T Watson-Crick base-pairing. Although RNA Fold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) predicted this variant to alter the secondary structure of *mt-tRNA*$^{Ser(AGY)}$[21], functional data indicated no obvious difference in *mt-tRNA*$^{Ser(AGY)}$ steady-state levels between mutant and control cell lines, strongly indicating that the *m.C12237T* variant did not affect *mt-tRNA*$^{Ser(AGY)}$ metabolism. Therefore, the *m.C12237T* variant most probably acts as a modifier of the *m.A5514G* variant in T2DM.

The perturbed tertiary structure caused by the *m.A5514G* variant made the mutant *mt-tRNA*^{Trp} more unstable and led to aberrant *mt-tRNA* metabolism, significantly reducing the activities of Complex I and IV in mutant cells, which was consistent with the clinical phenotypes of the affected patients. As a result, the RCC deficiency caused by the *m.A5514G* and *m.C12237T* variants may result in down-regulated mitochondrial biogenesis and uncoupling of the oxidative pathway for ATP synthesis. Pancreatic beta cells have a high OXPHOS demand and would, therefore, be primarily affected[48]. In the current study, ATP production was reduced by 22.1% in mutant cells as compared with control cells. In addition, defectives OXPHOS complexes can result in impaired MMP production. Importantly, MMP is generated by proton pumps (Complex I, III, and IV), and is an essential in energy storage during OXPHOS[49]. The average MMP level in patients' cells was only 74.4% of that in control cells. As a result, the defective OXPHOS in cells with *mt-tRNA* variants increased the production of ROS, 8-OhdG and MDA. Indeed, MDA is generated in the oxidative degradation of polyunsaturated lipids[50], whereas SOD is an antioxidant enzyme able to break down harmful oxygen molecules within cells and convert them into less toxic products[51]. Therefore, the increased oxidative damage and decreased antioxidant activity may be involved in the occurrence of oxidative stress. This would damage mitochondrial and cellular proteins, lipids, and nucleic acids *via* chemical modifications, including nitrosylation, peroxidation and carbonylation, and, in turn, promote pancreatic beta cell dysfunction and IR[52,53].

Reduced mtDNA copy number has been theoretically linked to increased oxidative stress *via* increased production of ROS[54]. Our data show no changes in mtDNA content in mutant and control groups. However, the precise mechanisms that led to variation in mtDNA copy number are uncertain and genetic and environmental factors have been hypothesized to interact to determine the number of mitochondria in a cell[55]. Therefore, although the *m.A5514G* and *m.C12237T* variants lead to mitochondrial dysfunctions, other factors such as nuclear background, restored the mtDNA content in the mutant cell lines.

Cellular NAD exists in two forms, oxidized (NAD+) and reduced (NADH). It plays essential roles in cellular redox reactions and is responsible for accepting high energy electrons and carrying them to the electron transport chain for the synthesis of ATP[56]. In diabetic patients, increased NADH levels cause reductive stress, which elevates ROS production and leads to IR and cell death[57]. In our study, mutant cell lines showed a marked decrease in the NAD^+/NADH ratio indicating that the *m.A5514G* and *m.C12237T* variants increases oxidative stress and mitochondrial dysfunction.

CONCLUSION

Our findings indicate that the *m.A5514G* variant reduced the steady-state level of *mt-tRNA*^{Trp}, which affected tRNA metabolism and led to mitochondrial dysfunctions involved in T2DM pathogenesis, whereas the *m.C12237T* variant had little impact on *mt-tRNA*$^{Ser(AGY)}$ stability and was probably a polymorphism with neutral effect on the for expressivity of the *m.A5514G* variant-induced T2DM phenotype. Furthermore, the incomplete penetrance of T2DM and variable clinical phenotypes indicate that *mt-tRNA* variants are not sufficient to produce clinical phenotypes. Therefore, other factors, such as environmental factors, nuclear genes or epigenetic modification, may contribute to T2DM progression. The main limitation of this study was the relatively small sample size, and further studies including more T2DM patients are needed to verify our conclusions.

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FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Intestinal glucagon-like peptide-1: A new player associated with impaired counterregulatory responses to hypoglycaemia in type 1 diabetic mice

Fang-Xin Jin, Yan Wang, Min-Ne Li, Ru-Jiang Li, Jun-Tang Guo

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Abstract

BACKGROUND

Impaired hypoglycaemic counterregulation has emerged as a critical concern for diabetic patients who may be hesitant to medically lower their blood glucose levels due to the fear of potential hypoglycaemic reactions. However, the pathogenesis of hypoglycaemic counterregulation is still unclear. Glucagon-like peptide-1 (GLP-1) and its analogues have been used as adjunctive therapies for type 1 diabetes mellitus (T1DM). The role of GLP-1 in counterregulatory dysfunction during hypoglycaemia in patients with T1DM has not been reported.

AIM

To explore the impact of intestinal GLP-1 on impaired hypoglycaemic counterregulation in type 1 diabetic mice.

METHODS

T1DM was induced in C57BL/6J mice using streptozotocin, followed by intraperitoneal insulin injections to create T1DM models with either a single episode of hypoglycaemia or recurrent episodes of hypoglycaemia (DH5). Immunofluorescence, Western blot, and enzyme-linked immunosorbent assay were employed to evaluate the influence of intestinal GLP-1 on the sympathetic-adrenal reflex and glucagon (GCG) secretion. The GLP-1 receptor agonist GLP-1(7-36) or the antagonist exendin (9-39) were infused into the terminal ileum or injected

intraperitoneally to further investigate the role of intestinal GLP-1 in hypoglycaemic counterregulation in the model mice.

RESULTS

The expression levels of intestinal GLP-1 and its receptor (GLP-1R) were significantly increased in DH5 mice. Consecutive instances of excess of intestinal GLP-1 weakens the sympathetic-adrenal reflex, leading to dysfunction of adrenal counterregulation during hypoglycaemia. DH5 mice showed increased pancreatic δ-cell mass, cAMP levels in δ cells, and plasma somatostatin concentrations, while cAMP levels in pancreatic α cells and plasma GCG levels decreased. Furthermore, GLP-1R expression in islet cells and plasma active GLP-1 levels were significantly increased in the DH5 group. Further experiments involving terminal ileal infusion and intraperitoneal injection in the model mice demonstrated that intestinal GLP-1 during recurrent hypoglycaemia hindered the secretion of the counterregulatory hormone GCG *via* the endocrine pathway.

CONCLUSION

Excessive intestinal GLP-1 is strongly associated with impaired counterregulatory responses to hypoglycaemia, leading to reduced appetite and compromised secretion of adrenaline, noradrenaline, and GCG during hypoglycaemia.

Key Words: Glucagon-like peptide-1; Impaired hypoglycaemic counterregulation; Type 1 diabetes; Intestine; Pancreas

Core Tip: Glucagon-like peptide-1 (GLP-1) is an incretin hormone primarily produced by specific enteroendocrine L-cells in the intestines. The role of GLP-1 in impaired hypoglycaemic counterregulation in type 1 diabetes mellitus (T1DM) has not been reported. In this study, a model of recurrent hypoglycaemia in T1DM mice was established, and it was found that excessive intestinal GLP-1 is strongly associated with impaired counterregulatory responses to hypoglycaemia, leading to reduced appetite and compromised secretion of adrenaline, noradrenaline, and glucagon during hypoglycaemia.

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INTRODUCTION

Patients with type 1 diabetes mellitus (T1DM) are prone to iatrogenic hypoglycaemia due to the administration of insulin or sulfonylureas, which occur an average of 1-2 times per week. Notably, approximately 40% of T1DM patients experience severe hypoglycaemia[1-3]. Severe hypoglycaemia events can manifest as symptoms such as confusion, coma, seizures, and, in extreme cases, death[4]. Studies indicate that hypoglycaemia contributes to up to 10% of fatalities in individuals living with diabetes[5].

When hypoglycaemia occurs in healthy individuals, the body perceives a decrease in blood glucose levels and initiates counterregulatory responses to restore glucose levels. Insulin secretion decreases when glucose levels decrease to 4.4-4.7 mmol/L, while glucagon (GCG) and adrenaline secretion begin to increase when glucose levels reach 3.6-3.9 mmol/L. At approximately 3.2 mmol/L, symptoms such as palpitations, tremors, anxiety, sweating, and hunger manifest due to autonomic nerve excitement, prompting the body to take corrective measures for hypoglycaemia. These symptoms of autonomic hypoglycaemia are commonly referred to as hypoglycaemia warnings. When glucose drops to 2.8 mmol/L, neuroglycopenic symptoms, such as cognitive impairment, epilepsy, or coma, appear[6].

Recurrent hypoglycaemia[7], defined as multiple episodes within a day or consecutive episodes across multiple days, especially if each episode lasts for more than 30 min, can lead to impaired hypoglycaemic counterregulation. This impairment includes decreased GCG secretion and compromised sympathetic nerve excitation, ultimately affecting the body's ability to regulate hypoglycaemia[6]. Impaired hypoglycaemic counterregulation has emerged as a critical concern for diabetic patients who may be hesitant to medically lower their blood glucose levels due to the fear of potential hypoglycaemic reactions. Clinical studies have demonstrated that hypoglycaemic warning symptoms can be reversed after 2 wk of hypoglycaemia prevention in T1DM patients. After preventing hypoglycaemia for 3 mo, neuroendocrine counterregulation, such as GCG and adrenaline secretion, normalizes[8]. However, the pathogenesis of impaired hypoglycaemic counterregulation is still unclear, making it challenging to develop effective prevention and treatment strategies for hypoglycaemia.

GCG-like peptide-1 (GLP-1) is an incretin hormone primarily produced by specific enteroendocrine L-cells in the terminal ileum and colon through the preproglucagon (*PPG*) gene[9,10]. When GLP-1 in the intestines binds to its receptor (GLP-1R), it can activate brain neurons related to autonomic nerves, stimulate adrenal medulla secretion, and

elevate blood catecholamine levels[11,12]. Additionally, GLP-1 can enhance insulin release and suppress GCG secretion by interacting with the GLP-1R on pancreatic islet cells[8,13]. Moreover, by influencing the GLP-1R on vagal afferent nerve fibers and afferent neurons in the gastrointestinal tract, GLP-1 can delay gastric emptying, reduce intestinal peristalsis and gastric acid secretion, and stimulate a satiation signal, thereby leading to weight loss and an improvement in body mass index[8,14,15]. Therefore, GLP-1 and its analogues are commonly used in the clinical treatment of type 2 diabetes mellitus and as adjuvant therapies for T1DM. Given that GLP-1 can induce hypoglycaemia and satiety, what role does GLP-1 play in counterregulation dysfunction and even loss of hunger awareness during hypoglycaemia in patients with T1DM? There are currently no reports available on this topic. Therefore, this study aimed to establish a recurrent hypoglycaemia model in T1DM mice to investigate changes in GLP-1 expression in the intestinal tract, explore the mechanisms underlying hypoglycaemic counterregulatory dysfunction and awareness impairment, and further provide experimental evidence for the prevention and treatment of hypoglycaemia in T1DM patients.

MATERIALS AND METHODS

Establishment of a recurrent hypoglycaemia model in T1DM mice

Male C57BL/6J mice aged 10 wk and weighing 22.5 ± 0.5 g were obtained from the Experimental Animal Center at Shandong Second Medical University (No. 2019SDL029; Shandong Province, China). The mice were allowed to acclimate and fed at room temperature (approximately 25 °C) for 1 wk prior to the study. To induce T1DM, all mice were fasted for 6 h, followed by intraperitoneal administration of 220 mg/kg streptozotocin (STZ) dissolved in 0.01 mol/L citrate buffer (pH 4.2). The successful establishment of the T1DM model was confirmed on the third day post-STZ injection when postprandial blood glucose levels in the tail vein blood of mice reached ≥ 16.7 mmol/L, indicating the onset of diabetes. A model of recurrent hypoglycaemia in T1DM mice was created using a standardized protocol with modifications[13-15]. T1DM mice were injected with short-acting insulin after a 6-h fast, resulting in blood glucose levels decreasing to less than 3.9 mmol/L (approximately 3.3 ± 0.5 mmol/L) for more than 60 min, representing a single episode of hypoglycaemia. Beginning on day 15 of diabetes, hypoglycaemia was induced once every 3 d for a total of 5 consecutive episodes to establish a recurrent hypoglycaemic model in T1DM mice (DH5 group). On the 27th day of diabetes, coinciding with the final hypoglycaemia episode in the DH5 group, a second group of T1DM mice underwent the same procedure to induce hypoglycaemia once, creating the T1DM one hypoglycaemia model (DH1 group).

Throughout the modeling process, the activity status of the model mice was closely monitored, and the plasma adrenaline and pancreatic noradrenaline levels were measured using enzyme-linked immunosorbent assay (ELISA) to evaluate the hypoglycaemic counterregulatory response. Pentobarbital sodium was administered intraperitoneally, followed by the collection of retroocular plasma, ileum, colon, and pancreas samples from the mice. Some tissue samples were fixed in 4% paraformaldehyde and processed into paraffin sections, while others were stored in a freezer at -80 °C.

Intraterminal ileum catheterization and infusions

On the 25th day after diabetes induction, following the 4th hypoglycaemic episode, the mice in the DH1 and DH5 groups were subcutaneously administered 0.05 mg/kg buprenorphine for preoperative analgesia. Anaesthesia was induced with 3% isoflurane and maintained at 2%. The abdomen and neck were prepared by shaving and disinfecting with iodine. A midline incision was made in the abdomen to remove the ileum. A 25 G needle was used to puncture the end of the ileum 5 cm from the ileocecal valve, and Micro-Renathane Tubing (Braintree Scientific, United States) was inserted into the puncture hole. Purse-string sutures were placed around the catheter. A small incision was made on the back of the neck, and the catheter was subcutaneously introduced, fixed in the interscapular area, and sutured. Subcutaneous administration of 0.05 mg/kg/d of buprenorphine was provided for analgesia on the day of surgery and for 2 d postoperatively. Exendin (9-39) amide (Abcam, United Kingdom) or GLP-1(7–36) amide (AnaSpec, United States) was freshly prepared in sterile saline. They were infused at a dose of 100 pmol/kg in 1 mL at a rate of 0.5 mL/min through an external catheter and administered 10 min before the conclusion of the final hypoglycaemic session.

Intraperitoneal injections

Mice in the DH5 group were injected intraperitoneally with either 5 μg of exendin (9-39) or 5 μg of GLP-1(7-36) 20 min before the conclusion of the final hypoglycaemic session. Plasma samples were then collected and prepared for subsequent experiments.

ELISA analysis

Adrenaline ELISA kit (Sin-troch, China), GLP-1-Active Form Assay Kit (Immuno-Biological Laboratories, Japan), GCG-ELISA Kit (Cloud-Clone, China), and SST-ELISA Kit (Cloud-Clone, China) were used to analyze the levels of adrenaline, active GLP-1, GCG, and somatostatin (SST) in the plasma of the mice in each group, respectively. A noradrenaline ELISA kit (Sin-troch, China) was used to assess the level of noradrenaline in pancreatic tissue homogenates of the model mice. ELISA was conducted following the instructions of the respective kits.

Immunofluorescence staining

Intestinal paraffin sections (5 μm thick) were deparaffinized in water, microwaved for repair, and blocked with 10% goat serum (Solarbio, China) at 37 °C for 30 min, after which mouse anti-GLP-1 antibody (Abcam, United Kingdom, 1:200) was added at 4 °C overnight. Subsequently, the sections were incubated in the dark with a fluorescently labeled secondary

antibody at 37 °C for 50 min and mounted with DAPI (Solarbio, China) sealing reagent.

Five fluorescently stained sections were extracted from mouse tissue (0.2 mm between sections), and ten randomly selected fields of view from each section were observed under a 200 × fluorescence microscope. The integrated optical density (IOD) values in each image were subsequently calculated using Image-Pro Plus 6.0.

Multilabel immunofluorescence staining with tyramide signal amplification

Pancreatic paraffin sections (5 μm thick) were deparaffinized in water, microwaved for repair, and blocked with 3% hydrogen peroxide and 10% goat serum at 37 °C for 30 min. Subsequently, rabbit anti-cAMP antibody (Abcam, United Kingdom, 1:100) was added, and the sections were incubated overnight at 4 °C. The sections were incubated with a biotin-labeled goat anti-rabbit secondary antibody (ZSGB-BIO, China) at 37 °C for 50 min, followed by incubation with horseradish peroxidase-labeled streptavidin for 30 min. Tyramide working solution (APExBIO, United States, 1:800 dilution, containing 0.3% hydrogen peroxide) was then added, and the sections were incubated at room temperature for 10 min. After washing with PBS, the sections were blocked with serum for 50 min and incubated with mouse anti-GCG (BOSTER, United States, 1:200) or mouse anti-SST (Santa Cruz Biotechnology, United States, 1:200) antibody at 37 °C for 1.5 h, followed by incubation with a fluorescently labeled secondary antibody at 37 °C for 50 min. Finally, the sections were mounted with DAPI sealing reagent.

The statistical analysis was in line with the immunofluorescence staining process.

Western blot analysis

Frozen intestinal tissues were homogenized and ultrasonically lysed to extract proteins, which were quantified using the bicinchoninic acid method. Each sample contained 30 μg of protein, which was transferred to a PVDF membrane using a wet method and then blocked with 5% skim milk for 2 h at room temperature on a shaker. Mouse anti-GLP-1 (1:2000), mouse anti-GLP-1R (DSHB, United States, 1:1000), or mouse anti-β-actin (Proteintech, United States, 1:10000) antibody was individually added and incubated overnight at 4 °C with shaking. The next day, the membranes were incubated with a secondary antibody (Proteintech, United States, 1:5000) for 2 h at room temperature and then visualized using enhanced chemiluminescence reagent. Data analysis was conducted using Image-Pro Plus software, and the relative expression of the target protein was calculated as the ratio of the optical density of the target protein to that of β-actin.

Statistical analysis

Statistical analyses were conducted using SPSS 25.0 software, while GraphPad Prism 9.0 software was utilized for creating visual representations. Normally distributed measurement data are expressed as the mean ± SE. Comparisons between two groups were assessed using the *t* test, while comparisons among multiple groups were analyzed using one-way ANOVA. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

A recurrent hypoglycaemia model is successfully established in T1DM mice

The blood glucose and body weight of model mice meet the modeling criteria: The flow diagram of the modeling process is presented in Figure 1A. Prior to STZ injection, there were no significant differences in blood glucose levels among the groups ($P > 0.05$). On the first day of diabetes, blood glucose levels significantly increased and exceeded 16.7 mmol/L ($P < 0.01$). By the seventh day of diabetes, a significant decrease in body weight was observed in each group ($P < 0.01$). These elevated blood glucose levels and decreased body weight align with the criteria for diabetes modeling, as shown in Table 1. On the 15th day of diabetes, fasted diabetic mice experienced hypoglycaemia, with 1 episode every 3 d, for a total of 5 episodes. The fluctuations in blood sugar levels are illustrated in Figure 1B. Following insulin injection during these episodes, blood glucose levels in the DH5 and DH1 groups decreased to 3.3 ± 0.5 mmol/L within 60 min. Since fasting blood glucose levels < 3.9 mmol/L can induce hypoglycaemia syndrome in diabetic patients, the blood glucose levels during repeated hypoglycaemic episodes in this study met the criteria for postdiabetes hypoglycaemia modeling.

Activity state, plasma adrenaline, and pancreatic noradrenaline levels in DH5 model mice reflect the development of impaired hypoglycaemic counterregulation: This study revealed activity changes in DH5 model mice during episodes of hypoglycaemia, as indicated by increased activity and foraging behaviors during the first two episodes, which gradually decreased during subsequent episodes. This decrease in activity is consistent with the decrease in hypoglycaemic warning symptoms resulting from impaired counterregulation.

To further clarify whether activity changes in DH5 mice are linked to impaired hypoglycaemic counterregulation, sympathetic response indices such as plasma adrenaline and pancreatic noradrenaline were measured using ELISA. The results indicated that plasma adrenaline and pancreatic noradrenaline levels were significantly lower in the DH5 group than in the diabetic mice (DM) and DH1 groups ($P < 0.01$; Figure 1C and D), suggesting that DH5 group mice exhibit impaired hypoglycaemic counterregulation.

Recurrent hypoglycaemia leads to an increase in expression of intestinal GLP-1 and its receptors

GLP-1 is predominantly present in L cells in the distal small intestine and colon. This study aimed to assess changes in GLP-1 expression in the distal ileum and colon using immunofluorescence staining and Western blot analysis. Active GLP-1-positive cells were primarily located in the glandular epithelium, with occasional presence in the covering

Table 1 Blood glucose levels on the first day of diabetes and body weight on the seventh day of diabetes (mean ± SE)

Group	Blood glucose (mmol/L)		Body weight (g)	
	Before diabetes	On the 1st day of diabetes	Before diabetes	On the 7th day of diabetes
DM	7.2 ± 0.4	22.4 ± 0.8[a]	23.3 ± 0.2	20.5 ± 0.6[b]
DH1	7.3 ± 0.3	25.3 ± 0.7[a]	23.4 ± 0.2	19.6 ± 0.8[b]
DH5	7.5 ± 0.4	23.4 ± 1.8[a]	22.9 ± 0.3	19.9 ± 0.3[b]

[a]$P < 0.01$ *vs* blood glucose before diabetes.
[b]$P < 0.01$ *vs* body weight before diabetes.
DM: Diabetic mice; DH1: Diabetic mice with a single hypoglycemic episode; DH5: Diabetic mice with five hypoglycaemic episodes.

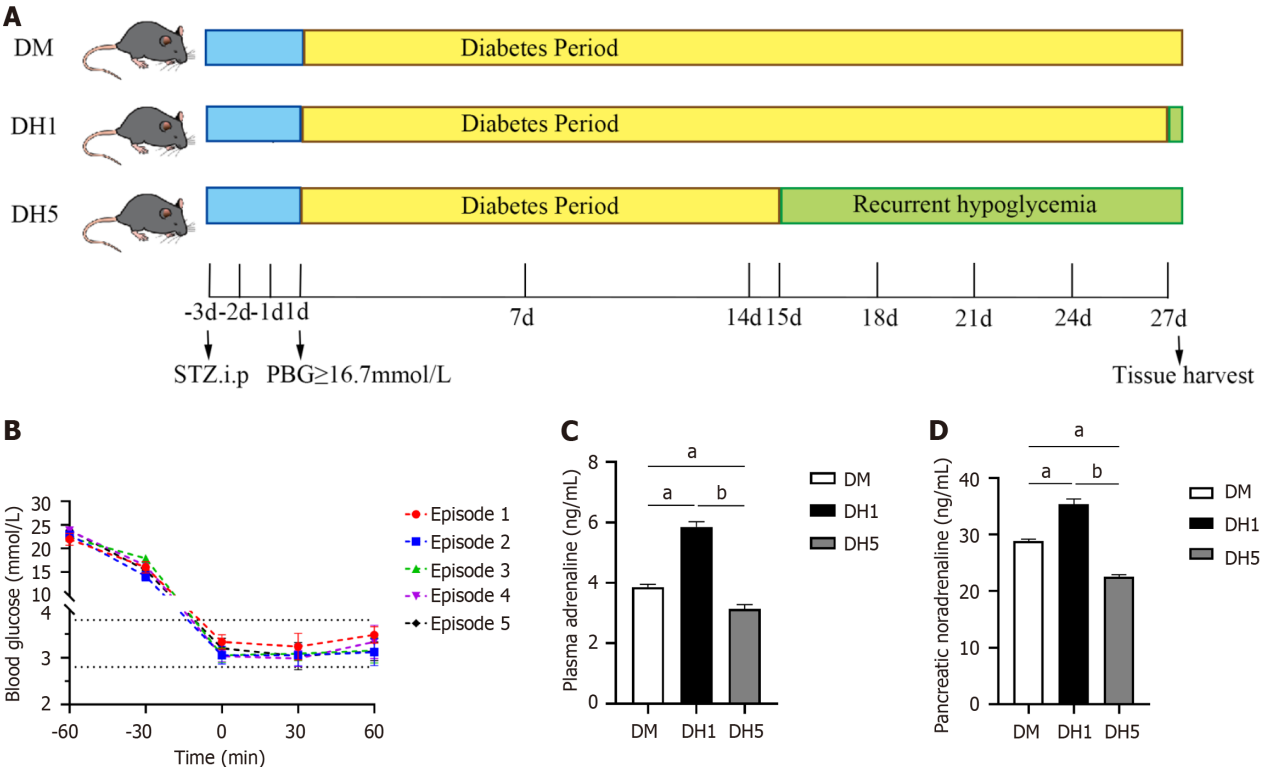


Figure 1 Diabetic mice with five hypoglycaemic episodes exhibit impaired hypoglycaemic counterregulation. A: Flow chart depicting the modeling process, with some content sourced from Scidraw; B: Fluctuations in blood glucose levels in diabetic mice following repeated insulin interventions; C: Alterations in plasma adrenaline levels; D: Changes in pancreatic noradrenaline levels. All the data are presented as the the mean ± SE. [a]$P < 0.01$ *vs* diabetic mice; [b]$P < 0.01$ *vs* diabetic mice with a single hypoglycaemic episode; PBG: Postprandial blood glucose; DM: Diabetic mice; DH1: Diabetic mice with a single hypoglycemic episode; DH1: Diabetic mice with a single hypoglycaemic episode; DH5: Diabetic mice with five hypoglycaemic episodes.

epithelium. The fluorescence images revealed a greater number of GLP-1-positive cells in the DH1 and DH5 groups than in the DM group. The GLP-1 IOD in the colon was significantly greater in the DH1 and DH5 groups than in the DM group ($P < 0.01$; Figure 2A). Since the ileum and colon are relatively long, further verification is needed to determine the total amount of GLP-1 reflected by the GLP-1 IOD in these sections. Therefore, Western blot experiments were conducted, and the results indicated that GLP-1 levels tended to increase sequentially in the DM, DH1, and DH5 groups, with a significant increase in the colon ($P < 0.01$; Figure 2B and C, Supplementary material).

Given that intestinal GLP-1 primarily acts through neighboring GLP-1Rs, this study aimed to investigate changes in intestinal GLP-1R expression through Western blot analysis. The results indicated a sequential increase in GLP-1R levels in the DM, DH1, and DH5 groups, with a significant increase observed in the colon of the DH5 group ($P < 0.01$; Figure 2B and C). These results suggest heightened levels of intestinal GLP-1 and GLP-1R expression in the DH1 and DH5 groups, indicating an enhanced paracrine effect, especially in the DH5 group.

Excessive intestinal GLP-1 is closely associated with impaired secretion of the counterregulatory hormones adrenaline and noradrenaline during hypoglycaemia

Adrenaline and noradrenaline serve as vital markers of the body's stress response. To investigate the role of intestinal GLP-1 in the stress response through paracrine effects, the levels of plasma adrenaline and pancreatic noradrenaline were

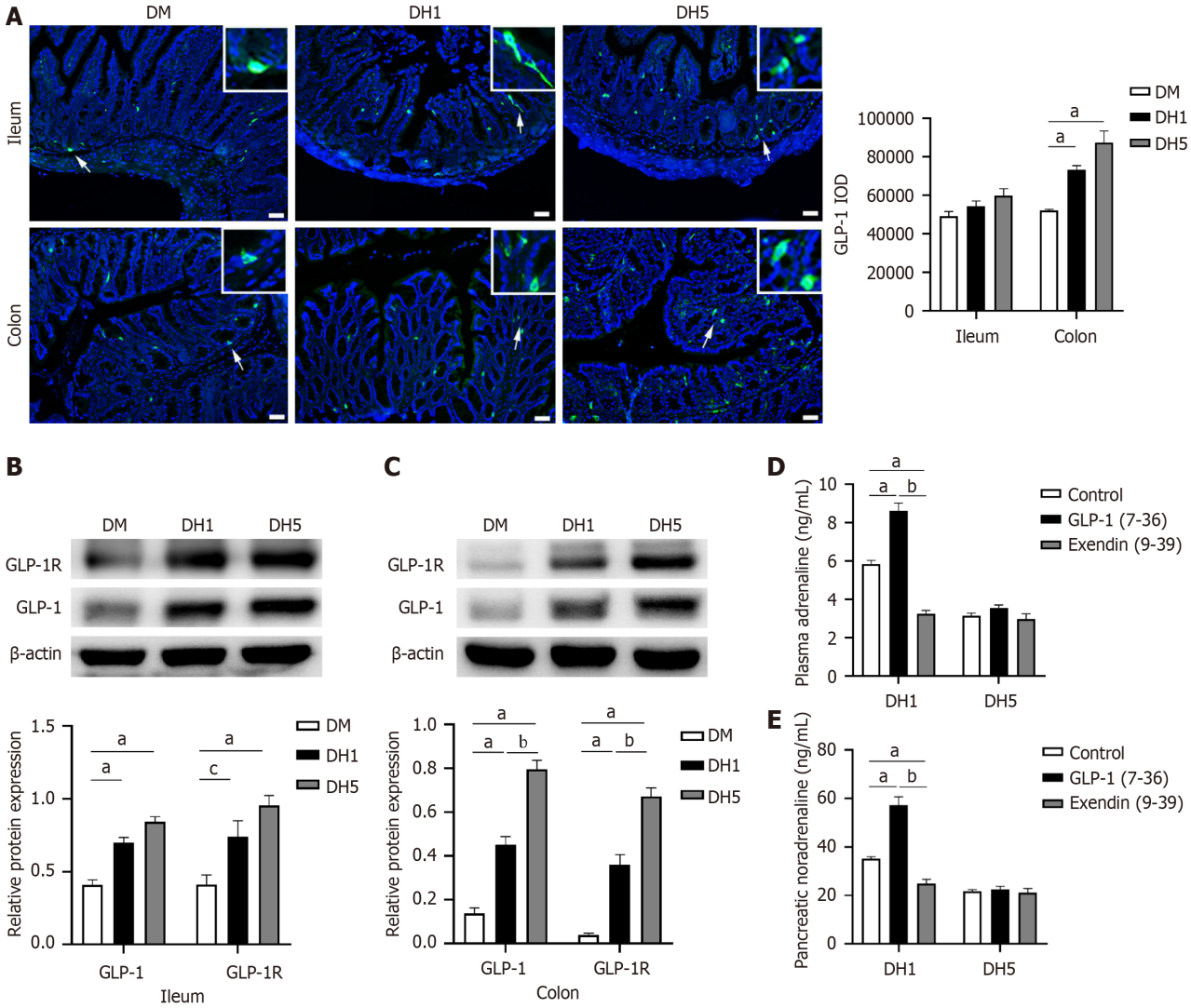


Figure 2 Elevated levels of glucagon-like peptide-1 and glucagon-like peptide-1 receptor in the intestines in response to hypoglycaemia. A: Immunofluorescence staining showing glucagon-like peptide-1 (GLP-1) expression in the ileum and colon, with GLP-1 in green and cell nuclei in blue. Scale bars = 50 μm; B: Western blot analysis of GLP-1 and its receptor (GLP-1R) protein expression in the ileum; C: Western blot analysis of GLP-1 and GLP-1R protein expression in the colon; D: Enzyme-linked immunosorbent assay (ELISA) results showing changes in plasma adrenaline levels after terminal ileal infusions in diabetic mice with a single hypoglycemic episode (DH1) and diabetic mice with five hypoglycaemic episodes (DH5); E: ELISA results showing changes in pancreatic noradrenaline levels after terminal ileal infusions in the DH1 and DH5 groups. The data are presented as the mean ± SE. $^{a}P < 0.01$ *vs* diabetic mice (DM); $^{b}P < 0.01$ *vs* DH1 group; $^{c}P < 0.05$ *vs* DM group; DM: Diabetic mice; DH1: Diabetic mice with a single hypoglycemic episode; DH5: Diabetic mice with five hypoglycaemic episodes; GLP-1: Glucagon-like peptide-1; GLP-1R: Glucagon-like peptide-1 receptor; IOD: Integrated optical density.

measured following infusions of the GLP-1R agonist GLP-1(7-36) or the antagonist exendin (9-39) into the terminal ileum. GLP-1(7-36) increased plasma adrenaline and pancreatic noradrenaline levels in DH1 mice ($P < 0.01$), while exendin (9-39) decreased adrenaline and pancreatic noradrenaline levels ($P < 0.01$). In contrast, neither GLP-1(7-36) nor exendin (9-39) affected plasma adrenaline or pancreatic noradrenaline levels in DH5 mice ($P > 0.05$; Figure 2D and E). These results indicate that heightened intestinal GLP-1 expression in DH1 mice can enhance sympathetic nerve excitation and adrenaline secretion, whereas excessive intestinal GLP-1 expression in DH5 mice hinders the activation of the sympathetic response following repeated hypoglycaemia. Excessive intestinal GLP-1 expression in DH5 mice appears to be linked to dysfunction in the secretion of hypoglycaemic counterregulatory hormones such as adrenaline and noradrenaline.

Excessive intestinal GLP-1 is closely related to impaired secretion of the counterregulatory hormone glucagon during hypoglycaemia

Intestinal GLP-1 participates in secretion of the counterregulatory hormone glucagon during hypoglycaemia through its endocrine effect: To further confirm impaired hypoglycaemic counterregulation in DH5 mice, the plasma level of GCG, another indicator of hypoglycaemic counterregulation, was measured using ELISA. The results showed a significant increase in plasma GCG levels in the DH1 group and a significant decrease in the DH5 group ($P < 0.01$; Figure 3A), supporting the conclusion of impaired hypoglycaemic counterregulation in DH5 mice.

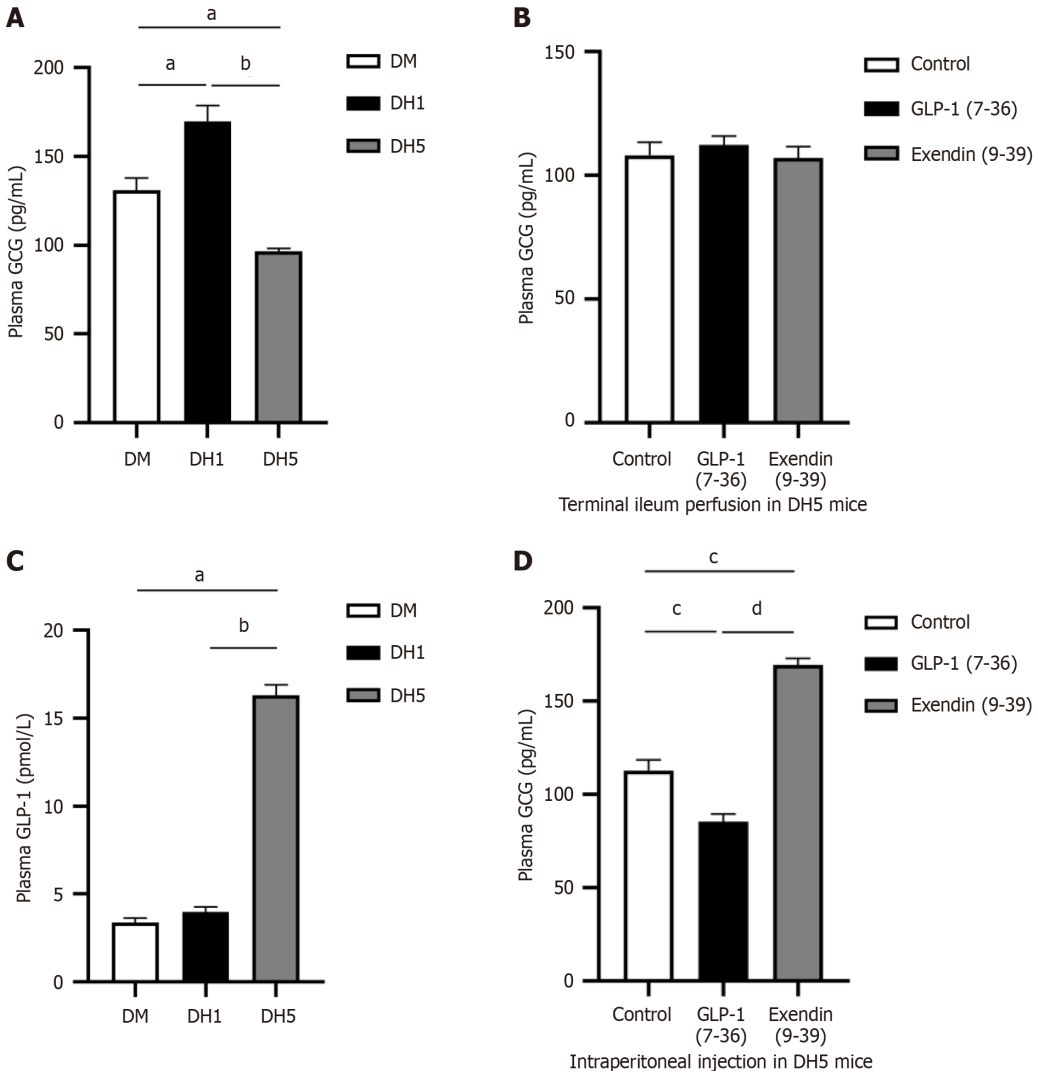
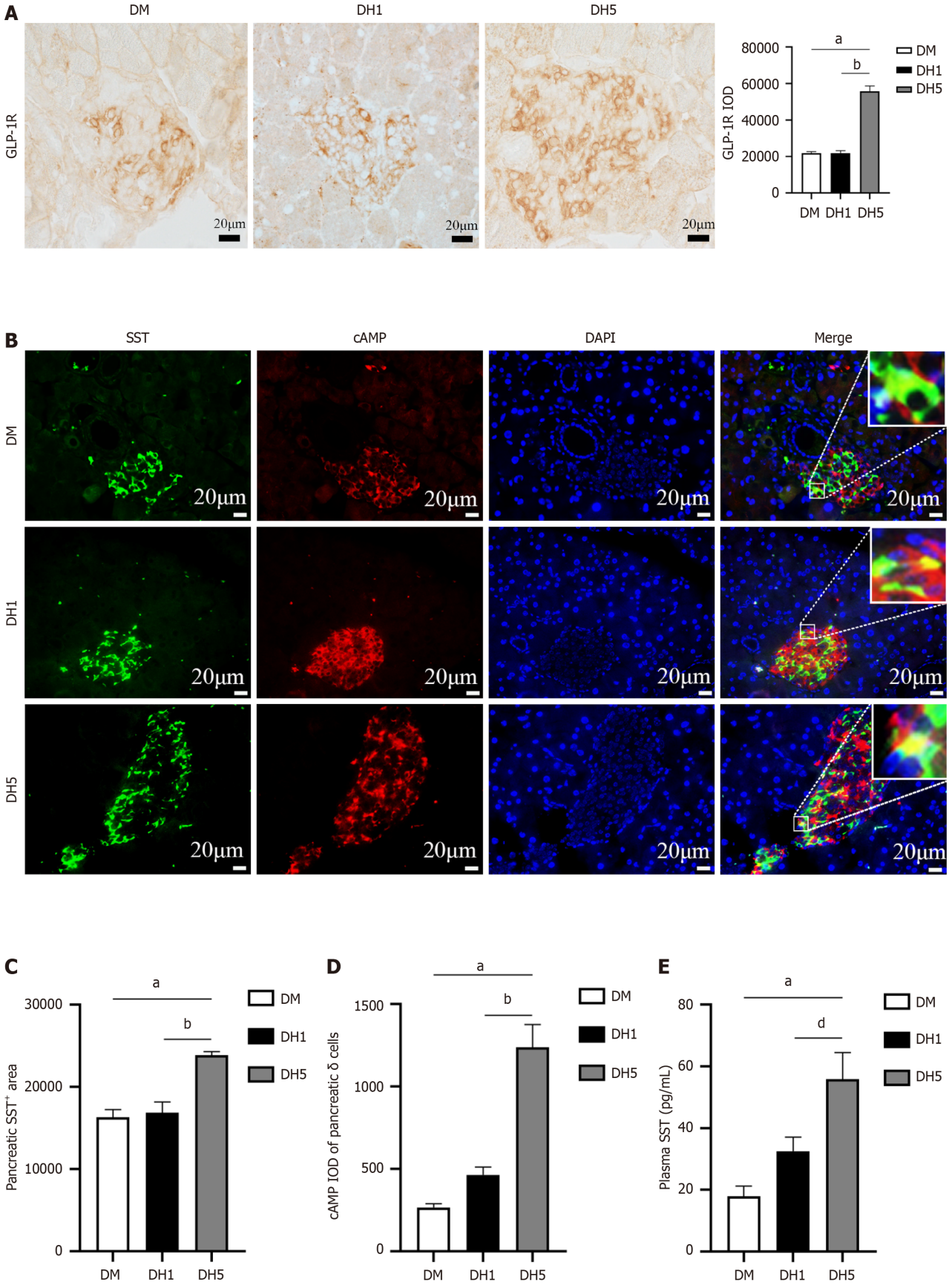


Figure 3 Intestinal glucagon-like peptide-1 regulates plasma glucagon levels through endocrine pathways. A: Changes in plasma glucagon (GCG) levels measured by enzyme-linked immunosorbent assay (ELISA); B: Alterations in plasma GCG levels measured by ELISA after terminal ileal infusion in diabetic mice with five hypoglycaemic episodes (DH5); C: Changes in plasma GCG-like peptide-1 (GLP-1) levels measured by ELISA; D: Alterations in plasma GCG levels measured by ELISA after intraperitoneal injection in the DH5 group. All the data are presented as the mean ± SE. $^{a}P < 0.01$ *vs* diabetic mice; $^{b}P < 0.01$ *vs* diabetic mice with a single hypoglycemic episode; $^{c}P < 0.01$ *vs* control group; $^{d}P < 0.01$ *vs* group receiving intraperitoneal GLP-1(7-36) injections; DM: Diabetic mice; DH1: Diabetic mice with a single hypoglycemic episode; DH5: Diabetic mice with five hypoglycaemic episodes; GLP-1: Glucagon-like peptide-1; GCG: Glucagon.

This study investigated the relationship between plasma GCG levels and intestinal GLP-1 secretion in DH5 mice. ELISA tests conducted after infusion of GLP-1(7-36) or exendin (9-39) revealed no significant changes in plasma GCG levels in the DH5 group ($P > 0.05$; Figure 3B), suggesting that the reduction in plasma GCG levels in the DH5 mice was not due to the paracrine effect of intestinal GLP-1. However, higher plasma GLP-1 levels were detected in the DH5 group than in the DM and DH1 groups ($P < 0.01$; Figure 3C), indicating an enhanced endocrine effect of GLP-1 in the DH5 group. Further experiments using intraperitoneal injections demonstrated that GLP-1 has an effect on GCG secretion in DH5 mice through endocrine pathways. Following the injection of GLP-1(7-36), there was a significant decrease in plasma GCG levels, whereas injection of exendin (9-39) led to a significant increase ($P < 0.01$; Figure 3D).

Elevated plasma GLP-1 levels enhance function of pancreatic δ cells through endocrine pathways, leading to suppression of pancreatic α cell secretion: To investigate the endocrine impact of intestinal GLP-1 on the pancreas, the expression of pancreatic GLP-1R was assessed using immunohistochemistry. The DH5 group exhibited significantly greater GLP-1R expression than the DM and DH1 groups ($P < 0.01$; Figure 4A). Given the scarcity of pancreatic β cells in T1DM mice, GLP-1R is predominantly expressed on pancreatic δ cells. To demonstrate the influence of GLP-1 on δ cells through its receptor, the area of SST-positive cells was measured. Surprisingly, the area of SST+ cells in the pancreas was significantly greater in the DH5 group than in the DM and DH1 groups ($P < 0.01$; Figure 4B and C). The signaling molecule cAMP plays a crucial role in the secretion of pancreatic SST. To investigate changes in SST secretion in pancreatic δ cells, the cAMP IOD values of these cells were assessed using immunofluorescence. The results revealed a significantly greater cAMP IOD in the pancreatic δ cells in the DH5 group than in the DM and DH1 groups ($P < 0.01$; Figure 4D). SST secreted by pancreatic δ cells can enter the bloodstream. To further evaluate SST secretion from pancreatic δ cells, the concentration of SST in plasma was measured using ELISA, and the results were consistent with the


A
DM
DH1
DH5
GLP-1R
20μm
GLP-1R IOD
80000
60000
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0
a
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DM
DH1
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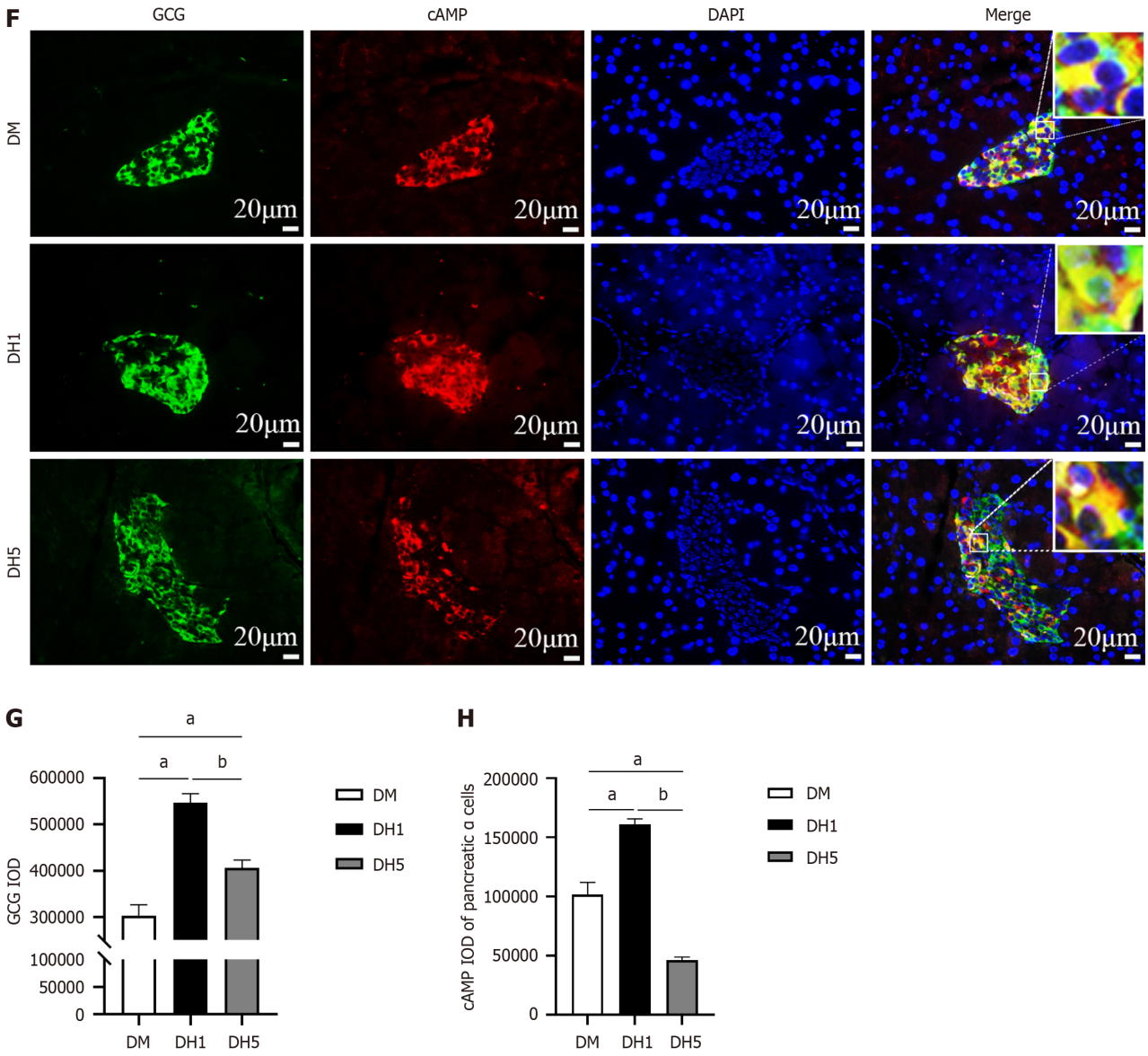

Figure 4 Intestinal glucagon-like peptide-1 inhibits glucagon secretion through endocrine effects. A: Immunohistochemistry showing pancreatic glucagon-like peptide-1 receptor (GLP-1R) levels, with GLP-1R in brown. Bars = 20 μm; B: Immunofluorescence staining showing somatostatin (SST) + cAMP expression in the pancreas, with SST in green, cAMP in red, and cell nuclei in blue. Bars = 20 μm; C: Pancreatic SST$^+$ cell area; D: CAMP integrated optical density (IOD) in pancreatic δ cells; E: Enzyme-linked immunosorbent assay results showing changes in plasma SST levels; F: Immunofluorescence staining showing GCG + cAMP expression in the pancreas, with GCG in green, cAMP in red, and cell nuclei in blue. Bars = 20 μm; G: Pancreatic GCG+ IOD; H: CAMP IOD in pancreatic α cells. All the data are presented as the mean ± SE. $^aP < 0.01$ *vs* diabetic mice; $^bP < 0.01$ *vs* diabetic mice with a single hypoglycemic episode (DH1); $^dP < 0.05$ *vs* DH1 group; DM: Diabetic mice; DH1: Diabetic mice with a single hypoglycemic episode; DH5: Diabetic mice with five hypoglycaemic episodes; GLP-1: Glucagon-like peptide-1; GLP-1R: Glucagon-like peptide-1 receptor; IOD: Integrated optical density.

cAMP findings (Figure 4E). Taken together, these results indicate that the elevated levels of intestinal GLP-1 in DH5 mice enhance SST secretion in pancreatic δ cells through endocrine mechanisms.

SST can inhibit GCG secretion from pancreatic α cells through paracrine effects. Next, the synthesis and secretion of GCG by pancreatic α cells were assessed using immunofluorescence staining and ELISA. The results of immunofluorescence staining revealed a significant difference in the pancreatic GCG IOD between the DH1 and DH5 groups and the DM group. Specifically, the DH5 group exhibited lower GCG levels than the DH1 group ($P < 0.01$; Figure 4F and G), suggesting a reduction in GCG synthesis in pancreatic α cells of the DH5 mice. Additionally, the cAMP IOD in pancreatic α cells was greatest in the DH1 group but significantly lower in the DH5 group than in the DM and DH1 groups ($P < 0.01$; Figure 4H). cAMP is a crucial indicator of GCG secretion, suggesting reduced GCG secretion from pancreatic α cells in DH5 mice. Furthermore, plasma GCG levels were significantly decreased in the DH5 group (Figure 3A). These results showed that GCG synthesis and secretion by pancreatic α cells were increased in the DH1 group but decreased in the DH5 group. In summary, the elevated levels of intestinal GLP-1 in the DH5 group may suppress GCG secretion from pancreatic α cells *via* SST released by pancreatic δ cells through endocrine pathways, leading to impaired hypoglycaemic counterregulatory responses.

DISCUSSION

Establishment of an impaired hypoglycaemic counterregulatory model

Numerous studies have demonstrated that diabetic mice induced by a high dose of STZ injection are commonly utilized as models for T1DM[16,17]. While most research on recurrent hypoglycaemia typically focuses on nondiabetic animals, there have also been reports of recurrent hypoglycaemia in T1DM rats[18-20]. Sankar *et al*[7] investigated different animal species, onset methods, and nervous system changes during hypoglycaemia, highlighting that recurrent insulin-induced hypoglycaemia in rodents (rats and mice) is the optimal approach for establishing a model of recurrent hypoglycaemia with impaired hypoglycaemic counterregulation. They observed that episodes of hypoglycaemia lasting 30 min or longer, totaling 90 min, can notably alleviate autonomic symptoms, leading to impaired hypoglycaemic counterregulation[7]. In this study, based on previous modeling experiences, male C57BL/6J mice were chosen as model organisms. Blood glucose levels on the third day after intraperitoneal STZ injection increased by ≥ 16.7 mmol/L, remaining consistently above this threshold thereafter, accompanied by a significant reduction in the weight of the mice. These findings strongly indicate the successful establishment of diabetic models.

In this experiment, rapid-acting insulin was administered intraperitoneally to lower blood glucose levels to 3.3 ± 0.5 mmol/L for 60 min, meeting the criteria for diabetic hypoglycaemia with fasting blood glucose levels ≤ 3.9 mmol/L. Previous studies have indicated that experiencing 2-3 episodes of hypoglycaemia per week can result in impaired hypoglycaemic counterregulation[6,18-20]. In this study, a hypoglycaemic frequency of 1 episode every 3 d was utilized for modeling, with the number of hypoglycaemic episodes increasing to 5 instead of the typical 3 reported in previous literature[20]. This adjustment aimed to further solidify and enhance the impaired hypoglycaemic counterregulation, creating a more robust model.

Observations following 3 episodes of hypoglycaemia revealed that DH5 mice exhibited reduced activity, excitement, and foraging behavior during hypoglycaemia. To investigate the potential link between changes in mouse activity in the DH5 group and impaired hypoglycaemic counterregulation, ELISA analyses were performed, which revealed lower levels of plasma adrenaline and pancreatic noradrenaline in the DH5 group than in the DM and DH1 groups. Given that adrenaline plays a significant role in increasing blood glucose levels and that sympathetic axons can stimulate GCG secretion by contacting pancreatic α cells[21,22], the decreased levels of plasma adrenaline and pancreatic noradrenaline in DH5 mice suggest the development of impaired hypoglycaemic counterregulation.

Excessive intestinal GLP-1 is closely associated with impaired secretion of the counterregulatory hormones adrenaline and noradrenaline during hypoglycaemia

Research has shown that the primary source of endogenous GLP-1 is predominantly L-cells in the terminal ileum and colon, particularly in the terminal colon. GLP-1 secretion is typically low in the fasting and interprandial states but increases rapidly during meal intake, depending on the meal size, and is closely linked to gastric emptying[15,23]. In this study, immunofluorescence and Western blot analyses revealed an increase in intestinal GLP-1 levels in T1DM mice following a single hypoglycaemic episode in the absence of food intake, with a further significant increase observed after multiple hypoglycaemic events. The question arises as to why intestinal GLP-1 levels rise in a fasting state.

Several studies have demonstrated a correlation between hypoglycaemia and oxidative stress[24]. Hypoglycaemia has been found to increase oxidative stress markers in nondiabetic individuals, individuals with diabetes, individuals who have undergone bariatric surgery, and in experimental cell cultures[24-27]. Furthermore, existing knowledge from both experimental and clinical studies indicates that GLP-1 or GLP-1 analogues play a significant role in antioxidant activity, offering therapeutic potential against micro- and macrovascular diabetic complications[28,29]. In this study, we investigated whether the elevated intestinal GLP-1 levels observed in DH1 and DH5 mice are linked to hypoglycaemic oxidative stress or whether they represent the body's protective response to such stress. Further research is needed to address this question.

However, recent research has shown that GLP-1 can serve as a crucial neuromodulator in the body's response to stress. Activation of intestinal GLP-1R leads to c-Fos expression in neurons located in autonomic control regions in the rat brain and adrenal medulla, which in turn promotes adrenal medullary secretion and increases the circulating levels of catecholamines[11,30,31]. The nucleus tractus solitarius (NTS) is identified as a crucial region involved in processing autonomic stress responses in both acute and chronic situations[32]. Through various techniques such as optogenetics and *in vivo* ganglion imaging, Erika found that the NTS plays a crucial role in processing visceral afferent information and transmitting it to spinal cord nuclei[12]. PPG neurons in the NTS receive monosynaptic input from vagal sensory neurons in the nodose ganglion and act directly on the sympathetic nuclei of the spinal cord to regulate adrenal secretion activity [33-36]. Studies have indicated the presence of GLP-1Rs in vagal afferent fibers and vagal neurons in the lamina propria and submucosa of the gastrointestinal tract[15]. Chemogenetics-mediated activation of GLP-1Rs in vagal afferent fibers can trigger c-Fos expression in NTS neurons, while blocking sympathetic nerve activity can diminish or eliminate the GLP-1R stress response[11,37]. Therefore, Diz-Chaves *et al*[38] proposed the concept of an intestinal GLP-1-neural reflex pathway, suggesting that intestinal GLP-1 can act on GLP-1R to activate the autonomic nerve and upload signals to the NTS PPG neurons, which in turn relay the signals to the sympathetic nerve, contributing to stress responses. Thus, intestinal GLP-1 has emerged as a critical neuromodulator that enhances the body's stress responses[38].

Hypoglycaemia, a detrimental condition of the body, can induce stress by activating the autonomic sympathetic nervous system[38]. In this study, after inducing stress from a single hypoglycaemic episode, the DH1 group showed higher levels of intestinal GLP-1 expression than the DM group, as evidenced by immunofluorescence and Western blot analysis. Moreover, Western blot analysis also demonstrated a noticeable upwards trend in GLP-1R expression in the intestine. Additionally, ELISA results indicated a significant increase in plasma adrenaline and pancreatic noradrenaline

levels in DH1 mice, which exhibited hyperactive and agitated behaviors. These findings imply that increased intestinal GLP-1 levels resulting from hypoglycaemia can initiate an intestinal GLP-1-neural reflex, triggering stress responses and activating the autonomic sympathetic nervous system. To validate the involvement of intestinal GLP-1 in the stress response to hypoglycaemia-induced sympathetic activation, the GLP-1R agonist GLP-1(7-36) or the antagonist exendin (9-39) was infused into the terminal ileum. The findings indicated that GLP-1(7-36) increased plasma adrenaline levels in DH1 mice, while exendin (9-39) had an inhibitory effect, further supporting the role of intestinal GLP-1 in stress responses to hypoglycaemia-induced sympathetic stimulation.

In addition, this study revealed that under conditions of recurrent hypoglycaemia, the expression of intestinal GLP-1 and GLP-1R in DH5 mice was greater than that in the other groups. Interestingly, the plasma adrenaline levels in the DH5 mice were significantly lower, and the mice did not exhibit typical excitement or foraging behavior during hypoglycaemia. These results indicate that intestinal GLP-1 levels continue to rise under recurrent hypoglycaemic stress, while sympathetic excitatory responses are significantly reduced. The discrepancy between single and recurrent hypoglycaemia may be partially explained by the impact of excessive stress on the adrenal gland, resulting in its hypertrophy and decreased sensitivity. Previous studies have shown that recurrent hypoglycaemic stress can induce adrenal gland hypertrophy and reduce sensitivity[39]. Ma *et al*[40] showed that a single hypoglycaemic stress can irritate the sympathoadrenal reflex, but recurrent stimulation can decrease adrenaline secretion and increase neuropeptide Y (NPY) secretion from chromaffin cells in the adrenal glands. Additionally, inhibition of NPY or NPY Y1 receptor signaling in adrenal chromaffin cells, either transgenically or pharmacologically, prevented the attenuated adrenaline release induced by recurrent hypoglycaemia[40]. Therefore, it is speculated that excessive intestinal GLP-1 due to recurrent hypoglycaemia may overly stimulate the sympathetic-adrenal reflex, leading to decreased plasma adrenaline levels in DH5 mice. Further experiments using ileum infusions of GLP-1(7-36) or exendin (9-39) in DH5 mice did not reveal a significant impact on adrenaline secretion, further confirming the above speculation. In summary, excessive intestinal GLP-1 due to recurrent hypoglycaemia can lead to overactivation of the sympathoadrenal reflex, resulting in impaired hypoglycaemic sympathetic-adrenal counterregulation.

Excessive intestinal GLP-1 is closely related to impaired secretion of the counterregulatory hormone glucagon during hypoglycaemia

The major hormones involved in hypoglycaemic counterregulation include adrenaline and GCG. In this study, the plasma GCG levels of the DH5 mice were significantly lower than those of the DH1 mice. To investigate the reason behind this decrease, this study measured plasma adrenaline and pancreatic noradrenaline levels in DH5 mice following terminal ileum infusion of GLP-1(7-36) or exendin (9-39). There were no significant changes in plasma epinephrine or pancreatic norepinephrine levels. Therefore, changes in GCG levels in DH5 mice experiencing recurrent hypoglycaemia are not related to the paracrine effect of intestinal GLP-1, indicating that alterations in GCG levels in DH5 mice are not due to the paracrine effect of intestinal GLP-1.

Previous research has demonstrated that peripheral dipeptidyl peptidase-4 (DPP-IV) degrades intestinal GLP-1, and the remaining undegraded GLP-1 enters the liver through the portal vein, with only approximately 10%-15% entering the systemic bloodstream in the active form to act on pancreatic islets[9,10]. In this study, the DH5 mice exhibited a significant increase in plasma levels of active GLP-1, which was more than 4 times greater than that in the DH1 group, indicating that intestinal GLP-1 has enhanced endocrine effects on DH5 mice. Furthermore, intraperitoneal injection of GLP-1(7-36) or exendin (9-39) in DH5 mice resulted in a decrease in plasma GCG levels following GLP-1(7-36) injection and an increase after exendin (9-39) injection. These findings suggest that GLP-1 can potentially suppress GCG secretion through endocrine pathways in DH5 mice.

To explore the underlying mechanism of GCG reduction in DH5 mice, this study focused on endocrine cells in the pancreas. Given the scarcity of pancreatic β cells in patients with T1DM, more attention has been given to the effect of GLP-1 on pancreatic α and δ cells. Previous research has shown that GLP-1R expression in δ cells is significantly greater than that in α cells, with only approximately 1% of α cells expressing GLP-1R[41]. The increased expression of GLP-1R in the pancreas of the DH5 mice in this experiment indicated an enhanced effect of GLP-1 on pancreatic δ cells. Studies by Ørgaard *et al*[42] using pancreatic perfusion demonstrated that GLP-1 can boost SST secretion while inhibiting GCG secretion through GLP-1R[42,43]. In contrast, blocking SST receptor 2, which is primarily found in α cells, can abolish the paracrine effect of GLP-1 on GCG secretion. Therefore, it is hypothesized that the suppressive effect of recurrent hypoglycaemia on GCG secretion might be due to the endocrine activity of GLP-1, which promotes SST release from pancreatic δ cells, ultimately leading to the suppression of GCG secretion from pancreatic α cells.

In this study, it was unexpectedly discovered for the first time that the pancreatic δ-cell mass in DH5 mice was significantly greater than that in DM and DH1 mice, indicating that the increase in pancreatic δ cells is additional new evidence of impaired hypoglycaemic counterregulation. However, an increase in pancreatic δ cells may not necessarily improve function. As cAMP is the main signaling molecule that plays a dominant role in SST secretion, this study revealed that the cAMP levels in the pancreatic δ cells of DH5 mice were significantly elevated, accompanied by a noticeable increase in plasma SST levels, indicating a significant increase in SST secretion. Moreover, in the present study, the cAMP levels in pancreatic α cells were reduced in DH5 mice, indicating that GCG secretion in DH5 mice decreased. Overall, the increase in pancreatic δ cells in DH5 mice, under the influence of the oversecretion of GLP-1, enhances SST secretion, which in turn inhibits adjacent pancreatic α cells and decreases their GCG secretion. Ultimately, excessive intestinal GLP-1 induced by recurrent hypoglycaemia may impair the secretion of the counterregulatory hormone GCG during hypoglycaemia.

Excessive intestinal GLP-1 is closely related to impaired appetite during hypoglycaemia

The NTS can receive signals from the visceral vagus nerve and plays a crucial role in regulating food intake[44]. Rats treated with intravenous GLP-1 demonstrate a reduction in food intake, which is reversed after transection of the subphrenic vagus nerve[45]. Acting as a prandial satiation signal to suppress food intake, intestinal GLP-1 reduces meal size and increases intermeal intervals[15,46]. In addition, recent studies using optogenetic and chemogenetic approaches in transgenic mice have shown that intestinal GLP-1 causes gastric distention and reduces appetite[47]. Intravital measurements of calcium transients have confirmed that ileal myenteric neurons expressing GLP-1 receptors are robustly responsive to GLP-1[47]. By the selective ablation of ileal myenteric neurons, the effects of intestinal GLP-1 can be eliminated, while chemogenetic stimulation of these neurons is sufficient to recapitulate the gastric anorectic effects of GLP-1[47]. Therefore, it is speculated that the decrease in foraging behavior during hypoglycaemia observed in the DH5 mice in this study is related to satiety induced by excessive intestinal GLP-1 and intestinal GLP-1R. The decreased appetite during hypoglycaemia in DH5 mice may be closely related to the activation of the central response through the neural pathway of intestinal GLP-1 and GLP-1 receptors to suppress food intake.

Currently, decreased awareness of hunger during hypoglycaemia in patients is generally believed to be linked to alterations in energy sources within brain neurons. As glucose levels in the brain repeatedly decrease, there is a shift in the energy supply from glucose to lactate or ketones, resulting in reduced motivation to take the initiative to eat[48]. This study, along with related research on the connection between GLP-1 and appetite, provides new experimental evidence on the excessive increase in intestinal GLP-1 and expands on the theoretical understanding of the lack of hunger awareness during hypoglycaemia.

CONCLUSION

In conclusion, the increased expression of intestinal GLP-1 in the DH5 model of T1DM mice is a crucial factor contributing to impaired counterregulatory responses to hypoglycaemia. The administration of GLP-1 or its agonists in T1DM treatment should be carefully monitored to avoid adverse effects. Proper dosing is crucial for effective T1DM therapy, as excessive amounts may worsen the impaired counterregulatory response to hypoglycaemia, leading to decreased appetite and compromised secretion of adrenaline, noradrenaline, and GCG during hypoglycaemia.

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FOOTNOTES

Author contributions: Li RJ and Jin FX designed the research study and prepared the manuscript; Guo JT, Li RJ, and Jin FX participated in the data analysis, interpretation of the results, and preparation of the draft manuscript; Jin FX, Wang Y, and Li MN performed the research and participated in the animal work and sample collection. All authors have read and approved the final manuscript. Both Li RJ and Guo JT have played important and indispensable roles in the experimental design, data interpretation, and manuscript preparation as the co-corresponding authors. Li RJ applied for and obtained the funds for this research project. Li RJ proposed and designed the established the animal model of type 1 diabetes mellitus with impaired hypoglycaemic counterregulation, performed data analysis, and prepared the first draft. Guo JT was responsible for mouse grouping and specimen collection. Guo JT was instrumental and responsible for data re-analysis and re-interpretation, and figure preparation, and participated in data analysis and interpretation. This collaboration between Li RJ and Guo JT is crucial for the publication of this manuscript.

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ORIGINAL ARTICLE

Basic Study

Mitigating diabetes-related complications: Empowering metformin with cholecalciferol and taurine supplementation in type 2 diabetic rats

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Abstract

BACKGROUND

Type 2 diabetes is one of the most prevalent chronic diseases worldwide, significantly impacting patients' quality of life. Current treatment options like metformin (MET) effectively counteract hyperglycemia but fail to alleviate diabetes-associated complications such as retinopathy, neuropathy, nephropathy, hepatopathy, and cardiovascular diseases.

AIM

To propose the supplementation of cholecalciferol (CHO) and taurine (TAU) to enhance MET efficacy in controlling diabetes while minimizing the risk of associated complications.

METHODS

The study involved sixty rats, including ten non-diabetic control rats and fifty experimental rats with type 2 diabetes induced by streptozotocin. The experimental rats were further subdivided into positive control and treatment subgroups. The four treatment groups were randomly allocated to a single MET

treatment or MET combined with supplements either CHO, TAU, or both.

RESULTS

Diabetic rats exhibited elevated levels of glucose, insulin, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), glycated hemoglobin percentage, lipid markers, aspartate aminotransferase, and malondialdehyde, along with reduced levels of antioxidant enzymes (catalase and superoxide dismutase). The administration of CHO and TAU supplements alongside MET in diabetic rats led to a noticeable recovery of islet mass. The antioxidative, anti-inflammatory, and anti-apoptotic properties of the proposed combination therapy significantly ameliorated the aforementioned abnormalities.

CONCLUSION

The supplementation of CHO and TAU with MET showed the potential to significantly improve metabolic parameters and protect against diabetic complications through its antioxidative, anti-inflammatory, and anti-apoptotic effects.

Key Words: Diabetes complications; Metformin; Cholecalciferol; Taurine; Glycated hemoglobin%; Antioxidant

Core Tip: Supplementation of cholecalciferol and taurine in conjunction with metformin shows promise in enhancing the management of type 2 diabetes by addressing hyperglycemia and minimizing the risk of associated complications. This combination therapy demonstrates antioxidative, anti-inflammatory, and anti-apoptotic properties, leading to improvements in glucose and insulin levels, Homeostasis Model Assessment of Insulin Resistance, glycated hemoglobin%, lipid profiles, liver function markers, and oxidative stress markers in diabetic rats. The proposed regimen also promotes recovery of pancreatic islet mass, highlighting its potential to mitigate diabetic complications.

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INTRODUCTION

Diabetes mellitus (DM) poses a substantial global health threat because of the high prevalence with different etiologies and increased risk of mortality[1]. This metabolic disorder's hallmarks are chronic hyperglycemia, hyperlipidemia, and insulin resistance, contributing to macrovascular and microvascular events[2]. Atherosclerotic cardiovascular disease is a prominent global cause of morbidity and mortality, with approximately 70% to 80% of deaths in individuals with diabetes attributed to vascular disorders[3]. Other serious diabetic complications may damage many vital organs, which can result in more severe and irreversible pathological illnesses, including nephropathy, retinopathy, vasculopathy, neuropathy, cardiovascular diseases, and hepatopathy[4].

The hyperglycemic state leads to complications, especially atherosclerotic cardiovascular disease, by affecting lipoproteins, vascular matrix components, and cellular metabolism. This results in the excessive formation of free radicals due to glucose autoxidation and non-enzymatic protein glycation[5]. Over time, it damages cellular organelles and enzymes, leading to increased lipid peroxidation[6]. A complete understanding of the precise mechanisms underlying oxidative stress in diabetes requires further exploration, which may involve multiple pathways. In diabetes, antioxidant defenses are compromised, and free radical and reactive oxygen species (ROS) levels are elevated, suggesting the potential antioxidant supplements[7]. Various ROS, originating mainly from mitochondrial electron transport chains, cause damage to vascular endothelial cells, disrupt junctions, and increase permeability, which in turn accelerates microvascular disease progression[8].

Despite advances in DM research, insights into its etiology, effects, and progression, and developments in insulin and its analogs, optimal glycemic control remains challenging. For patients with type 2 DM (T2DM), lifestyle modifications like diet and exercise are important, yet they are challenging to follow consistently[9]. There are currently two existing primary treatment protocols for T2DM: Injections of insulin or insulin-like peptides and oral administration of hypoglycemic agents. Although these therapies are essential for the treatment of T2DM, they also bear adverse effects[10]. For instance, several prescribed medications, such as metformin (MET), sulfonylureas, GLP-1 receptor agonists, and SGLT2 inhibitors, are involved with gastrointestinal issues, risk of hypoglycemia, weight gain, and increased susceptibility to infection[11]. Aside from this, these medications can become less effective over time as the disease progresses, necessitating the need for dose adjustments or combination therapy, which complicates treatment regimens and, in turn, leads to poor adherence. Additionally, MET has been a standard treatment for T2DM due to its remarkable ability to

lower plasma glucose levels[12]. Despite this, MET suffers from the lowest compliance of all oral antidiabetic medications due to minor to severe adverse effects that impair patient compliance. While MET controls diabetes and provides hepatoprotection, patients are still experiencing liver-related complications, necessitating a more effective therapeutic option [13].

Taurine (TAU), a semi-essential amino acid relatively abundant in the brain, the heart, and muscles, is active in several cellular processes and has anti-inflammatory and antioxidant properties[14,15]. Regarding health benefits, TAU controls glucose and lipid metabolism, promotes energy metabolism, and reduces inflammation while acting as an antioxidant by acting as a ROS scavenger[16]. TAU depletion causes numerous pathological changes, including severe cardiomyopathy, renal and pancreatic ailments, as well as loss of retinal photoreceptors. The use of TAU supplements has shown promise in preventing complications of diabetes, including endothelial dysfunction, nephropathy, retinopathy, and cardiomyopathy[17].

Earlier studies have demonstrated TAU's critical role in endocrine pancreas development and insulin production and secretion. A diet containing TAU was found to increase pancreatic islet mass while simultaneously reducing beta cell apoptosis, suggesting it protects beta cells from damage[18]. TAU also mitigates the effects of cytokine-induced apoptosis in pancreatic beta cells, which suggests it could be beneficial to the anti-inflammatory processes associated with autoimmune diabetes[19]. Considering TAU's potential for preventing or alleviating diabetes, particularly in early life stages, further research into its role in improving pancreatic function and mitigating diabetic complications is necessary. Taking into account the negative aspects, numerous studies have been conducted on the effects of high TAU intake but have not reported serious adverse effects[20,21]. There could be potential unwanted effects of TAU on the nervous system, cardiovascular system, and muscle emerging from its activity in regulating calcium release[22]. Despite that, TAU appears to be safe for humans at doses as high as 10 g/day for six months[23]. However, as with any dietary supplement, TAU should be consumed moderately. When TAU is overconsumed, side effects may arise as nausea, vomiting, diarrhea, dizziness, tremors, and headache[24]. Furthermore, caution must be exercised due to the potential interactions between TAU and cardiovascular and nervous system drugs, which may exacerbate the effects of such treatments and increase the risk of hypotension[25], altered heart rhythms, or neurological concerns[26].

In addition, cholecalciferol (CHO), the major form of vitamin D in humans, plays a significant role in bone health by increasing intestinal absorption of calcium, magnesium, and phosphate[27]. Aside from promoting bone health, research suggests that CHO also possesses anti-inflammatory and antioxidant properties[28]. This antioxidant role is relevant in insulin resistance and diabetes, where oxidative stress has been linked to their related complications[29]. Supplementation with CHO is unlikely to cause severe adverse reactions, even at high doses[30]. The excessive intake of this nutrient, on the other hand, may lead to hypercalcemia, which may cause calcium deposits in soft tissues or lead to kidney stones and cardiovascular complications[31,32]. Moreover, hypercalcemia can be exacerbated by CHO in individuals with high calcium levels, as well as hypertensive patients on thiazide diuretics[33]. In this regard, blood calcium levels are advised to be monitored to prevent CHO toxicity.

The current study aimed to determine if CHO and TAU supplements could enhance MET's effectiveness in treating diabetes while minimizing the associated pathological risks, including hepatitis, dyslipidemia, and oxidative stress tissue damage. If so, it may be possible to anticipate improvements in patient compliance with MET.

MATERIALS AND METHODS

Chemicals

CHO (Vidrop®) was purchased from Medical Union Pharmaceuticals (MUP, Ismailia, Egypt). MET (Cidophage®) was obtained from Chemical Industries Development Co. (CID; Giza, Egypt). TAU powder was obtained from BDH Chemicals Ltd. (Poole, Dorset, United Kingdom). Streptozotocin (STZ) was purchased from Sigma-Aldrich Chemical Corporation (St Louis, MO, United States). Assay kits for antioxidant and oxidative biomarkers [superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA)] as well as low-density lipoprotein cholesterol (LDL-C) assay kit were purchased from Biodiagnostic Co. (Dokki, Giza, Egypt). Kit for serum glucose, triglyceride, and high-density lipoprotein cholesterol (HDL-C) were obtained from BioMed Diagnostics (EGY-CHEM for lab technology, Badr city, Egypt).

Animals and experimental design

The animal study was conducted under the guiding principle for the care and use of research animals reviewed and approved by the ZU-IACUC committee, with ethical sanction number ZU-IACUC/2/F/47/2022. Initially, the experiment started with 75 healthy male albino rats (Sprague Dawley) weighing approximately 200-250 gm. Rats were obtained from the animal house at the Faculty of Veterinary Medicine of Zagazig University, where animals were kept in cages under standard conditions of temperatures around 20-25 °C with proper ventilation during light and dark cycles. Rats were left to acclimatize for one week before the experiment, during which they were fed with rat normal pellet diet (kcal percentage from fat approximately 12%) and tap water before they were simply randomized into two main groups, namely:

Non-diabetic group (control): Ten healthy adult albino rats were intraperitoneally injected with a single dose of citrate buffer solution (0.1 mmol/L) and continued their normal diet.

Diabetic group: The rats were fed a high-fat diet containing an approximate kcal percentage of 58% fat, 25% protein, and 17% carbohydrates for four weeks before receiving a single intraperitoneal dose of STZ (45 mg/kg) dissolved in sodium

citrate buffer as described in previous studies[34,35]. Rats were fed fructose solution 20% (w/v) directly after receiving STZ to prevent hypoglycemia. MET and the proposed supplements were administered to rats on the third day following T2DM induction. Prior to the onset of diabetes, blood glucose levels were assessed, and thereafter, weekly monitoring for six weeks was carried out using a glucometer (GlucoDrTM, All Medicus Co. Ltd, Gyeonggi, Korea). Induction of diabetes was confirmed two days following STZ administration. Fifty rats were deemed diabetic whose blood-glucose levels exceeded 250 mg/dL and were selected for further investigation. This group was further randomly subdivided into five subgroups of ten diabetic rats treated for six weeks each, as follows: (1) STZ group: Rats served as the non-treated diabetic group after experimental induction of diabetes; (2) MET-treated group: Diabetic rats received a daily dose of MET (250 mg/kg) for six weeks, administered orally using a metallic stomach tube[36]; (3) TAU + MET-treated group: A combination of TAU (100 mg/kg) and MET (250 mg/kg) was orally co-administered daily to diabetic rats[37]; (4) CHO + MET-treated group: A combination of CHO (7500 IU/kg) and (250 mg/ kg) of MET were orally co-administered daily to diabetic rats[38]; and (5) TAU + CHO + MET-treated group: A mixture of CHO (7500 IU/kg), TAU (100 mg/kg), and (250 mg/ kg) of MET was orally co-administered daily to diabetic rats.

Blood collection

Upon completing the ten-week experiment, rats were fasted for 12 hours and then sedated with ketamine-xylazine, and blood samples were collected by orbital puncture using heparinized microhematocrit capillary tubes. Two blood samples were taken from each rat to evaluate oxidative stress markers and biochemical parameters. The first was collected in a clean tube with anticoagulant (EDTA), then centrifuged to separate plasma for measuring glycated hemoglobin (HbA1c). The serum collected from the second sample was used for further biochemical analysis.

Biochemical analysis

As described by Ng *et al*[39], blood glycated hemoglobin (HbA1c%) levels were measured using an immunoassay kit (ELISA; Cusabio, Houston, TX, United States). Serum glucose levels were measured using the BioMed Diagnostic kits following the method described by Young *et al*[40]. In rat serum, insulin levels were measured using a radioimmunoassay kit (insulin-I125 kit, Radio Assay System Laboratories Inc., England) as outlined by Woodhead *et al*[41]. The obtained glucose and insulin data were used to calculate the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), a measure of insulin resistance and beta cell function, using the following equation[42].

HOMA-IR = Serum insulin (μIU/mL) × serum glucose (mg/dL)/405.

Serum HDL-C levels were determined following the method by Castelli *et al*[43] (1977) using the BioMed HDL-C-cholesterol kit. Moreover, LDL-C-cholesterol was measured using a perciptation assay kit according to the manufacturer's (Biodiagnostics) instructions. Furthermore, serum triglyceride levels were determined by enzyme colorimetry using the BioMed kit, whereas the colorimetric kit (Spinreact, Santa Coloma, Girona, Spain) was used to assess serum cholesterol levels following the manufacturer's instructions. Consequently, very-low-density lipoprotein cholesterol (VLDL-C) was determined according to Friedewald equation: VLDL-C = TG/5.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using the kinetic UV KIT (EliTech group Clinical Systems, Seppim SAS, Sées, France), while serum lactate dehydrogenase (LDH) activity was determined using the kinetic UV assay kit (Spectrum Diagnostics, Cairo, Egypt) according to Young[44]. Additionally, SOD activity was evaluated using the commercially available Biodiagnostic kit, following Nishikimi *et al* [45]. The analysis of CAT activity was performed using Aebi's method[46]. In addition, MDA levels were determined using the colorimetric end-point kit, following the Ohkawa method[47].

Tissue collection and histological study

Following blood collection, animals were euthanized by cervical dislocation directly after receiving a high anesthetic dose. Then, the animals were dissected, and the pancreas and liver were fixed in 10% formalin for histopathological and immunohistopathological studies. After ethanol dehydration, the samples were prepared as a paraffin block. A thin section of the paraffin block was stained with hematoxylin and eosin (H&E) and then observed under the microscope (Amscope) for detailed information about the tissue. Photomicrographs were captured at different magnifications 100 × and 400 ×.

Immunohistochemistry was conducted according to the standard procedures. Tissue sections were subjected to routine microwave treatment to distinguish antigen epitopes[48]. Immunostaining is a two-step process: Initially, the primary antibody binds to the related antigen, followed by interaction with a specific secondary antibody carrying an amplifying enzyme system to visualize this reaction. The Biotin-Streptavidin system was used to visualize the markers, whereas diaminobenzidine was used as a permanent chromogen[49]. Counterstaining was carried out with hematoxylin. Photomicrographs were captured at different magnifications 40 × and 400 ×. Fiji software was used to analyze the stain intensity, as well as number, and size of the stained islets per 40 × field[50]. In order to evaluate stain intensity, images of test groups were first deconvoluted, inverted, then analyzed, and compared with control measurements.

Statistical analysis

Data analyses were conducted using the SPSS for Windows, version 26 software. SPSS was used to perform the normality test, and parametric tests were conducted on the normally distributed data. Data were analyzed by ANOVA except for baseline-corrected blood glucose changes, which were analyzed by repeated-measures two-way analysis of variance and compared by Tukey's test. The results' significance was tested at a P value of ≤ 0.05, and the data were expressed as mean ± standard error. The graphs were created with the aid of GraphPad Prism 9.

RESULTS

Changes in blood glucose levels

Blood glucose levels were sequentially recorded from the first to the sixth week of the experiment, and changes were then calculated by comparing them with the initial measurements. Changes in blood glucose levels among the STZ group were significantly greater than the control group. Compared to the STZ group, the MET-treated group showed no significant changes until the sixth week, when their glucose level decreased significantly. Meanwhile, glucose levels decreased significantly in the three treated groups, CHO + MET, TAU + MET, and CHO + TAU + MET, during the six-week experiment compared to STZ and MET groups. Eventually, the glucose level in the CHO + TAU + MET group decreased significantly, reaching a level close to normal.

Serum glucose, insulin, HOMA-IR levels, and HbA1c%

As shown in Figure 1, the STZ group showed significant increases in serum glucose, insulin, HOMA-IR, and HbA1c% compared with the control group. Yet, serum insulin levels in the MET group did not differ significantly from those in the STZ group, whereas serum glucose and HOMA-IR levels declined significantly. A pronounced reduction in serum glucose and insulin levels occurred in the CHO + MET-treated group compared with the STZ and MET groups. Although serum glucose and insulin levels were significantly lower in the TAU + MET-treated group than in the STZ group, they were comparable to the MET-treatment group. The combined treatment of CHO, TAU, and MET resulted in a significant 1.98-fold decrease in serum glucose levels compared to the MET-treated group. The HOMA-IR level receiving a single TAU or CHO-treated group supplement declined significantly compared to the STZ and MET groups. The combined use of CHO, TAU, and MET significantly reduced insulin and HOMA-IR levels by 1.97-fold and 3.91-fold, respectively, *vs* MET alone.

Moreover, the HOMA-IR level for this group was comparable to the control group. MET treatment did not exert any significant difference from the diabetic control. Compared to the STZ group, there was a significant difference in HbA1c% between the supplemented MET-treated groups. With the abovementioned combination, HbA1c% significantly declined by 1.58 times concerning STZ-induced diabetics.

Lipid profile

As can be observed in Figure 2, untreated diabetic rats did not demonstrate any significant differences in serum cholesterol levels compared to the control group. Similarly, none of the treated groups showed significant changes in serum cholesterol levels compared to the STZ group. In the meantime, the STZ group showed a significant increase in triglycerides (TGs), LDL-C, and VLDL-C levels and a significant decline in HDL-C levels compared with the control group. HDL-C levels increased significantly in all treated rats relative to STZ but failed to reveal significance compared to the MET-treated group.

Neither TAU nor CHO alone nor their combination showed any significant difference in VLDL-C levels compared to MET-treated groups. Compared to the sole MET treatment, there were non-significant differences in TG levels except for TAU and CHO co-treatment, which showed a significant drop in TG levels. Also, neither the MET, CHO + MET, or TAU + MET-treated groups exhibited any significant differences in TGs or VLDL-C levels from the STZ group. However, compared to the STZ group, the CHO + TAU + MET group showed significant declines in TG and VLDL-C levels.

Although the MET-treated group alone failed to demonstrate a significant change in LDL-C levels compared with the STZ group when TAU or CHO were used alone or in combination, a significant reduction in LDL-C levels was observed. Meanwhile, comparing the three treated groups with the MET-treated group showed no significant differences in LDL-C levels.

Serum aminotransferase enzymes (AST, ALT) and LDH activities

Figure 3 shows a significant increase in AST levels among the STZ group in comparison to the control group. Meanwhile, neither the MET, the CHO + MET, nor the TAU + MET groups showed significant differences in AST levels from the STZ group. Conversely, the AST level decreased significantly following the coadministration of CHO, TAU, and MET compared to both STZ and MET-treated groups. All experimental groups had no statistically significant changes in ALT and LDH levels.

Antioxidants and oxidative stress markers

In the STZ group, the activity of antioxidant markers (SOD and CAT) was significantly decreased compared to the control group, as presented in Figure 4, whereas there was a significant increase in oxidative stress marker (MDA) level. In contrast, the MET-treated group did not exhibit significant differences in SOD, CAT, and MDA levels when compared to the STZ group. Meanwhile, the daily intake of CHO + MET and CHO + TAU + MET significantly ameliorated the reduction of SOD and CAT levels while increasing the MDA level as compared to the STZ group, reaching near to normal estimates and slightly better than the control group.

Histopathology

Histopathology of pancreatic sections: In the control group, the pancreatic tissue exhibited well-defined normal islets of Langerhans with granulated cytoplasm and a regular lining of acinar cells around the islets (Figure 5A and B). However, the untreated diabetic rats displayed a marked reduction in the sizes of islets, the irregular acinar cell lining around the islets, and a notably thickened blood vessel (Figure 5C and D). Treatment with MET in diabetic rats reduced islet size, degenerative changes in acinar cells, and perivascular inflammation (Figure 5E and F). Conversely, MET-treated diabetic

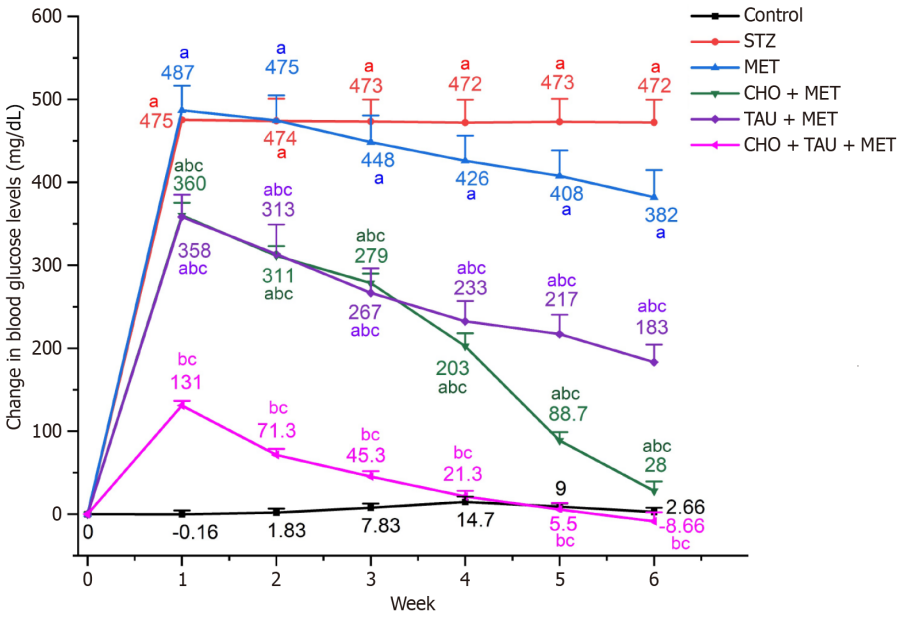

Figure 1 Changes in blood glucose levels in the control and experimental groups. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; TAU: Taurine. [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [C]Significance compared to the metformin-treated group.

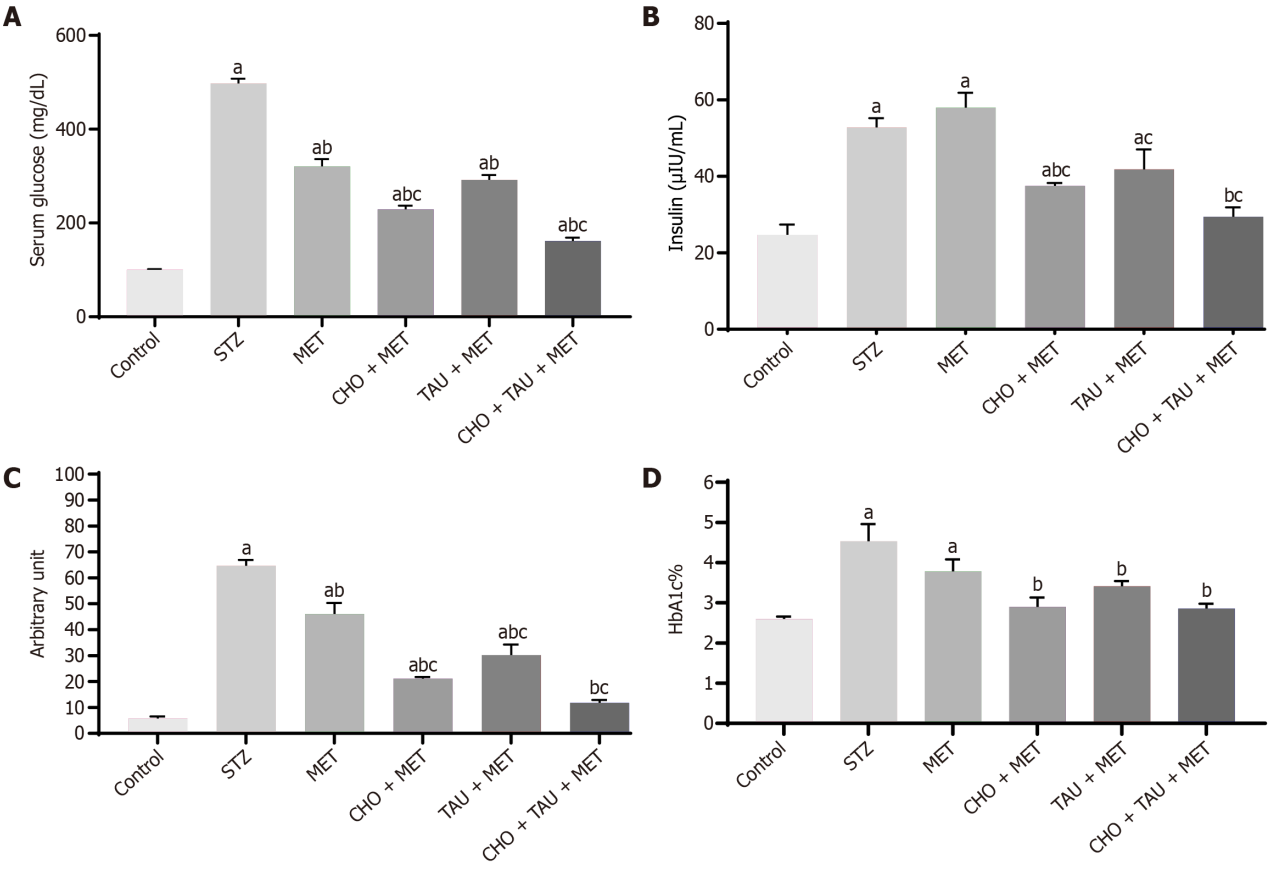

Figure 2 Assessment of glycemic profiles. A-D: Measurement of serum glucose level (A), insulin level (B), Homeostasis Model Assessment of Insulin Resistance levels (C), glycated hemoglobin% (D) in the control group and other experimental groups. The data, presented as means ± SE (n = 6), indicate significant differences at $P < 0.05$. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; TAU: Taurine; HbA1c: Glycated hemoglobin. [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [c]Significance compared to the metformin-treated group.

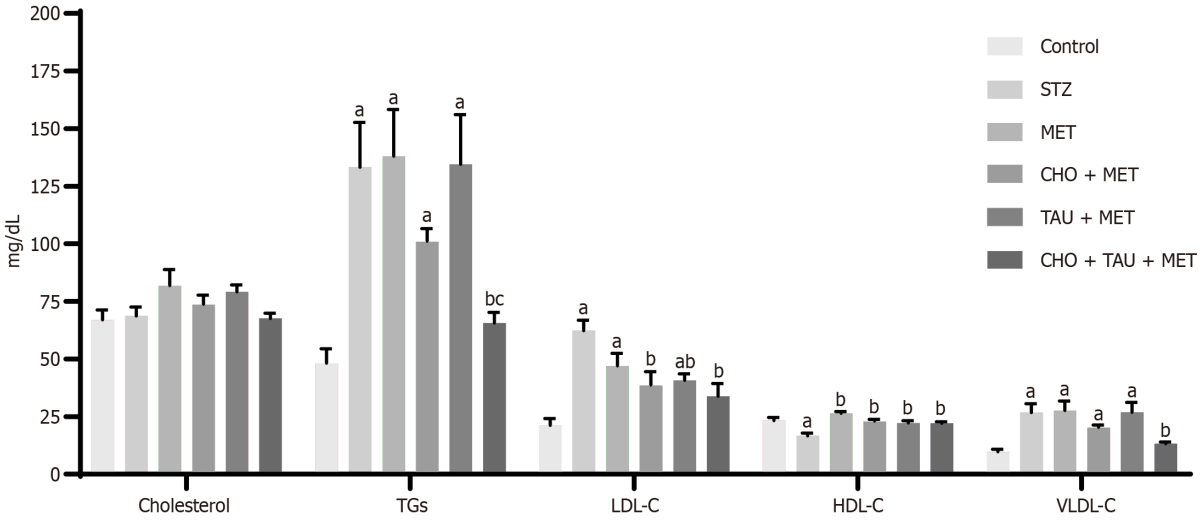

Figure 3 Lipid markers measurements in the control group and other experimental groups. The data, presented as means ± SE (n = 6), indicate significant differences at $P < 0.05$. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; TAU: Taurine; TGs: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; VLVL-C: Very low-density lipoprotein cholesterol. [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [c]Significance compared to the metformin-treated group.

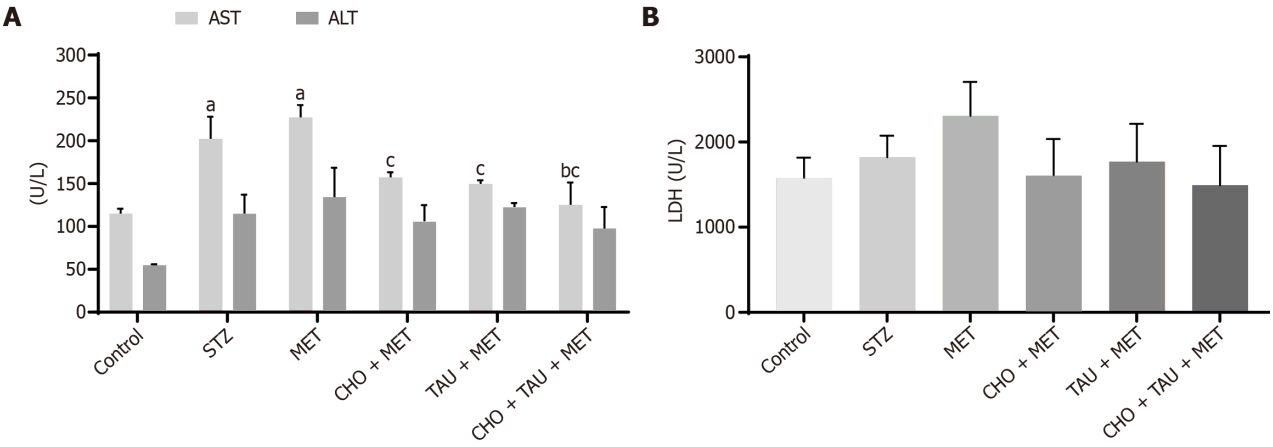

Figure 4 Measurement of liver enzymes. A and B: Serum aminotransferase enzymes (aspartate aminotransferase, alanine aminotransferase; A) and Serum lactate dehydrogenase (LDH; B) levels in the control group and other experimental groups. The data, presented as means ± SE (n = 6), indicate significant differences at $P < 0.05$. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; TAU: Taurine; TGs: Triglycerides; LDH: Lactate dehydrogenase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [c]Significance compared to the metformin-treated group.

rats that received CHO had restored islet structure (Figure 5G and H), while the combination of TAU showed a similar effect but without inflammation (Figure 5I and J). In the meantime, the coadministration of CHO and TAU to the MET-treated diabetic rats showed a slightly increased size of islets surrounded by acinar cells (Figure 5K and L).

Histopathology of liver sections: The histopathological examination of rat liver sections stained with H&E revealed distinct features in each experimental group. In the control group, the liver exhibited normal morphology. In contrast, in the STZ group, there were signs of lobular inflammation (Figure 6A and B), fibrous portal expansion, sinusoidal dilatation, and hydropic degeneration of hepatocytes with areas of necrosis (Figure 6C and D). Treatment with MET partially mitigated these effects, showing hydropic degeneration of hepatocytes with necrotic regions (denoted by lost nuclei) with congested central veins and lobular inflammation but no fibrosis (Figure 6E and F). The CHO + MET-treated group displayed preserved hepatocyte arrangement with binucleation of some hepatocytes and mild lobular inflammation (Figure 6G and H). In contrast, TAU supplementation showed hydropic degeneration of hepatocytes with areas of necrosis with lobular inflammation (Figure 6I and J). The supplementation of CHO and TAU in the MET-treated group exhibited preserved hepatocyte arrangement with minimal signs of inflammation and fibrosis (Figure 6K and L).

Immunohistochemical observations

As can be seen in Figure 7A, the control group contains a well-defined typical Langerhans islet with positive insulin staining, surrounded by acinar cells with negative insulin staining. Comparatively, STZ diabetic rats showed decreased

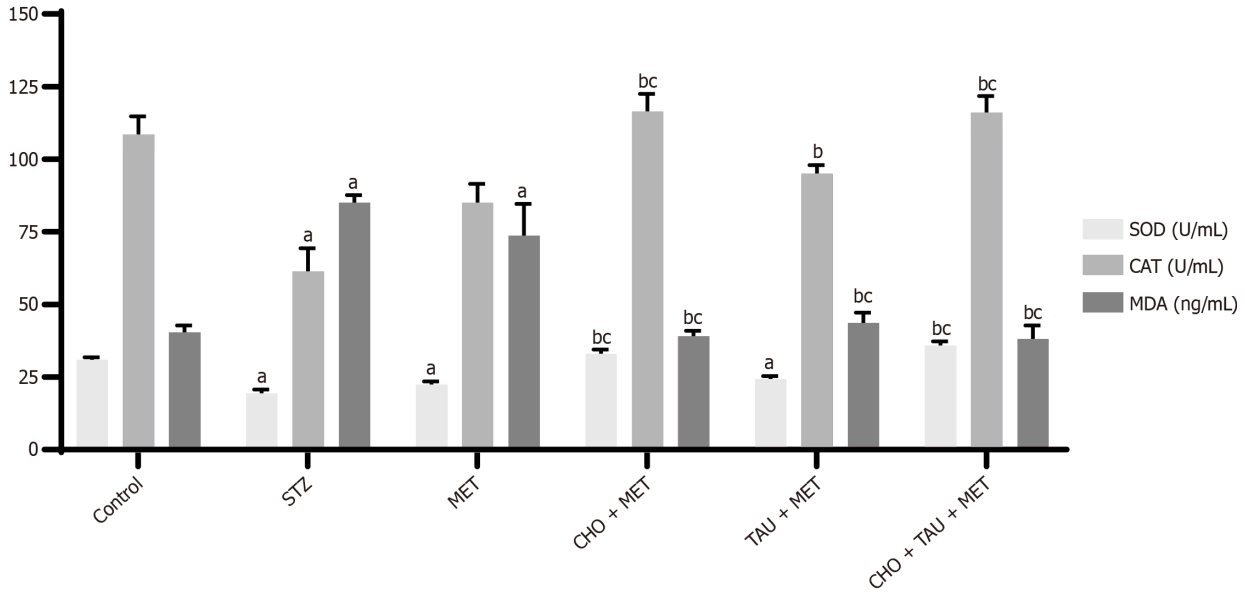

Figure 5 Measurement of antioxidant biomarkers in the control group and other experimental animals. The data, presented as means ± SE (n = 6), indicate significant differences at $P < 0.05$. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; TAU: Taurine; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde. [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [c]Significance compared to the metformin-treated group.

positive (brown) and very weak insulin staining in their islets and markedly decreased mass of their islets (Figure 7B). Also, in the MET-treated group, weak insulin staining of the islets with a marked decrease in islet mass was observed, which was relatively higher than in the STZ group (Figure 7C). The CHO + MET-treated group showed a significant restoration of insulin staining on the islet, whereas treatment with TAU and CHO + TAU groups (Figure 7D) exhibited a more substantial recovery of staining intensity with increased area and number of stained islets (Figure 7E and F).

In comparison with the control, the relative stain intensity was reduced by 32.7 and 24.3% in the STZ and MET groups, respectively (Figure 8A). Also, the number and size of islets were significantly lower than those of the control group (Figure 8B). Meanwhile, TAU exerted prominent restoration of insulin staining of the islets with restored and enhanced islets staining intensity with regular outlining comparable to control (Figure 8A). Also, the number of stained islets was increased to 5 stained areas per field, whereas the area was increased to (7102 μm^2). Eventually, rats supplemented with CHO and TAU experienced a significant rise in relative insulin stain intensity by 13.8% (Figure 8A), with greater stained islet size (8747 μm^2; Figure 8B).

DISCUSSION

T2DM exhibits a progressive nature with evolving pathological implications over time. Initial monotherapy becomes less effective, thereby requiring combination therapy for efficient management[51]. While several studies have introduced the beneficial effects of dietary supplements (TAU, CHO)[18,29], our study specifically aimed to explore their synergistic effects in enhancing the effectiveness of MET for T2DM treatment.

To experimentally validate the induction of T2DM, the group treated with STZ demonstrated a notable and significant rise in the mean random blood glucose levels throughout the six-week experiment. This increase was observed in comparison to the control non-diabetic group. Further evidence supporting these findings comes from histological examination, through which T2DM was evident by the cytotoxic effects of STZ on pancreatic β-cells.

Nevertheless, the proposed treatment groups exhibited a significant reduction in mean random blood glucose compared to the STZ and MET-treated groups throughout the experimental period. As seen in Figure 9, both supplements exerted an effect alone in counteracting the STZ impact on the glycemic profile. The effect of CHO on blood glucose was prominent and was in alignment with a previous report by Santos and Vianna, who reported the administration of 12.5 $\mu g/kg^{-1}$ of CHO to Wistar rats led to a reduction of 40% in blood glucose[52]. Also, Yousefi Rad *et al*[53] have reported the beneficial effect of CHO on the control of blood glucose levels as well as insulin resistance in T2DM patients. This could be attributed to the stimulating effect of CHO on insulin receptors in beta cells, leading to insulin gene expression, as well as its role in promoting intestinal transport of glucose molecules[54]. Aside from this, calcitriol, the active form of CHO, participates in calcium absorption, an essential step in insulin release from beta cells[55].

Additionally, TAU also influenced blood glucose levels when combined with MET. This effect was also evident in earlier preclinical studies and clinical trials. For instance, in a trial by Maleki *et al*[14], TAU demonstrated a significant reduction in fasting blood glucose, insulin, and HOMA-IR. The cause of this may be linked to the reported effect it has on the liver, in which it reduces the phosphorylation of the insulin receptor β subunit (IRβ) and hepatic Akt, thereby reducing glucose production as well as glucagon activity[56,57]. Also, TAU was reported to improve beta-pancreatic cell

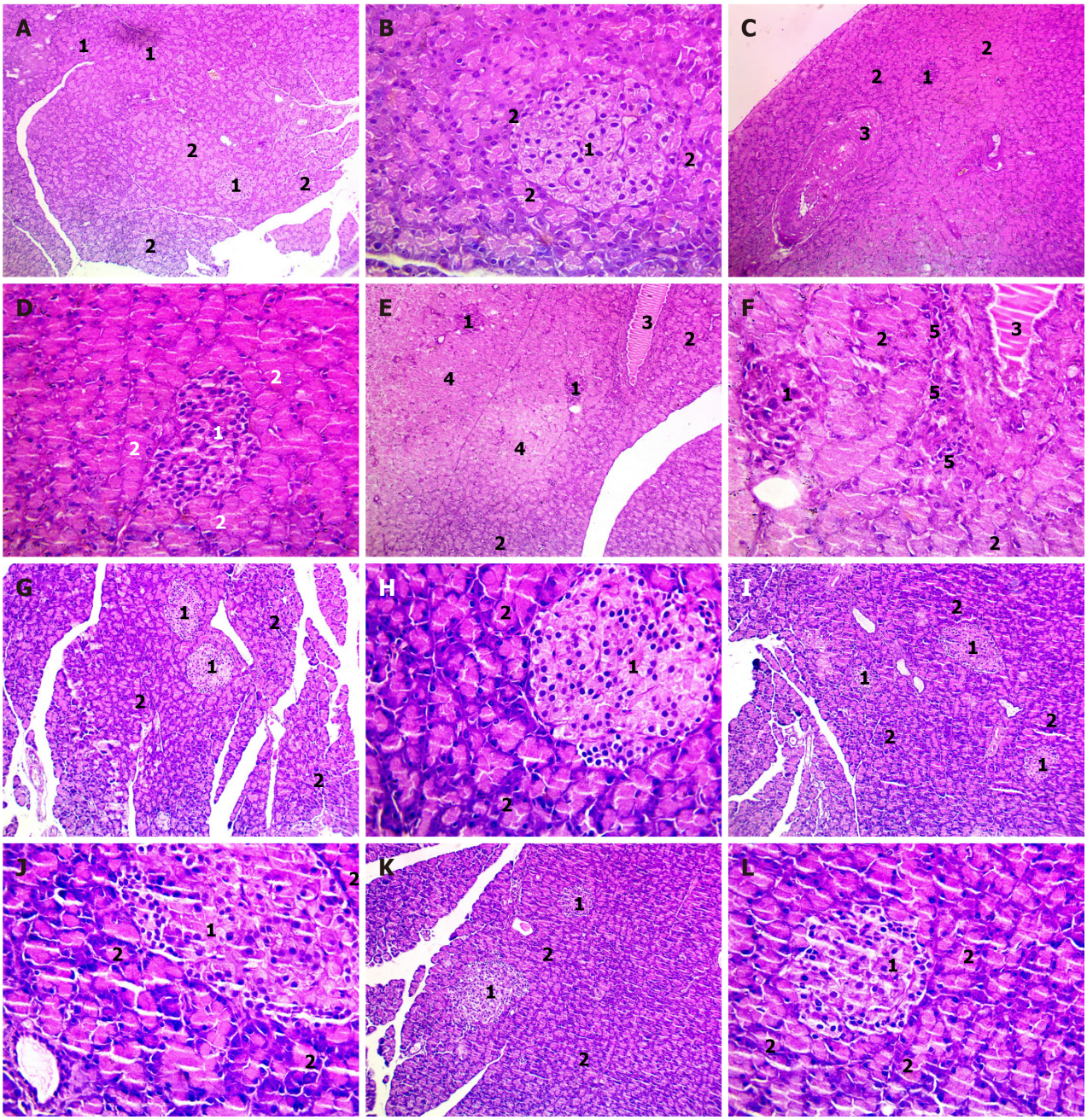

Figure 6 Examination of histopathological changes in rat pancreatic sections using HandE staining. A-I: Photomicrographs of different magnifications showing pancreatic sections of control group (A and B), Streptozotocin diabetic group (C and D), metformin (MET)-treated group (E and F), cholecalciferol (CHO) + MET-treated group (G and H), taurine (TAU) + MET-treated group (I and J), and TAU + CHO + MET-treated group (K and L). [1]Normal structure of the islet of Langerhans. [2]Normal structure of acinar cells. [3]Normal structure of a thickened blood vessel. [4]Normal structure of areas of degenerative changes in acinar cells. [5]Normal structure of perivascular inflammation.

function and insulin clearance while enhancing AMPK activity in muscles that help maintain glucose hemostasis[18,58]. Also, the immunohistochemical localization of insulin antibodies supported the microscopic H and E histopathological results of pancreas tissues (Figure 8). TAU treatment prominently restored insulin staining and islet size, with regular outlining comparable to the control. These findings were in line with those of Arany *et al*[18], who reported the increased pancreatic islet mass in TAU-supplemented rats, which could explain the augmented stain intensity for rats co-supplemented with CHO and TAU. Eventually, TAU and CHO held great potential on the glycemic profile, and as shown in Figures 1 and 9, the synergy resulted in a significant reduction in blood glucose levels, serum insulin, HOMA-IR, and HbA1C comparable to those of non-diabetic rats.

Considering the lipid profile evaluation, it can be concluded that MET intake contributes to ameliorating STZ effects on HDL levels (Figure 2). These effects stem from control over the hyperglycemia-induced ROS generation. As a result, the formation of advanced glycation end-products is reduced, and consequently, oxidative stress and inflammation[59]. The CHO supplement, on the other hand, was the primary supplement that affected the lipid profile in a positive way, specifically through reducing LDL-C and TG. However, CHO is thought to directly reduce lecithin-cholesterol acyltransferase activity, directly influencing TGs, total cholesterol, and LDL cholesterol. Also, as it enhances calcium absorption,

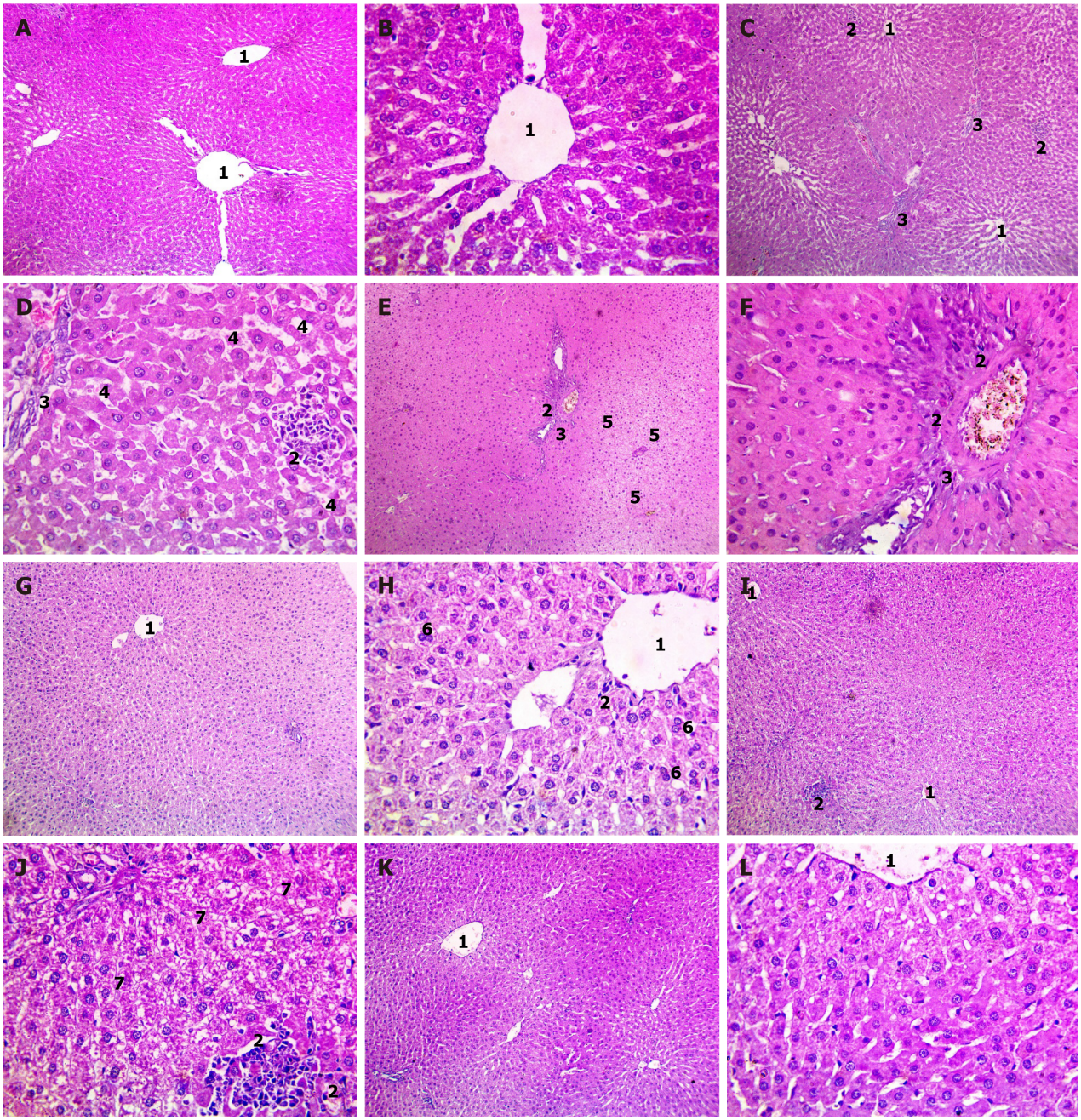

Figure 7 Examination of histopathological changes in rat liver sections using HE staining. Photomicrographs of different magnifications showing pancreatic sections of the control group (A and B), Streptozotocin diabetic group (C and D), metformin (MET)-treated group (E and F), cholecalciferol (CHO) + MET-treated group (G and H), taurine (TAU) + MET-treated group (I and J), and TAU + CHO + MET-treated group (K and L). [1]The central vein. [2]Lobular inflammation. [3] Fibrous portal expansion. [4]Sinusoidal dilatation. [5]Hydropic degeneration. [6]Preserved hepatocyte arrangement with binucleation of some hepatocytes. [7]Hydropic degeneration of hepatocytes with areas of necrosis indicated by lost nuclei.

CHO can be indirectly implicated by impacting cholesterol absorption and hepatic bile acid conversion by decreasing fat absorption[60].

MET treatment appears to be the contributor to the reversal of STZ effects on HDL levels (Figure 2). Treatment with CHO, TAU, MET, and their combination resulted in significant improvements in levels of lipid markers, supporting Malek *et al*[61] and Asemi *et al*[62], who revealed that CHO, TAU, and MET together have therapeutic roles in modification of lipid abnormalities *via* lipid peroxidation prevention and reduction of the atherosclerotic inflammatory process, which in turn may help to prevent the onset of plaque and all subsequent disorders.

Elevated levels of ALT and AST in the STZ group are related to insulin resistance and fatty liver, while elevated LDH levels interfere with glucose metabolism and insulin production and play a role in increasing cytosolic free ATP and Ca^{2+} levels within islet beta cells[63]. These effects were exacerbated by MET treatment, which is in line with previous hepatotoxicity reports[64]. It is clear from Figure 3 that the hepatocellular necrosis induced by STZ was reversed by CHO, as evidenced by the decline in AST, ALT, and LDH levels. The findings were in accordance with those reported by Lorvand Amiri *et al*[65], who found that vitamin D led to a significant reduction in liver aminotransferase levels. Additionally,

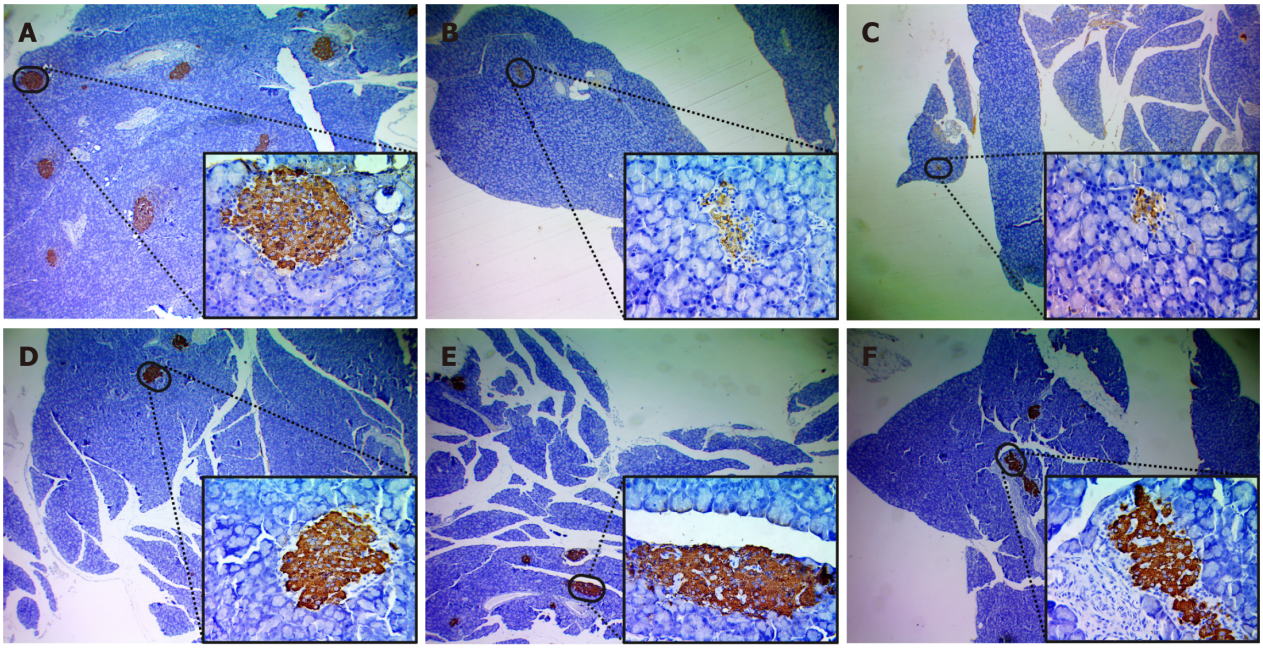

Figure 8 Photomicrographs from immunohistochemistry assays of insulin staining for the pancreas in the six study groups at a Magnification power of × 400. A: Control group; B: Streptozotocin group; C: metformin (MET)-treated group; D: Cholecalciferol (CHO)+ MET- treated group; E: Taurine (TAU)+ MET- treated group; F: CHO + TAU + MET-treated group. The brown color indicates positive insulin staining (diaminobenzidine).

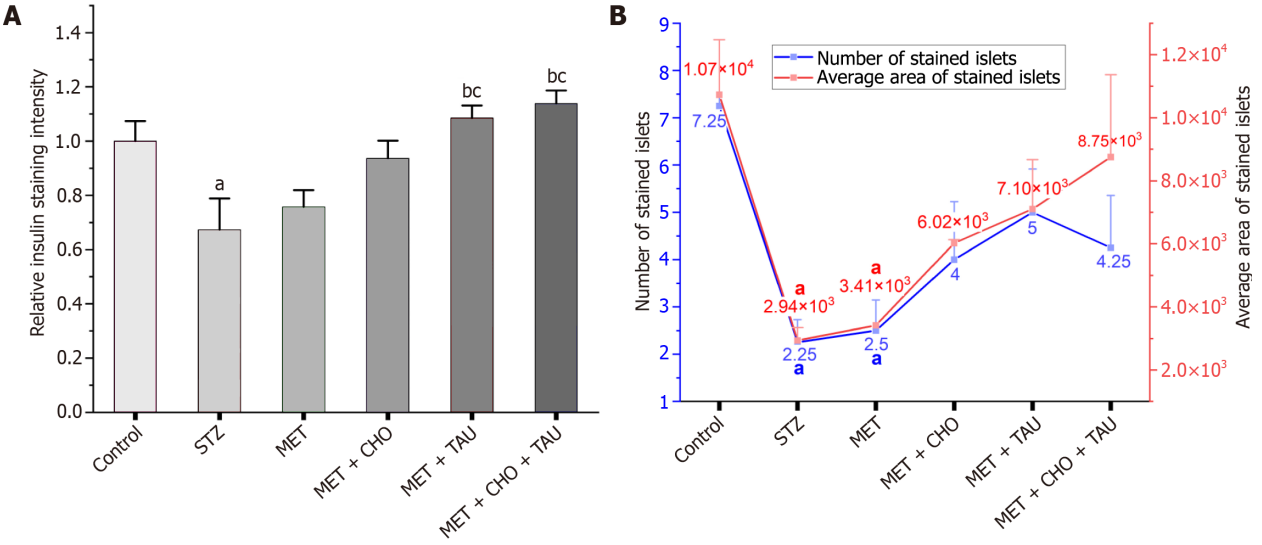

Figure 9 Analysis of immunohistochemical results. A: The relative islet staining intensity of stained islets; B: The number and area of the stained islets. The data, presented as means ± SE (n = 6), indicate significant differences at $P < 0.05$. STZ: Streptozotocin; MET: Metformin; CHO: Cholecalciferol; [a]Significance compared to the control group. [b]Significance compared to the Streptozotocin diabetic group. [c]Significance compared to the metformin-treated group.

histopathological examination of liver tissues in the STZ diabetic group revealed lobular inflammation, sinusoidal dilatation, and fibrous portal expansion. The combination therapy (CHO + TAU + MET) demonstrated preserved hepatocyte arrangement with no inflammation or fibrosis, surpassing the effects of MET alone.

A significant part of the protective action of CHO is to enhance the activities of key antioxidant enzymes, SOD, and CAT (Figure 4). Also, CHO exerted a significant negative effect on MDA levels, which were in alignment with a meta-analysis of clinical data[66]. This can be explained by the fact that the CHO supplement interacts with Vitamin D receptors, which in turn activates the Vitamin D Response Elements in the nucleus, leading to the transcription of antioxidant genes[66]. Moreover, CHO also stimulates antioxidant pathways such as nuclear factor erythroid-2-related factor 2 (Nrf2)/Keap1[67] and ROS-scavenging enzymes (Glucose-6-phosphate dehydrogenase)[68] while reducing ROS-generating enzymes (NADPH oxidase)[69], thus indirectly reducing the risk of oxidative damage.

Despite the significance of preclinical studies, animal models offer limited predictive power[70]. This is attributed to the biological and physiological differences due to the discrepancy in how disease manifests and how treatment responds between rodents and humans. In contrast to animal models, the high genetic variation in drug metabolism and immune

activity between individuals potentially affects estimates of treatment efficacy and toxicity[71]. Even so, it may still be necessary to conduct animal studies of TAU interaction prior to clinical studies to detect any potential pharmacokinetic interactions between the suggested combination therapy. Also, more parameters can be included in future glucose-insulin dynamic studies to directly assess insulin sensitivity (hyperinsulinemic-euglycemic clamp) or indirectly (glucose tolerance tests). It will, therefore, be crucial to conduct dose-finding trials to identify effective and well-tolerated dosages of CHO and TAU to minimize MET's adverse effects and optimize therapeutic outcomes in patients with diabetes. Furthermore, conducting clinical trials on diabetic patients can offer insight into the long-term benefits, safety profiles, and complications associated with the proposed co-therapy.

CONCLUSION

The study highlighted hyperglycemia, oxidative stress, and insulin resistance as contributors to pathological tissue changes and abnormal biochemical parameters in diabetic rats. MET, while possessing mild antioxidant and anti-inflammatory properties, was deemed insufficient to prevent diabetic complications. The combined therapy involving MET, CHO, and TAU proved more effective in mitigating apoptosis and ameliorating the biochemical, histological, and immunohistochemical changes associated with diabetes. This supplementation approach effectively restored these parameters to normal levels. The current study findings demonstrate the importance of a synergistic therapeutic approach to alleviating diabetes-related complications through treating oxidative stress-induced tissue damage and achieving metabolic balance.

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FOOTNOTES

Author contributions: Attia MaS and Ayman F contributed equally to this experimental study as they are co-first authors of this manuscript. Attia MaS, Ayman F and Diab AA designed the study; Attia MaS and Ayman F conducted the research experiments, Attia MaS and FA performed data collection and statistical analysis; Attia MoS, Ayman F, Attia MaS wrote the original draft; Attia MoS, Ayman F and Yahya G rewrote and revised the manuscript after editing; Diab AA, Zahra MH, Yahya G and Khalil MMI provided supervision; all authors have read and agreed to the published version of the manuscript.

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SYSTEMATIC REVIEWS

Safety of teplizumab in patients with high-risk for diabetes mellitus type 1: A systematic review

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Novelty: Grade B, Grade B, Grade B, Grade C, Grade C

Creativity or Innovation: Grade B, Grade B, Grade B, Grade C, Grade C

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Abstract

BACKGROUND

The incidence of diabetes mellitus type 1 (DM1) has been rising worldwide because of improvements in diagnostic techniques and improved access to care in countries with lower socioeconomic status. A new anti-CD4 antibody, Teplizumab, has been shown to delay the progression of DM1 and is the only medication approved for this indication. However, more information is needed about the safety profile of this drug.

AIM

To identify the odds ratios (OR) of systems-based adverse effects for Teplizumab when compared to Placebo.

METHODS

An extensive systematic review was conducted from the inception of the medication until December 31, 2023. All clinical trials and studies that evaluated

Teplizumab *vs* placebo were included in the initial review. The study protocol was designed using Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines guidelines and was registered in PROSPERO (ID: CRD42024496169). Crude OR were generated using RevMan Software version 5.4.

RESULTS

After screening and review, 5 studies were selected to determine the risk of adverse effects of teplizumab compared to placebo. A total of 561 patients were included in the study population. Total adverse effects and system-based adverse effects were studied and reported. We determined that patients receiving Teplizumab had a higher risk of developing gastrointestinal (GI) (OR = 1.60, 95%CI: 1.01-2.52, P = 0.04), dermatological (OR = 6.33, 95%CI: 4.05-9.88, P < 0.00001) and hematological adverse effects (OR = 19.03, 95%CI: 11.09-32.66, P < 0.00001). These patients were also significantly likely to have active Epstein-Barr Virus infection (OR = 3.16, 95%CI: 1.51-6.64, P < 0.002). While our data showed that patients receiving Teplizumab did have a higher incidence of total adverse effects *vs* placebo, this finding did not reach statistical significance (OR = 2.25, 95%CI: 0.80-6.29, P = 0.12).

CONCLUSION

Our systematic review suggests that Teplizumab patients are at risk for significant adverse effects, primarily related to GI, dermatological, and hematological systems. The total adverse effect data is limited as study populations are small. More studies should be conducted on this medication to better inform the target population of potential adverse effects.

Key Words: Teplizumab; Diabetes mellitus type 1; Adverse effects; Monoclonal antibody; Systematic review

Core Tip: Teplizumab is an anti-CD4 antibody that has been shown to delay the clinical onset of diabetes mellitus type 1. Our systematic review evaluates the incidence of total adverse effects and a systems-based risk reported in various clinical trials. Our review shows an increased incidence of adverse effects in patients receiving Teplizumab compared to placebo, but this risk is not statistically significant. There is also a statistically significant increased risk of gastrointestinal, dermatological, and hematological adverse effects in the Teplizumab group compared to placebo. More trials need to be conducted to understand better the risk of side effects related to Teplizumab.

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INTRODUCTION

Diabetes mellitus type 1 (DM1) is an autoimmune disease that has been rising in incidence around the world[1]. In 2021 alone, the estimated number of cases was 8.5 million, projected to increase by about 2% to 4% over the next 20 years[1,2]. While the exact cause of this rise is unknown, a reasonable assumption is the improvements in detection in countries of lower socioeconomic status as they gain access to better resources. The condition, characterized by autoimmune destruction of the insulin-producing pancreatic beta cells, often manifests in early childhood or during puberty (10 to 14)[3]. There is strong evidence to support the idea that young patients with DM1 often demonstrate clinical antibodies for many years prior to developing clinical symptoms[4]. This time frame is key for preventative therapies that can ideally halt further immune destruction. As a result, the Endocrine Society and the American Diabetes Association have released a staging system for classifying patients with DM1 based on a combination of immune response and clinical symptoms[5]. This classification is shown in Figure 1.

In the past, significant research has been performed on human leukocyte antigen proteins that conferred a genetic risk for DM1, hoping to identify a potential cure for this illness[6]. Even in high-risk patients, there wasn't a reliable method to predict conversion from asymptomatic to a symptomatic stage. More recently, the focus has shifted towards immunological markers that directly cause islet cell destruction. One of the key drivers of an autoimmune response is CD4+ and CD8+ T-cells that mark beta cells for destruction in patients with DM1. The CD3 surface protein of T-cells has been a popular target in autoimmune conditions, with various monoclonal antibody agents showing excellent efficacy against this protein in clinical trials[7]. The most promising one for patients with a risk of DM1 is Teplizumab, which has been shown to slow progression in patients in stage 2 of DM1[8]. This agent is a modified version of the OKT3 anti-CD3 antibody approved for transplant patients to prevent rejection[9]. The original OKT3 antibody was not extensively used due to its severe adverse effect profile of flu-like symptoms and cytokine storm reaction[10]. Thus, a newer version of the antibody was created by modifying the binding protein to maintain the anti-CD3 properties without causing an adverse reaction[11]. Initial Phase I studies of this new antibody showed that the autoimmune response was sufficiently

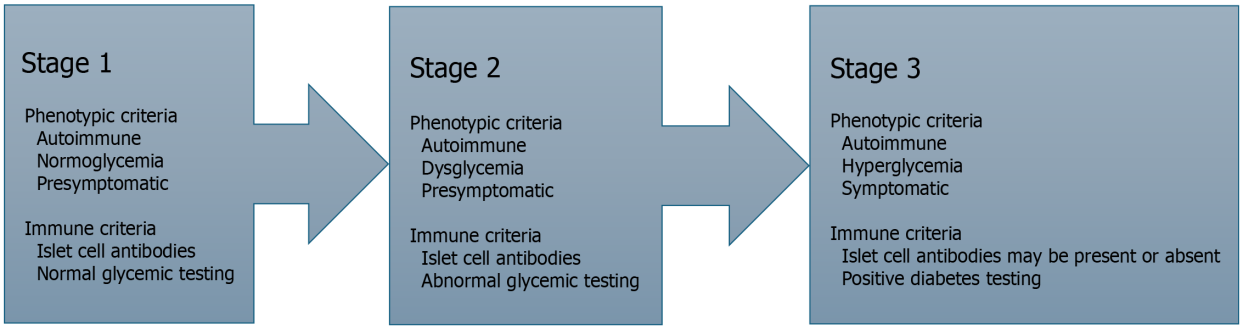

Figure 1 Staging system for diabetes mellitus type 1.

suppressed, and insulin production was maintained in patients over the course of 2 entire years[12].

As a result, the United States Food and Drug Administration (FDA) has approved Teplizumab for use in this target population. Due to this fast-tracked process, more significant data must be collected on the adverse effects of this medication. In this systematic review, we aim to study the incidence and severity of various side effects of Teplizumab as reported in clinical trials.

MATERIALS AND METHODS

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. A systematic literature review was conducted from the inception of the medication until December 31, 2023.

Search strategy

We searched the databases of PubMed, Scopus, and Cochrane Library for titles using the term 'teplizumab.' Only articles in the English language were selected for screening. The search dates from the inception of the medication in 2009 until December 31st, 2023. We also manually scanned ClinicalTrials.gov for clinical trials that were in progress or recently completed. The search approach and design are highlighted in Figure 2. The protocol of this systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO ID: CRD42024496169).

Eligibility criteria

All clinical trials that met the following criteria were included in this review: (1) Study design: randomized blinded; (2) Reported adverse effects of Teplizumab *vs* that of Placebo; and (3) Addressed specific adverse effects including but not limited to severe adverse effects, gastrointestinal (GI), and dermatological and hematological adverse effects. All observational studies, cohort studies, review articles, case reports, editorials, conference abstracts, and commentary articles were excluded from our study.

Study selection

All selected articles were compiled using Rayyan Software[13]. Four authors (Venkata Buddhavarapu, Gagandeep Dhillon, Harpreet Grewal and Pranjal Sharma) independently screened articles after eliminating duplicates to ensure that appropriate manuscripts were selected for final review. Two authors (Venkata Buddhavarapu and Gagandeep Dhillon) reviewed these manuscripts for eligibility in the final evaluation. A third party addressed any discrepancies or conflicts *via* arbitration.

Outcome measures

The primary outcome was the incidence of severe adverse effects in patients receiving Teplizumab when compared to placebo. The secondary outcome was systems-based adverse effects, including GI, dermatological, and hematological adverse effects.

Data extraction and quality assessment

The included articles underwent a thorough evaluation of the full text by two independent reviewers (Venkata Buddhavarapu and Gagandeep Dhillon) to determine eligibility. The articles were assessed using the Population, Intervention, Comparison, Outcomes tool for systematic reviews[14]. The patients selected were those in stage 2 of DM1 who were deemed to be candidates for the intervention, Teplizumab. Studies included matches these patients against placebo-controlled cohorts. The primary outcome was to evaluate the incidence of severe and specific adverse effects related to GI, dermatology, and hematology systems. The studies were evaluated in a blinded manner to control against bias using Rayyan Software. An independent third reviewer (Harpreet Grewal) resolved conflicts following the selection process.

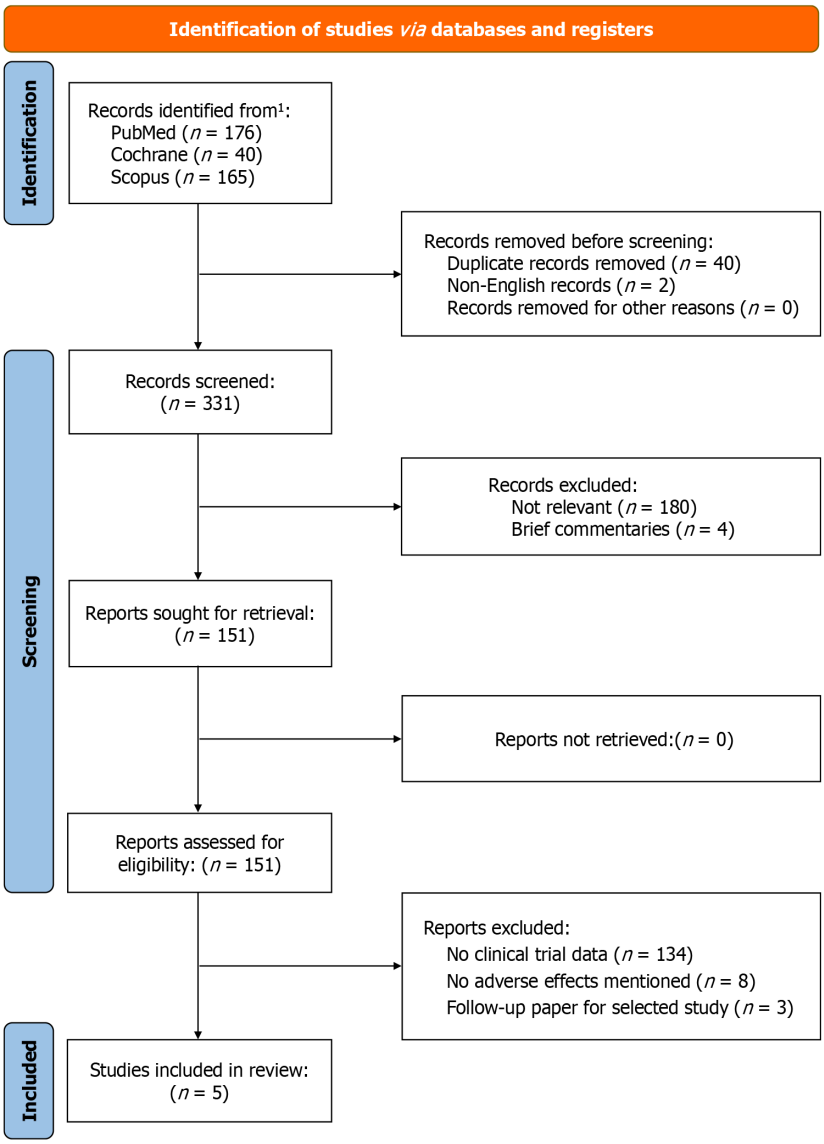

Figure 2 Preferred reporting items for systematic reviews and meta-analyses diagram for inclusion of studies discussing the adverse effects of teplizumab. [1]No automation tools used in record search.

Statistical analysis

The primary outcomes were analyzed using the RevMan software version 5.4 to generate all results. Crude odds ratios (OR) were calculated using a Random-effects model for each adverse outcome measured with 95%CI. A *P* value less than 0.05 was considered to be statistically significant. Forest plots were generated to determine the effect of each study. Study heterogeneity was determined using Cochrane *Q* and I^2 statistics, with a low-level heterogeneity being defined at I^2 less than 20%.

Risk of bias assessment

Risk of bias assessment was done by two reviewers (Venkata Buddhavarapu and Harpreet Grewal) using the Cochrane Database Risk of Bias Tool[15]. All studies were evaluated as 'high', 'low' or 'unclear' risk of bias using established domains of risk (Figure 3). All five studies had an overall low risk of bias with only Herold *et al*[16] in 2013[16], showing an area of concern due to being an open-label study. The authors expressed that the study results were randomized, which mitigated some of the risks.

RESULTS

After screening 331 search results from three databases, 151 articles were selected for secondary review. After reviewing all articles based on the eligibility criteria, a total of. Five studies were selected for final inclusion. These studies included 561 patients in the study group and 298 patients in the control group. The studies were conducted in various countries, including the United States, India, and the United Kingdom. A more detailed outline of the general characteristics of the

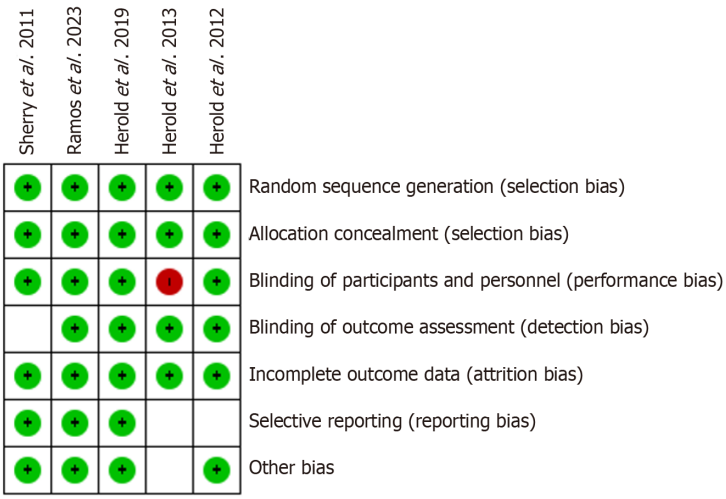

Figure 3 Risk of bias assessment using Cochrane's risk of bias tool. Green plus: Low risk; Red minus: High risk; Empty space: Unclear risk.

studies is shown in Table 1[16-20].

Table 1 General characteristics of studies

Ref.	Locations	Study design	No. of participants in teplizumab group	No. participants in placebo group	Mean age in teplizumab group	Mean age in placebo group	Male sex% teplizumab	Male sex% placebo
Ramos *et al*[17], 2023	United States, Canada, Europe	RCT	217	111	12	12.3	54.8	62.2
Herold *et al*[18], 2019	United States, Canada, Australia, Germany	RCT	44	32	14	13	57	53
Herold *et al*[16], 2013	United States	RCT	52	25	12.7	12.3	53.8	64
Herold *et al*[19], 2013	United States	RCT	33	27	12.9	12	52	63
Sherry *et al*[20], 2011	United States, Canada, Europe	RCT	209	99	18.5	18.2	62.8	62.2

RCT: Randomized controlled trial.

Odds ratio for adverse effects

The OR analysis showed that while total adverse effects were higher in the Teplizumab groups when compared to Placebo, this finding was not significant (OR = 2.25, 95%CI: 0.80-6.29, P = 0.12). The heterogeneity was low, with an I^2 of 9%. This is shown in Figure 4A.

GI adverse effects, which included nausea, vomiting, diarrhea, and abdominal pain, were more associated with Teplizumab as compared to placebo (OR = 1.60, 95%CI: 1.01-2.52, P = 0.04). Heterogeneity was low at I^2 = 5%. Figure 4B demonstrates this association.

There was a strong association with Teplizumab for both dermatological side effects (OR = 6.33, 95%CI: 4.05-9.88, P < 0.00001) and lymphopenia when compared to placebo (OR = 19.03, 95%CI: 11.09-32.66, P < 0.00001). Heterogeneity was also low, with I^2 = 32% and I^2 = 44%, respectively. Figure 4C and D demonstrate this.

Teplizumab was also shown to activate Epstein-Barr virus (EBV) infections as demonstrated by detectable EBV viral loads in those receiving Teplizumab (OR = 3.16, 95%CI: 1.51-6.64, P < 0.002, I^2 = 39%)

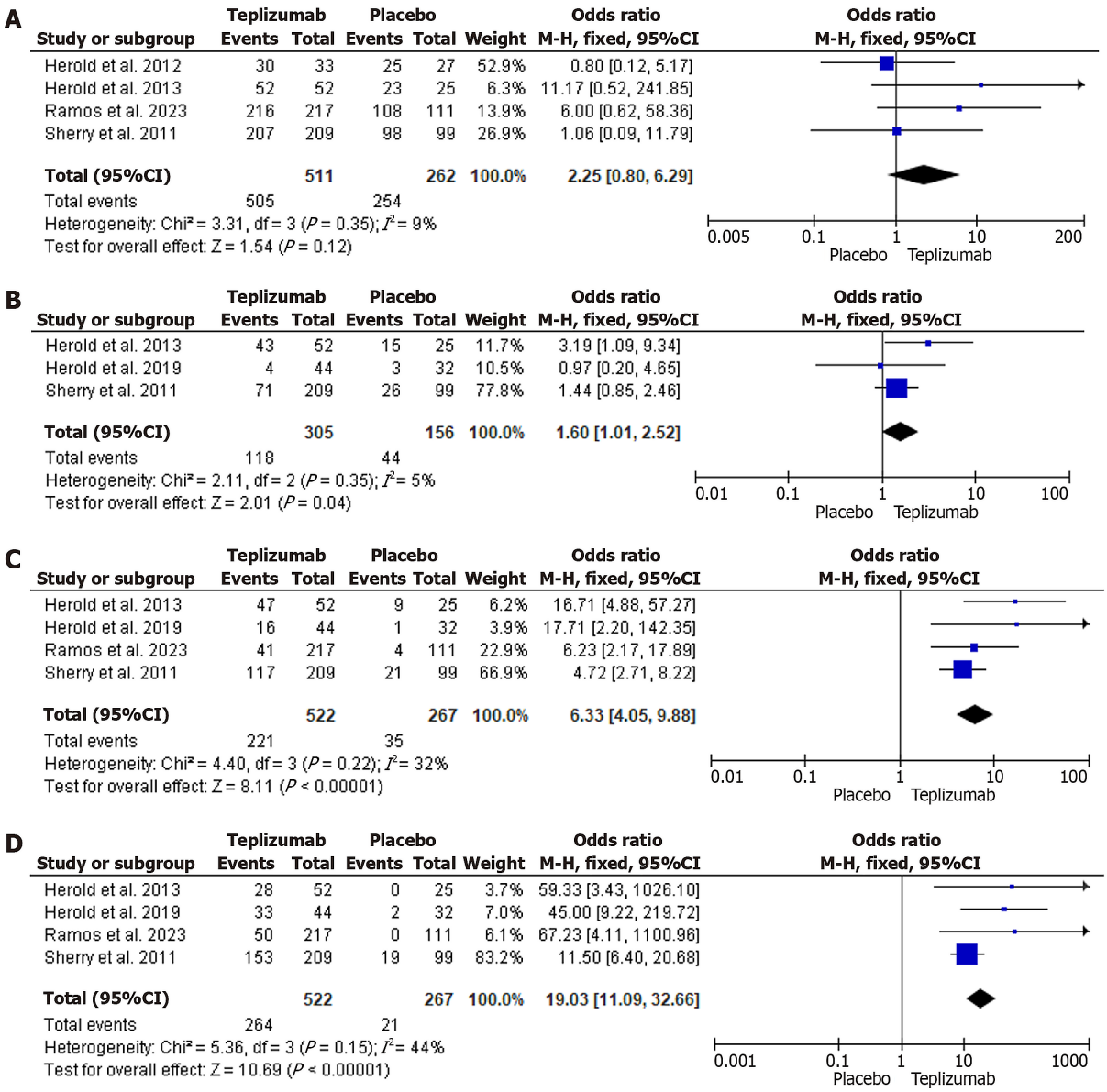

Figure 4 Teplizumab *vs* placebo. A: Pooled odds ratio (OR) of total adverse effects in teplizumab *vs* placebo; B: Pooled OR of gastrointestinal adverse effects in teplizumab *vs* placebo; C: OR of dermatological adverse effects in teplizumab *vs* placebo; D: OR of lymphopenia in teplizumab *vs* placebo.

DISCUSSION

Teplizumab has been shown to delay the symptomatic progression of patients with high risk for DM1[21] and is the only medication currently approved by the FDA for this indication[22]. This is achieved by its ability to alter activated T-cells, leading to a decreased immune response[23]. Due to its strong affinity, this response is maintained over an extended period, leading to this delay in progression[23]. Studies have shown that this medication is also associated with a certain side effect profile. While traditional anti-CD3 antibodies led to severe reactions such as fevers, chills, and headaches, the current versions of this antibody, such as Teplizumab, have an altered Fc receptor, which seems to lead to this particular reaction[24]. Due to this change, the side effect profile also appears to be different and has yet to be studied.

Our systematic review reveals that patients are at a much higher risk of developing GI, dermatological, and hematological adverse effects when taking this medication. GI effects included mainly nausea and emesis, the mechanism behind which is not well described. The control group also had many of these effects, an interesting factor related to the placebo. The dermatological adverse effects mainly include rashes and self-limited soft tissue infections. The mechanism behind this is not well described. Still, it could result from a change in the levels of activated CD4 T-cells and a decrease in interferon-gamma, which have been associated with skin conditions[25]. While the shift in ratios from predominantly CD4 T-cells to CD8 memory T-cells is the main factor in lymphopenia, this effect is transient, and the counts are restored to normal shortly after completing the treatment course[26]. During this phase, patients are more susceptible to all infections, with many patients experiencing respiratory and Upper respiratory infection symptoms. Teplizumab has also been shown to block the effect of regulatory and activated CD4 T-cells directly, which are responsible for the immune response against viral infections[21]. This effect explains the dramatic increase in EBV infections in patients receiving this

medication.

Our systematic review is the first study to evaluate Teplizumab's pooled risk of system-specific adverse events from the limited clinical data available. Liu *et al*[27] evaluated both Teplizumab and another CD3 antibody Otelixizumab and also found a higher incidence of total adverse effects but failed to show statistical significance. As their analysis included both drugs, a direct comparison cannot be made with our study. A recent systematic review by Nourelden *et al*[28] also found lymphopenia and skin disorders to be the most common adverse effects. Still, it did not go into further detail about the incidence and risk of these findings.

The Strengths of our paper mainly involve the significance of the adverse events described with low heterogeneity. The findings are consistent across all clinical trials and should be predictable in any newer studies. The main limitation is that the number of patients enrolled in these studies remains low, which may lead to overestimating adverse effects, especially in systems with lower incidence, such as EBV infections and dermatological events. Additionally, the papers in our review were inconsistent in describing adverse events as systems and often included individual events rather than patients. Some papers described adverse effects of higher incidence, which may make the medication seem worse than predicted. More studies need to be done to describe all potential adverse effects of Teplizumab further. These are currently underway[29].

Teplizumab promises to be an exciting new development in type 1 diabetes and shows great promise in reducing the incidence of this condition in the target group. The type of adverse effects with higher risk may affect compliance with this treatment, especially in the pediatric population, which is more sensitive to these issues and should be weighed against the benefit of the medication. Teplizumab is also being studied in patients with active DM1 who have some preserved pancreatic beta-cell function to assess for reversal[30]. These studies should expand our knowledge of the efficacy and safety of this medication.

CONCLUSION

Patients on Teplizumab have a much higher risk of developing GI, dermatological, and hematological adverse effects when compared to placebo. These patients are also more likely to have EBV activation when receiving this medication. These adverse effects can lead to poor compliance with treatment protocols, especially in the pediatric population. More studies are needed to identify better the mechanisms behind these effects and other significant adverse effects.

FOOTNOTES

Author contributions: Buddhavarapu V, Dhillon G, Grewal HS and Sharma P designed the systematic review, acquired the data and interpreted the data; Buddhavarapu V, Kashyap R and Surani S drafted the article and made critical revisions; All authors were responsible for the final approval of the version submitted for publication.

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META-ANALYSIS

Circulating glycated albumin levels and gestational diabetes mellitus

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Abstract

BACKGROUND

Gestational diabetes mellitus (GDM) is characterized by glucose intolerance that is first diagnosed during pregnancy, making it the most common complication associated with this period. Early detection and targeted treatment of GDM can minimize foetal exposure to maternal hyperglycaemia and subsequently reduce the associated adverse pregnancy outcomes. Previous studies have inconsistently suggested that the level of glycated albumin (GA) might predict GDM.

AIM

To review and synthesize existing evidence to evaluate the relationship between GA levels and the development of GDM.

METHODS

We sought to compare GA levels between GDM and control groups in this meta-analysis by systematically searching the Web of Science, PubMed, Cochrane Library, and Embase databases for articles published up to June 2023. The analysis utilized the weighted mean difference (WMD) as the primary metric. The data were meticulously extracted, and the quality of the included studies was assessed. Additionally, we conducted a subgroup analysis based on study region and sample size. We assessed heterogeneity using I^2 statistics and evaluated publication bias through funnel plots. Additionally, trim-and-fill analysis was employed to detect and address any potential publication bias.

RESULTS

The meta-analysis included a total of 11 studies involving 5477 participants, comprising 1900 patients with GDM and 3577 control individuals. The synthesized results revealed a notable correlation between elevated GA levels and increased susceptibility to GDM. The calculated WMD was 0.42, with a 95% confidence interval (95%CI) ranging from 0.11 to 0.74, yielding a P value less than 0.001. Concerning specific GA levels, the mean GA level in the GDM group was

12.6, while for the control group, it was lower, at 11.6. This discrepancy underscores the potential of GA as a biomarker for assessing GDM risk. Moreover, we explored the levels of glycated haemoglobin (HbA1c) in both cohorts. The WMD for HbA1c was 0.19, with a 95%CI ranging from 0.15 to 0.22 and a *P* value less than 0.001. This observation suggested that both GA and HbA1c levels were elevated in individuals in the GDM group compared to those in the control group.

CONCLUSION

Our meta-analysis revealed a substantial correlation between elevated GA levels and increased GDM risk. Furthermore, our findings revealed elevated levels of HbA1c in GDM patients, emphasizing the significance of monitoring both GA and HbA1c levels for early GDM detection and effective management.

Key Words: Glycated albumin; Gestational diabetes mellitus; Diabetes mellitus; Meta-analysis; Weighted mean difference

Core Tip: The aim of this investigation was to elucidate the relationship between glycated albumin (GA) levels and the development of gestational diabetes mellitus (GDM), a prevalent gestational complication characterized by glucose intolerance. Emphasizing the importance of early diagnosis and intervention, this study used a meta-analytical approach to consolidate existing evidence, thereby performing a comprehensive examination of GA as a potential biomarker for GDM. By aggregating data from diverse studies, this research not only corroborated the association of elevated GA levels with increased GDM risk but also highlighted the significance of glycated haemoglobin (HbA1c) in this context. Consequently, the findings advocate for the inclusion of GA and HbA1c monitoring in prenatal care practices as a means to enhance early detection and management strategies for GDM, aiming to mitigate the adverse outcomes associated with this condition.

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INTRODUCTION

Gestational diabetes mellitus (GDM) involves a range of abnormal glucose metabolism observed in pregnant women. It is defined as hyperglycaemia first diagnosed during pregnancy, typically in the second or third trimester, and is one of the most frequent medical complications associated with pregnancy[1]. GDM affects approximately 14.8% of pregnant women in China and is a considerable public health concern[2]. Indeed, it has been suggested that one in every six pregnancies worldwide is impacted by GDM[3]. The complications associated with GDM are numerous and can affect both the mother and foetus. Foetal complications include macrosomia, shoulder dystocia, newborn asphyxia, neonatal respiratory distress syndrome, neonatal hypoglycaemia, and premature birth. In contrast, maternal complications include preeclampsia, an increased risk of caesarean section, an increased risk of type 2 diabetes later in life, and a twofold increase in the risk of developing hypertension and ischaemic heart disease[4].

Research indicates that effective GDM treatment can decrease the incidence of short-term perinatal complications and enhance maternal quality of life[5]. Given these implications, it is crucial to systematically identify at-risk individuals and accurately diagnose GDM[6]. Currently, the oral glucose tolerance test (OGTT), conducted between the 24th and 28th weeks of gestation, is the standard diagnostic method for GDM, with universal screening recommended in populations with a high prevalence of type 2 DM (T2DM)[7]. However, the OGTT is cumbersome, time-consuming, and necessitates a fasting state[8], leading to interest in identifying reliable, easily measurable biomarkers that could supersede the traditional OGTT.

Glycated albumin (GA) is a primary precursor of advanced glycation end products and reflects mean glucose levels over the past 2-3 weeks[9]. GA, a specific glycated product of albumin, has emerged as a vital indicator of blood glucose control[10]. Importantly, it is not influenced by serum albumin levels, as it is expressed as a ratio to total serum albumin [11]. Thus far, serum GA has been proposed as a reliable, specific, and sensitive serological diagnostic test and marker to supersede haemoglobin (HbA1c) levels in diabetic patients with chronic kidney disease[12] owing to its independence from anaemia and associated treatments. Furthermore, relative to HbA1c levels, GA levels can more rapidly reflect changes or fluctuations in blood glucose levels, which is particularly advantageous for patients with wide blood glucose variations or those at an elevated risk for hypoglycaemia[13]. However, these benefits have yet to be confirmed in large-scale clinical trials and systematic meta-analyses. Therefore, a contemporary meta-analysis to consolidate these findings is warranted.

Motivated by this context, we performed the present study. Our goal was to explore the relationship between GA levels and GDM risk.

MATERIALS AND METHODS

Search strategy

We searched for articles investigating GA levels in GDM patients and control individuals in electronic databases, including Web of Science, PubMed, the Cochrane Library, and Embase. This search adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring transparency, accuracy, and comprehensiveness in the systematic review process[14]. We aimed to identify articles published up until June 2023. Our search strategy incorporated various combinations of the following medical subject headings: "diabetes, gestational", "gestational diabetes mellitus", "pregnancy-induced diabetes", and "glycated albumin". In addition to this electronic search, we manually searched the reference lists of selected articles to identify any further studies potentially eligible for inclusion. The detailed retrieval strategy is presented in Supplementary material.

Inclusion and exclusion criteria

To be included in the present meta-analysis, studies had to be observational studies, including prospective and retrospective cohort studies, case-control studies, and cross-sectional studies of women diagnosed with diabetes during pregnancy, with an explicit assessment of GA levels. The exclusion criteria were as follows: (1) Studies including women who were diagnosed with type 1 or 2 diabetes before pregnancy; (2) Laboratory studies, nonhuman research, letters, and review articles; and (3) Studies failing to report the women's GA levels and/or the incidence of GDM.

According to the selection criteria for our meta-analysis, the control group comprised pregnant women who were confirmed to not have GDM, either by a normal OGTT or by not observing any signs of gestational diabetes during pregnancy. The control individuals were carefully matched with the GDM patients in terms of age, body mass index, and gestational week to minimize potential confounding factors. Furthermore, we excluded studies in which the control subjects had any other form of diabetes or significant medical conditions that could interfere with GA levels.

Data extraction

The data were meticulously extracted using a standardized, predetermined data collection form. These data included: (1) Study characteristics (author, year, study design, and country); (2) Study groups (sample size); (3) Methods (GDM screening/diagnostic criteria); and (4) GA levels in GDM patients and control subjects.

Quality assessment

The methodological quality of the included studies was assessed utilizing the Newcastle-Ottawa Scale[15], a validated tool designed to evaluate the quality of nonrandomized studies in meta-analyses. This scale allows for the assessment of a study from three broad perspectives: The selection of the study groups, comparability of the groups, and ascertainment of either the exposure or outcome of interest. A maximum score of 9 points can be achieved, considering selection, comparability, the exposure (for case-control studies), or the outcome (for cohort studies). Studies scoring over 7 points are classified as high-quality studies.

Statistical analysis

Outcomes for continuous variables are expressed as weighted mean differences (WMDs) with 95% confidence intervals (CIs). Heterogeneity was evaluated using I^2 values, where values below 25% indicated low heterogeneity, values of 25% to 50% indicated moderate heterogeneity, and values exceeding 50% indicated high heterogeneity. For analyses with an $I^2 > 50\%$, a random-effects model was employed, whereas a fixed-effect model was used for analyses with an $I^2 \leq 50\%$. Subgroup analyses were conducted according to the country where patients were included and sample size. A sensitivity analysis was carried out to explore the robustness and stability of the study findings by excluding low-quality studies. A funnel plot was used to assess potential publication bias, with asymmetry in the plot serving as an indication of bias. All the statistical analyses were conducted using Stata (Stata SE, version 15).

RESULTS

Identification of studies

We identified 1036 studies using our search strategy. After deduplication, we screened titles and abstracts to exclude studies that were not relevant to our analysis. Subsequently, 11 studies[16-26] were found to meet the inclusion criteria. The flow chart of the study selection process is depicted in Figure 1.

Study characteristics

Adhering to the inclusion and exclusion criteria, a total of 11 articles involving 5477 patients diagnosed with GDM were included in our analysis. Patient characteristics and other pertinent information extracted from each study are summarized in Table 1. The quality assessment results indicated moderate quality across all 11 studies, as shown in Table 1. Notably, of the 11 studies selected, 5 were conducted in China. The measurement of GA in patients with GDM was primarily conducted between 24 and 28 weeks of gestation. There was only one study[27] that deviated from this range, where the GA levels were measured at 36-38 weeks of gestation. All included studies evaluated GA levels using standardized and internationally approved laboratory methodologies, specifically enzyme-linked immunosorbent assay (ELISA). One study reported the GA level in mmol/mL, while the others reported the GA level in mmol/mL (%). To

Table 1 Characteristics of available studies relating glycated albumin levels to gestational diabetes mellitus risk

No.	Country	Study design	GDM case	Control patients	NOS	Ref.
1	Brazil	Cross-sectional study	28	121	7	Chume *et al*[16], 2021
2	China	Prospectively	639	1479	8	Li *et al*[18], 2016
3	China	Prospectively	232	466	7	Zhu *et al*[19], 2018
4	Turkey	Cross-sectional case-control	40	40	7	Saglam *et al*[17], 2017
5	China	Cross-sectional	243	470	8	Pan *et al*[22], 2013
6	Japan	Retrospectively studied	25	17	8	Sugawara *et al*[23], 2016
7	China	Cross-sectional	376	743	8	Li *et al*[21], 2021
8	Japan	Retrospective	40	31	7	Sugawara *et al*[25], 2018
9	China	Prospective	225	125	8	Zhang *et al*[26], 2021
10	Italy	Multicenter study	22	32	7	Paroni *et al*[24], 2007
11	Italy	Cohort	30	53	7	Piuri *et al*[20], 2020

GDM: Gestational diabetes mellitus; NOS: Newcastle-Ottawa Scale.

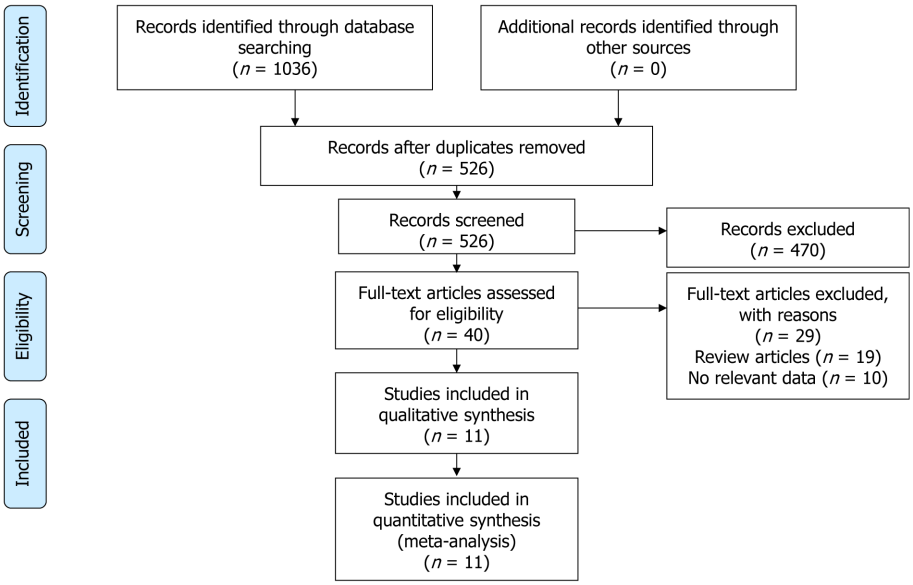

Figure 1 Flow of study selection.

address this issue and ensure consistency, we used a unit conversion equation. The conversion equation we used was GA (%) = 0.05652 × GA (mmol/mol) - 0.4217[28]. This equation enabled us to standardize the GA values into the same unit across all studies.

Meta-analysis

We initially carried out an analysis examining the correlation between GA levels and GDM risk across all patients. The pooled WMD for GA levels and GDM risk was 1.03 (95%CI: 0.33-1.74; I^2 = 98.1%, P < 0.001) (Figure 2A). As the pooled WMD exhibited significant heterogeneity, we proceeded to perform a sensitivity analysis and assessed publication bias using funnel plots (Figure 3A and B). Two studies[18,21] were identified as the primary sources of heterogeneity. Upon exclusion of these studies, the pooled WMD for GA levels and GDM risk in all patients decreased to 0.42 (95%CI: 0.11-0.74; I^2 = 75%, P < 0.001) (Figure 2B). We conducted a sensitivity analysis and evaluated publication bias by visually inspecting a funnel plot (Figure 3C and D). The sensitivity analysis confirmed the robustness of this result. The funnel plot displayed asymmetry, suggesting potential publication bias. Therefore, we employed the trim-and-fill method to reassess the pooled prevalence (Figure 4). The trim-and-fill analysis did not alter the summary effect estimate, indicating that publication bias is unlikely to significantly influence the results of our meta-analysis.

We also analysed the expression of glycated HbA1c in the two groups. The results showed that the pooled WMD for GA levels and GDM risk in all patients decreased to 0.19 (95%CI: 0.15-0.22; I^2 = 31.1%, P < 0.001) (Supplementary Figure

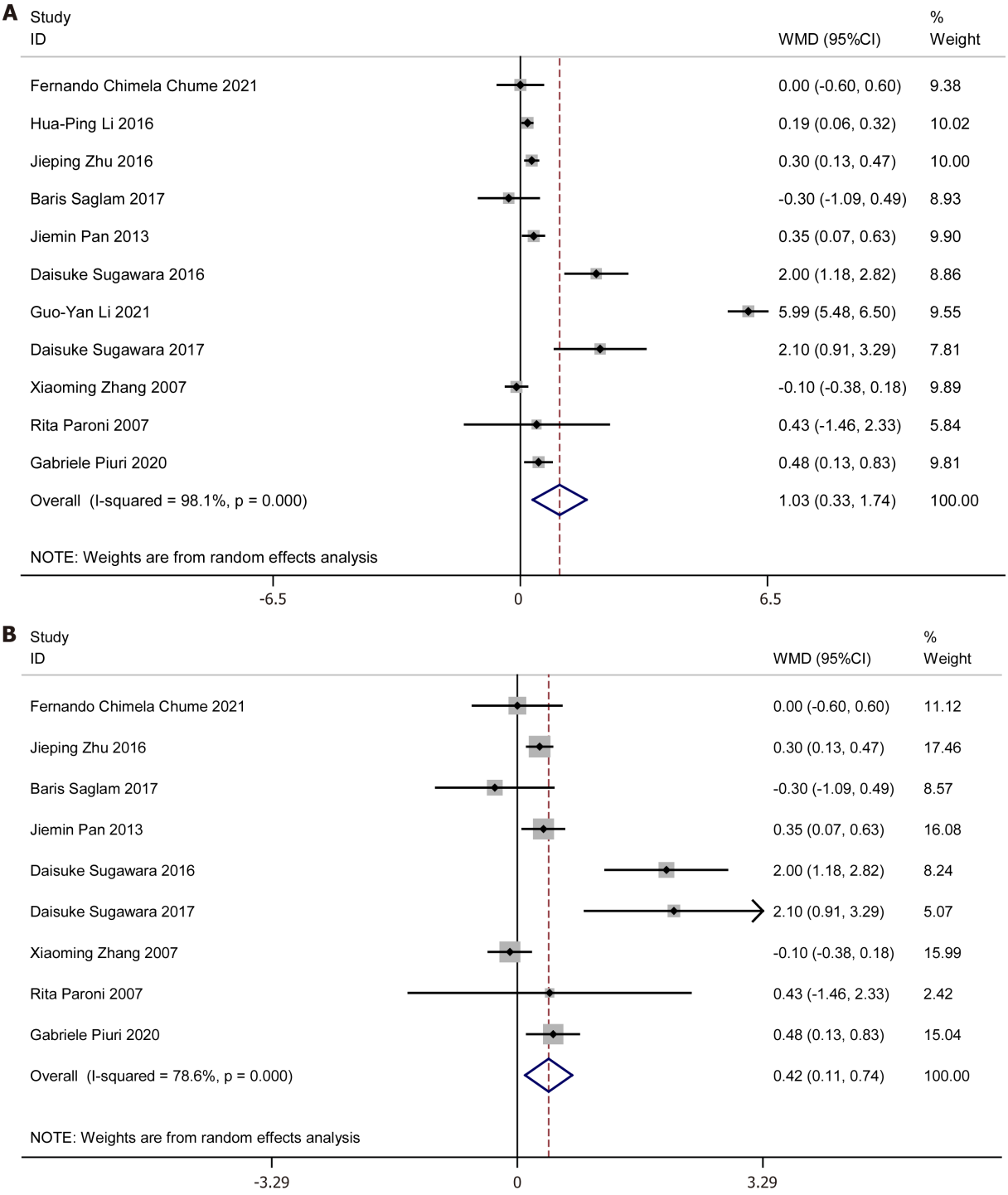

Figure 2 Forest plot evaluating glycated albumin levels in people with gestational diabetes mellitus versus those without gestational diabetes mellitus. A and B: The pooled results are expressed as weighted mean differences with 95% confidence interval. 95%CI: 95% confidence interval; WMD: Weighted mean differences.

1).

DISCUSSION

This meta-analysis revealed that there is a significant correlation between elevated levels of GA and an increased risk of GDM. The average GA level was notably greater in the GDM group, underscoring the potential of GA as a predictive biomarker for GDM.

GA has advantages over HbA1c as a biomarker. First, because the half-life of albumin is approximately 2-3 weeks, GA increases in response to hyperglycaemia and thereby represents the mean glucose level over a similar period[29]. Consequently, multiple clinical studies have underscored GA as a more effective indicator of short-term glycaemic

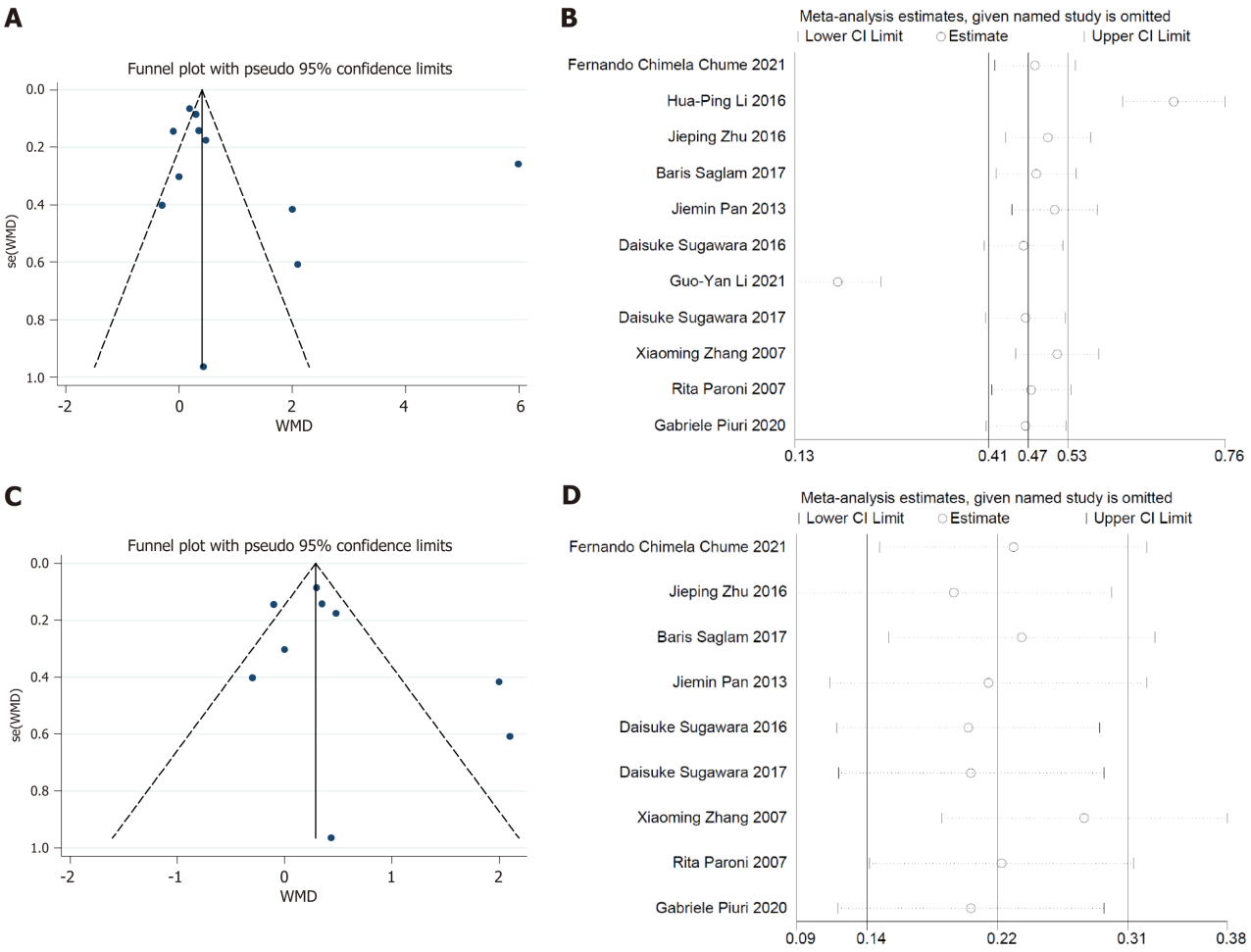

Figure 3 Sensitivity analysis and funnel plots for publication bias. A: Sensitivity analysis for the effects of glycated albumin levels on gestational diabetes mellitus risk; B: Publication bias analysis based on a funnel plot; C: Sensitivity analysis for the effects of glycated albumin levels on gestational diabetes mellitus risk; D: Publication bias analysis based on a funnel plot. WMD: Weighted mean differences.

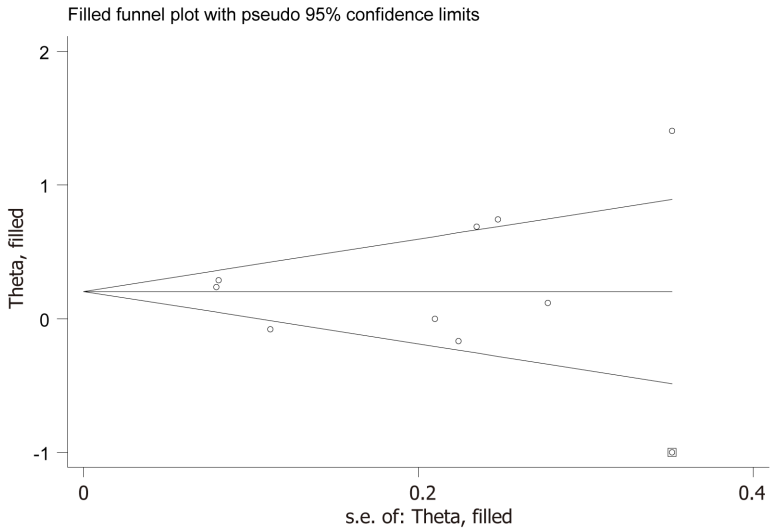

Figure 4 Trim-and-fill analysis of the studies included.

variability than HbA1c. Second, GA is unaffected by iron metabolism. In the later stages of pregnancy, iron deficiency anaemia often arises due to increased iron demand, which elevates HbA1c levels relative to the actual glycaemic state. As GA is not correlated with HbA1c, it remains uninfluenced by iron deficiency anaemia[30]. This study included several patients whose HbA1c levels were high but whose GA levels fell within normal ranges.

GA, similar to HbA1c, is strongly correlated with diabetic complications and even mortality in individuals with diabetes[31]. GA levels are independent of the serum albumin concentration, and fasting is not required for GA detection [32]. A recent study exploring the value of measuring GA in GDM patients reported that GA was less influenced by insulin resistance and diastolic blood pressure than was HbA1c. The authors proposed that GA might be superior to HbA1c for monitoring women with GDM[33]. Serum GA levels change swiftly in response to glucose fluctuations. Increased blood glucose levels hinder absorption from kidney tubules by competing with 1,5-AG.

Although our meta-analysis highlights the potential of GA as a predictive biomarker for GDM, we acknowledge that our results did not directly compare GA with traditional markers such as HbA1c and fasting plasma glucose (FPG). Numerous studies have shown that HbA1c levels reflect long-term glucose control, while GA levels might be more sensitive to short-term fluctuations in blood glucose levels[33,34]. Therefore, GA might offer a timelier indication of glycaemic status, which could be particularly useful in the dynamic physiological context of pregnancy. In terms of predicting GDM, some studies have found GA to be superior to HbA1c[22,23], while others have their predictive abilities to be similar[16,17]. We acknowledge that the relative performance of GA and HbA1c may depend on various factors, such as the timing of measurement and the specific population under study. However, the ability of GA to detect and predict GDM compared to that of FPG still needs further exploration. Some studies suggest that GA may be more sensitive than FPG in detecting early glucose intolerance due to its reflection of short-term glucose fluctuations, but more research is needed to fully understand this relationship. In conclusion, our study indicates the potential role of GA in predicting GDM, but further research is needed to compare its effectiveness and reliability with those of traditional biomarkers such as HbA1c and FPG.

In our study, we focused on the association between GA levels and GDM risk, whereas another published meta-analysis investigated the role of GA in predicting mortality risk in dialysis patients with DM. Although both meta-analyses involved GA as a key factor, the population and clinical outcomes examined in each study were distinct. Previous meta-analyses have also explored the role of GA in diabetic patients, particularly in predicting various clinical outcomes such as all-cause mortality, cardiovascular mortality, and cardiovascular events[34]. These studies have provided valuable insights into the role of GA as a potential biomarker in diabetic patients, particularly for those undergoing dialysis. However, our study specifically targeted pregnant women and the development of GDM, which is a separate clinical context with unique challenges and implications. It is important to acknowledge and discuss the literature on GA levels in diabetic patients, as it helps to contextualize our findings and contributes to a broader understanding of the potential role of GA as a biomarker in different patient populations. Although the results of the published meta-analysis on dialysis patients with DM suggest that GA may predict all-cause mortality risk, our study aimed to assess the association between GA levels and the development of GDM in pregnant women. Our findings indicate a possible relationship between higher GA levels and increased GDM risk, but further research is needed to confirm that GA is a potential biomarker for GDM.

The noteworthy correlation found in our meta-analysis between elevated GA levels and increased GDM risk offers valuable insights for clinicians in the identification and management of GDM. By utilizing GA as a potential biomarker in conjunction with existing diagnostic methods, health care professionals may be able to enhance the accuracy and efficiency of GDM detection and intervention. In clinical practice, accurately determining cut-off values is crucial for the appropriate application of biomarkers. Further research is warranted to establish optimal GA cut-off values for GDM prediction, considering factors such as population characteristics, ethnic backgrounds, and methodological variations in GA measurements. Establishing accurate cut-off values may contribute to improved clinical decision-making and targeted GDM management strategies, ultimately improving maternal and foetal health outcomes. It is important to emphasize that integrating the measurement of GA into clinical practice should be approached with caution until additional research provides more substantial evidence for its reliability and validity as a biomarker. Future studies should also address potential confounding factors and methodological issues that might influence the performance of GA in GDM prediction, ensuring its utility in diverse clinical settings. By building upon our findings and addressing these concerns, the scientific community can contribute to optimizing GDM identification and management and enhancing health care outcomes for both mothers and their offspring.

However, some inherent limitations of the study warrant consideration. First, several included studies had relatively small patient populations, limiting the strength of the conclusions drawn due to the limited data on the relationship between GA levels and GDM risk. The small patient numbers and ethnic variation are primarily responsible for the wide confidence intervals. Second, this meta-analysis relied on evidence from observational studies and was limited to English-language publications due to potential mistranslation issues. The absence of randomized controlled trials directly comparing the prognostic efficacy of GA with that of other glycaemic markers, alongside the inclusion of observational and cross-sectional studies, limited this analysis. Another confounding factor is the study population characteristics, as most studies in this meta-analysis were conducted in China. It remains uncertain whether these findings are applicable to European nations, given the substantial variations in dietary habits, cultural factors, comorbidities (such as metabolic syndrome or obesity), ethnicity, and genetic diversity in European countries compared to Asian countries. Third, there is no unified standard cut-off value. Although our results indicate that high GA values predict a greater GDM risk, the optimal cut-off value for prognosis prediction remains undetermined.

CONCLUSION

In conclusion, our findings underscore a significant association between GA levels and GDM risk. The incorporation of GA in early risk assessment holds promise for guiding informed treatment decisions, potentially impacting resource allocation, healthcare costs, and patient outcomes. Moving forward, there is a need for further research to explore GA's role in stratifying risk among GDM patients and to establish clinical decision limits, thereby advancing our understanding and enhancing clinical practice.

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FOOTNOTES

Author contributions: Xiong W contributed to conceptualization, methodology, and formal analysis; Zeng ZH and Xu Y contributed to software; Li H and Lin H contributed to validation; Zeng ZH contributed to investigation; Xu Y contributed to resources; Li H contributed to data curation and writing original draft preparation; Xiong W contributed to writing review and editing; and all authors have read and agreed to the published version of the manuscript.

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CASE REPORT

Transient diabetes mellitus with ABCC8 variant successfully treated with sulfonylurea: Two case reports and review of literature

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Abstract

BACKGROUND

Transient neonatal diabetes mellitus (TNDM) is a rare form of diabetes mellitus that usually presents within the first 6 mo of life. Patients often enter remission within several months, although relapse can occur later in life. Mutations in the *ABCC8* gene, which encodes the sulfonylurea receptor 1 of the ATP-sensitive potassium channel in pancreatic beta cells, are associated with TNDM and permanent neonatal diabetes. This study describes a novel *de novo* c.3880C>T heterozygous *ABCC8* variant that causes TNDM and can be treated with sulfonylurea therapy.

CASE SUMMARY

We retrospectively analyzed 2 Chinese patients with TNDM who were diagnosed, treated, or referred for follow-up between September 2017 and September 2023. The patients were tested for mutations using targeted next-generation sequencing. Patients with neonatal diabetes mellitus caused by a c.3880C>T heterozygous missense variant in the *ABCC8* gene have not been reported before. Both children had an onset of post-infectious diabetic ketoacidosis, which is worth noting. At a follow-up visit after discontinuing insulin injection, oral glyburide was found to be effective with no adverse reactions.

CONCLUSION

Early genetic testing of neonatal diabetes mellitus aids in accurate diagnosis and treatment and helps avoid daily insulin injections that may cause pain.

Key Words: Neonatal diabetes mellitus; ABCC8; Sulfonylurea receptors 1; KATP channels; Sulfonylurea; Glyburide; Insulinulin; Case report

Core Tip: To date there have been no reports of the c.3880C>T heterozygous variant of the *ABCC8* gene in patients with diabetes mellitus. After finding that this heterozygous variant can cause transient neonatal diabetes mellitus, we determined that oral sulfonylurea a was safe and effective therapy that successfully replaced insulin in 2 patients with the c.3880C>T heterozygous variant of the *ABCC8* gene. This genetic diagnosis will inform clinicians about the probable course and optimal management of diabetes in such patients.

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INTRODUCTION

Neonatal diabetes mellitus (NDM) is characterized by the onset of diabetes within the first 6 mo of life, but some cases are diagnosed between 6 mo and 12 mo of age. NDM is a type of monogenic diabetes that is very rare and difficult to treat, as reported by Kitsell in 1851[7]. The incidence of NDM is estimated to be approximately 1/89000-1:500000 live births[8], but it is not known in China. There are two clinical NDM subtypes depending on the permanence of hyperglycemia, transient (TNDM) and permanent (PNDM)[9]. The latter is a lifelong disease without remission; but TNDM, which accounts for 50%-60% of the NDM cases, usually enters remission after 6-12 mo. However, such patients may relapse in adolescence or early adulthood[10]. There are over 20 known genetic causes of NDM[11]. Approximately two-thirds of TNDM cases are related to abnormalities in an imprinted region on chromosome 6q24. Activating mutations in either of the genes encoding the two subunits of the ATP-sensitive potassium (KATP) channel of the pancreatic β-cell membrane [potassium inwardly rectifying channel subfamily j member 11 (*KCNJ11*) or *ABCC8*] are responsible in most of the remaining cases (KATP-NDM)[12,13]. Patients with KATP-NDM respond to sulfonylurea (SU) therapy, and approximately 90%-95% may be successfully transitioned to SU therapy, with complete discontinuation of insulin and a significant decrease in glycated hemoglobin levels[14,15]. In China, reports of the *ABCC8* gene variant causing TNDM are rare. Here we describe a clinical phenotype of TNDM caused by a novel *de novo ABCC8* gene c.3880C>T heterozygous variant and report effective SU treatment in 2 Chinese infants.

CASE PRESENTATION

Chief complaints

Case 1: A male infant 2 mo and 14 d of age was admitted to our hospital following a fever of 4 d, dyspnea of 3 d, and high blood glucose of 1 d duration.

Case 2: A male infant 7 mo and 20 d of age was admitted to our hospital following polydipsia and polyuria of 13 d, intermittent fever 6 d, and poor spirits of 5 d duration.

History of present illness

Case 1: Four days before admission, the parents complained of fever and increased water intake; however, the specific urine volume was not known. They reported that no chills, convulsions, nasal congestion, runny nose, cough, vomiting, abdominal distension, diarrhea, abnormal crying, or other symptoms had occurred. The patient had been given oral medication for a respiratory infection at a local clinic for 1 d. However, recurrent fever persisted. 3 d before admission he had dyspnea and was treated at a local hospital. But the response to atomization and oral medication was poor. One day earlier, he had an occasional cough and sputum in his throat accompanied by dyspnea, poor milk intake, poor spirits, and drowsiness. He was transferred to a superior local hospital for treatment. His blood glucose was high, and blood gas analysis revealed a pH of 7.06, a bicarbonate(HCO_3^-) level of 3.4 mmol/L, and a base excess(BE) of −24.8 mmol/L. The patient was treated with antibiotics, fluid replacement, and insulin therapy for 1 d. His breathing improved, and his body temperature was normal, but his blood glucose level was not well controlled. Therefore, he was transferred to our hospital for further treatment.

Case 2: Thirteen days before admission, the patient developed polydipsia and polyuria without evident cause, and the water intake and urine volumes were not known. Increased nocturia, evident overeating, or weight loss were not noted, but 6 d before admission, he had a fever with a peak temperature of 38 °C and occasional coughing. Oral ibuprofen effectively normalized the body temperature but was accompanied by frequent episodes of crying and agitation. He was taken to a local hospital and was treated with oral drugs for bronchitis. Furthermore, 5 d before admission, he manifested a poor spirit, mouth breathing, and tachypnea, with no nasal congestion, runny nose, cough, expectoration, rash, or convulsions. On a hospital revisit, he was considered to have severe pneumonia or respiratory failure. After receiving intravenous diazepam and treatment with cardiotonic drugs, his heart rate increased to 180-190 bpm, and he was transferred from the local facility to the intensive care unit of a tertiary hospital. His blood glucose was high, and blood gas analysis revealed a pH of 7.12, a $HCO3^-$ of 2.1 mmol/L, and a BE of −27 mmol/L. In addition, ventilator-assisted breathing, oxygen inhalation, sedation, anti-infectives, rehydration therapy, and other symptomatic treatments were administered. Insulin was given for 3 h but blood glucose levels remained at > 30 mmol/L. After the patient's condition stabilized, an insulin pump was implanted for continuous subcutaneous insulin infusion. During hospitalization, the patient had an intermittent fever with a peak of 38.5 °C, accompanied by a cough and expectoration. 2 d before admission, the treatment was switched to regular subcutaneous insulin injection of 1.2 IU every 6 h. The blood glucose level fluctuated between 4-19 mmol/L, and the patient's mental state and respiration were significantly improved. Subsequently, the patient was transferred to our hospital for further treatment.

History of past illness

Cases 1 and 2: The patients had no specific medical histories.

Personal and family history

Case 1: The patient was born at term by cesarean section, with a birth weight of 3.85 kg. The parents denied a history of asphyxia rescue. He was breastfed after birth, and his parents were not consanguineous. No family history of diabetes was reported.

Case 2: The patient was the second live birth of the mother (gravida 2, para 2), was delivered uneventfully at 38 wk, with a birth weight of 3 kg. The parents denied a history of asphyxia rescue. He was breastfed after birth and was not given complementary food. His parents were not consanguineous. No family history of diabetes was reported.

Physical examination

Case 1: Physical examination at admission showed a body temperature of 36.4 °C, pulse rate of 168 bpm, a respiratory rate of 42 breaths/min, blood pressure of 75/40 mmHg, height of 52 cm, and a weight of 7 kg. There was a poor spirit, no jaundice, no bleeding spots, normal skin elasticity, soft neck, normal fontanelle, equal pupils, sensitivity to light reflex, ruddy lips, smooth oral mucosa, rapid and regular breathing with thick breath sounds in both lungs, strong heart sounds, normal abdomen, normal spine and limbs, normal muscle strength, but poor peripheral circulation.

Case 2: Physical examination at admission showed a body temperature of 36.5 °C, pulse rate of 108 bpm, respiratory rate of 25 breaths/min, blood pressure of 80/45 mmHg, height of 66.8 cm (3^{rd}-10^{th} percentile), and weight of 10 kg. Clear consciousness, moderate nutrition, anemic appearance, pale complexion, no jaundice, no bleeding spots, normal skin elasticity, equal and round pupils, sensitivity to light reflex, soft neck, smooth breathing, thick breath sounds, and phlegm rales in both lungs, strong heart sounds, normal abdomen, normal spine and limbs, normal muscle strength, normal reflexes, slightly pale nail beds.

Laboratory examination

Case 1: Laboratory examinations were performed at admission. The venous blood glucose level was 11.15 mmol/L after admission and multiple random peripheral blood glucose levels were > 11.1 mmol/L. Blood gas analysis revealed a pH of 7.356, a $HCO3^-$ of 19.9 mmol/L, and a BE of 4.5 mmol/L. Urine glucose was markedly elevated (++++) and there was a weak positive result for urine ketone bodies. Blood ammonia and lactic acid levels were 67.8 μmol/L and 3.4 mmol/L, respectively. Routine blood tests revealed a white blood cell (WBC) count of 10.14×10^9/L, red blood cell (RBC) count of 3.3×10^{12}/L, Hb level of 99 g/L, and a platelet (PLT) count of 101×10^9/L. Blood biochemistry results were total cholesterol 6.27 mmol/L (reference range 0.36-6.2 mmol/L), triglycerides 2.46 mmol/L (reference range, 0.38-2.25 mmol/L), and normal liver function and renal function, myocardial enzyme levels, electrolyte levels, and thyroid function results were normal. The C-peptide level was 0.88 ng/mL (reference range, 1.1-4.4 ng/mL), and glycosylated Hb (HbA1c) was 9.52%. Insulinulin autoantibodies (glutamic acid decarboxylase, islet cell, and insulin antibodies) were negative.

Case 2: Laboratory examination was performed at admission. The venous blood glucose level was 13.26 mmol/L, and multiple random peripheral blood glucose samples were > 11.1 mmol/L. Blood gas analysis revealed a PH of 7.411, a $HCO3^-$ of −24.5 mmol/L, and a BE of 0.4 mmol/L.Urine glucose and urine ketone bodies were weakly positive, respectively. The blood ammonia level was 15.5 μmol/L and the lactic acid level was 2.4 mmol/L. Routine blood tests showed a WBC count of 11.57×10^9/L, RBC count of 4.14×10^{12}/L, Hb level of 66 g/L, and PLT count of 317×10^9/L. Blood biochemistry results were triglycerides 3.61 mmol/L (reference range, 0.38-2.25 mmol/L) and normal liver function, renal function, myocardial enzymes, electrolytes, and thyroid function. The C-peptide level was 1.44 ng/mL (reference range, 1.1-4.4 ng/mL), HbA1c was 12.63%; and five insulin antibodies (glutamic acid decarboxylase autoantibodies, islet cell antibodies, insulin autoantibody, zinc transporter-8 antibody, and tyrosine phosphatase

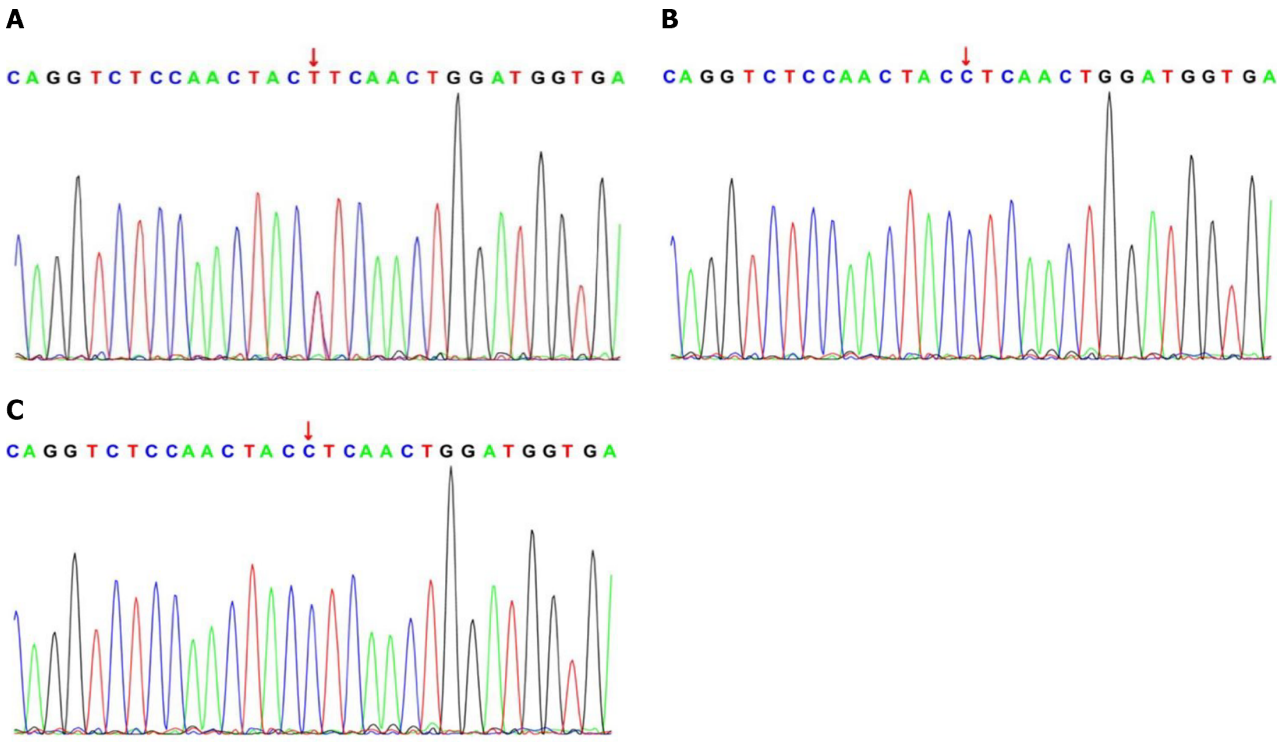

Figure 1 *ABCC8* gene. A: C.3880C>T heterozygous mutation; B and C: It was not observed in the father (B) and mother (C).

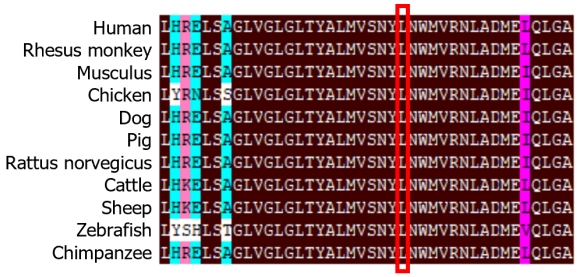

Figure 2 Amino acid conservation. Multiple alignments show the amino acid L at position 1294 of the human ABCC8 protein, indicating a high level of amino acid conservation with 10 mammalian proteins.

antibody) were negative.

Imaging examination

Genetic testing and mutation analysis: The Ethics Committee of the Children's Hospital Affiliated with Zhengzhou University approved this study (Approval No. 2023-K-123). Informed consent was obtained from the patients' guardians. DNA samples were sent to the hospital's Institute of Pediatrics for next-generation sequencing. The procedure was performed following the standard protocol. Sanger sequencing was used to verify the variant sites in the patients, and sequence analysis was performed on samples from the parents to determine the source of variation. A heterozygous missense variant in *ABCC8* was found in both patients, resulting in a C>T variant at position 3880 and the substitution of leucine (L) by phenylalanine (F) at amino acid position 1294 of the encoded protein. No corresponding gene variants were detected in the parents of either infant (Figure 1).

The *ABCC8* c.3880C>T variant has not been reported in PubMed or other databases such as the Human Gene Mutation Database, ClinVar, and the Database for Single Nucleotide Polymorphisms (dbSNP). Homologous sequence comparison using DNAMAN software (Lynnon Biosoft Bioinformatics Solutions, San Ramon, CA, United States) showed that amino acid L at position 1294 of the *ABCC8* protein was highly conserved (Figure 2). Bioinformatics software, such as Mutation Taster (https://www.mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), and SIFT (http://sift.bii.a-star.edu.sg/), predicted that the c.3880C>T variant was pathogenic or likely pathogenic. This variant changed the nonpolar hydrophobic aliphatic L to a nonpolar hydrophobic aromatic F at position 1294, altering the side chain structure with insignificant changes in hydrogen bonding (Figure 3). The c.3880C>T variant is classified as pathogenic (PM1 + PM2 + PP3 + PP4) by the American College of Medical Genetics and Genomics (ACMG) Classification of Genetic Variations[16].

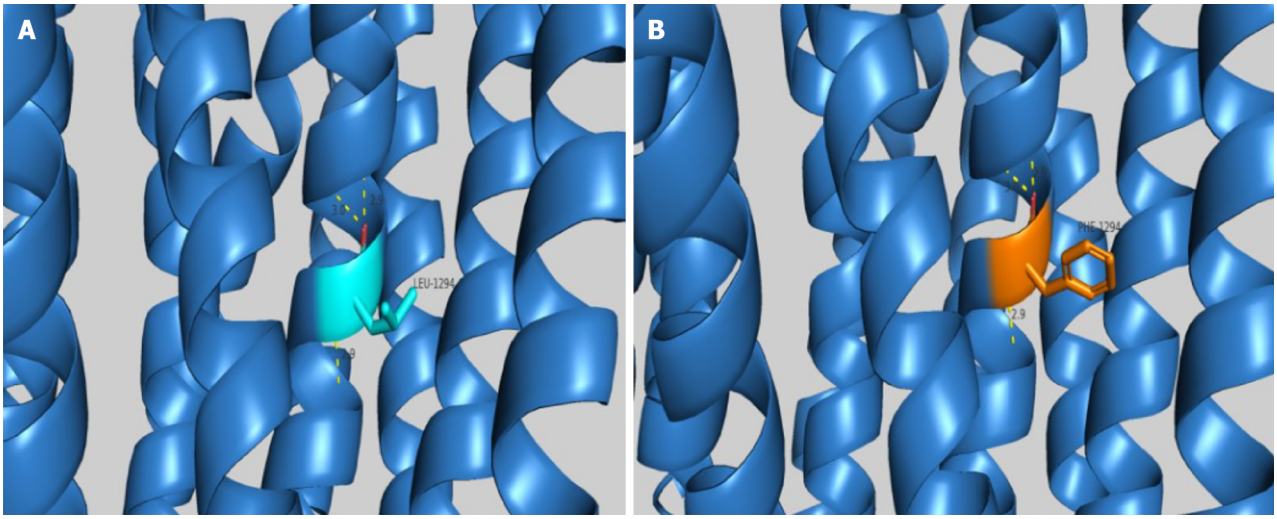

Figure 3 Predicted three-dimensional structure of the protein at c.3880C>T variant points of the *ABCC8* gene. A: Three-dimensional structure of the wild-type protein at the 1294 position is that of leucine; B: C.3880C>T variant protein becomes phenylalanine, altering the side chain structure, with no significant change in the hydrogen bonding.

FINAL DIAGNOSIS

The final diagnosis was TNDM.

TREATMENT

Both infants were treated with low-dose insulin after diabetic ketoacidosis. The initial dose was as follows: after ketoacidosis was corrected, neutral protamine Hagedorn insulin was administered three times, and glyburide was used as an experimental treatment when the blood glucose level was relatively stable. The initial dose of glyburide was 0.1-0.2 mg/kg/day. The glyburide dose was gradually adjusted by the results of blood glucose monitoring and insulin was gradually discontinued.

OUTCOME AND FOLLOW-UP

In both cases, blood glucose levels were in the normal range during outpatient follow-up. Glyburide was effective and was discontinued after 1 mo of oral administration in case 1 and after 1 year in case 2. No major side effects of glyburide were found. The growth and development of the 2 children were similar to that of their peers, and no neurological abnormalities were detected.

DISCUSSION

TNDM is a genetically heterogeneous form of NDM characterized by hyperglycemia and is usually diagnosed before 6 mo of age. It remits during infancy but recurs in later life in most patients[17,18]. Subcutaneous insulin was routinely used to treat NDM previously. However, establishing an effective long-term insulin therapy for NDM is a great challenge for pediatricians and parents because of the irregular feeding habits of infants. Consequently, oral SU therapy has been found to improve glycemic control and to be a more effective in improving the quality of life[19,20].

KATP channels are hetero-octameric complexes formed by four pore-forming Kir6.2 subunits and four SU receptor-1 (SUR1) regulatory subunits and encoded by the KCNJ11 and ABCC8 genes, respectively. In normal pancreatic beta cells, increased glucose across glucose transporter 2 is metabolized by the enzyme glucokinase, resulting in increased production of ATP. This closes the KATP channel, which in turn depolarizes the cell membrane and activates an influx of calcium through voltage-gated calcium channels that subsequently permit exocytosis of insulin granules. ABCC8 gene mutations cause the KATP channels to remain inappropriately open, even in the presence of hyperglycemia. Without channel closure, the cell membrane depolarizes and blocks insulin release from the beta cells, resulting in the clinical manifestations of diabetes mellitus. SU closes the KATP channel through an ATP-independent route, leading to increased insulin secretion[14,21]. Patients with NDM carrying ABCC8 variations have been successfully switched from insulin to oral SU treatment[20,22,23]. However, the appropriate treatment may differ owing to different types of variants and variable clinical phenotypes. In addition, the sensitivity to SU varied among patients with ABCC8 variants. Notably, most

Table 1 Clinical features of transient neonatal diabetes mellitus patients with *ABCC8* variants (including 2 patients in this case report)

Case	Age	DKA	PH	HCO_3^-	BE	HbA1c	C-peptide	Neurological symptoms	Mutation	Zygosity	Inheritance	Treatment	Ref.
1	36 d	Severe	7.08	3.3	−26	7.6	0.17	No	Arg1183Trp	Het	Paternal	Insulin → SU remission in 24 mo	Ngoc *et al*[29], 2021
2	50 d	Moderate	7.19	8	−19	7.58	0.41	Convultion	Glu1141Gly	Het	Paternal	Insulin → SU remission in 6 mo	Ngoc *et al*[29], 2021
3	35 d	Yes	NA	NA	NA	NA	NA	No	Arg216Cys	Hom	Both parents	Insulin → SU remission in 14 mo	Nayak *et al*[30], 2021
4	270 d	No	Normal	NA	NA	NA	NA	No	Leu1295Phe	Het	Paternal	Insulin remission in 48 mo	Nayak *et al*[30], 2021
5	4 d	No	Normal	NA	NA	NA	0.06	NA	Arg1380Cys	NA	NA	Insulin remission in 4 mo	Torbjörnsdotter *et al*[31], 2020
6	96 d	NA	NA	NA	NA	7.2	NA	Developmental delay	Arg653Gln	Het	NA	NA	Balamurugan *et al*[32], 2019
7	12 d	NA	NA	NA	NA	4	0.33	NA	Ile395Phe	NA	NA	Insulin → SU unresponsive remission in 5.5 mo	Li *et al*[28], 2018
8	60 d	NA	NA	NA	NA	7.2	< 0.5	NA	Arg877Gln	NA	NA	Insulin remission in 4 mo	Li *et al*[28], 2018
9	105 d	NA	NA	NA	NA	7.9	0.42	NA	Gly1255Ser	NA	NA	Insulin remission in 3.5 mo relapse in 8 yr	Li *et al*[28], 2018
10	35 d	No	7.459	22.5	−0.4	4.3	0.6	No	Gly832Cys	Het	*De novo*	Insulin → SU remission in 1 yr and 10 mo	Yamazaki M *et al*[33], 2017
11	74 d	Severe	7.06	3.4	−24.8	0.88	9.52	No	Leu1294Phe	Het	*De novo*	Insulin → SU remission in 1 mo	This study
12	230 d	Severe	7.12	2.1	−27	1.44	12.63	No	Leu1294Phe	Het	*De novo*	Insulin → SU remission in 12 mo	This study

HbA1c: Glycosylated hemoglobin; NA: Not available; SU: Sulfonylurea.

patients, but not all, were successful in transitioning from insulin therapy to SU[22]. The ABCC8 gene, encoding a 1582 amino acid protein, is located on the short arm of chromosome 11 (11p15.1) and comprises 39 exons. Over 700 pathogenic or likely pathogenic ABCC8 mutations have been identified in the ABCC gene, and these include point, missense, nonsense, frame, splicing, and deletion variations[24-26].

A large prospective cohort study by Busiah *et al*[27] of neonatal diabetes diagnosed before 1 year of age in 68 French centers reported that of 31 patients with NDM caused by an ABCC8 mutation, 11 (13%) were < 1 mo, 19 (27%) were between 1 mo and 6 mo, and 1 (6%) was between 6 mo and 1 year of age. At the end of follow-up, 24 cases were identified as TNDM (78%) and 5 as PNDM (16%)[27]. In 2020, De Franco *et al*[24] described a total of 748 ABCC8 pathogenic and likely pathogenic variants associated with NDM and congenital hyperinsulinism that had been identified in various countries. To date, the only patients with ABCC8 variants associated with TNDM in China were reported by Li *et al*[28]. We searched the literature in PubMed and Chinese literature databases, such as China National Knowledge Infrastructure and Wanfang. The ABCC8 gene variants associated with TNDM were reviewed and are summarized in Figure 4, with the majority of variants located in the coding region of the gene. A total of 12 cases with detailed clinical information and the outcomes of SU therapy transfer are summarized in Table 1. The c.3880C>T variation is in the TMD2 domain of the SUR1 subunit in exon 32 of ABCC8 and results in a change from amino acid L to amino acid F at the 1294 position in the encoded protein. According to the ACMG guidelines for classifying genetic variants, this variant is likely to be pathogenic (PM1 + PM2 + PP3 + PP4). So far, no reports of the ABCC8 c.3880C>T variation have been reported, and further functional studies are needed. The age at onset in case 1 was 2 mo, and that in case 2 was 7 mo and 20 d. Both

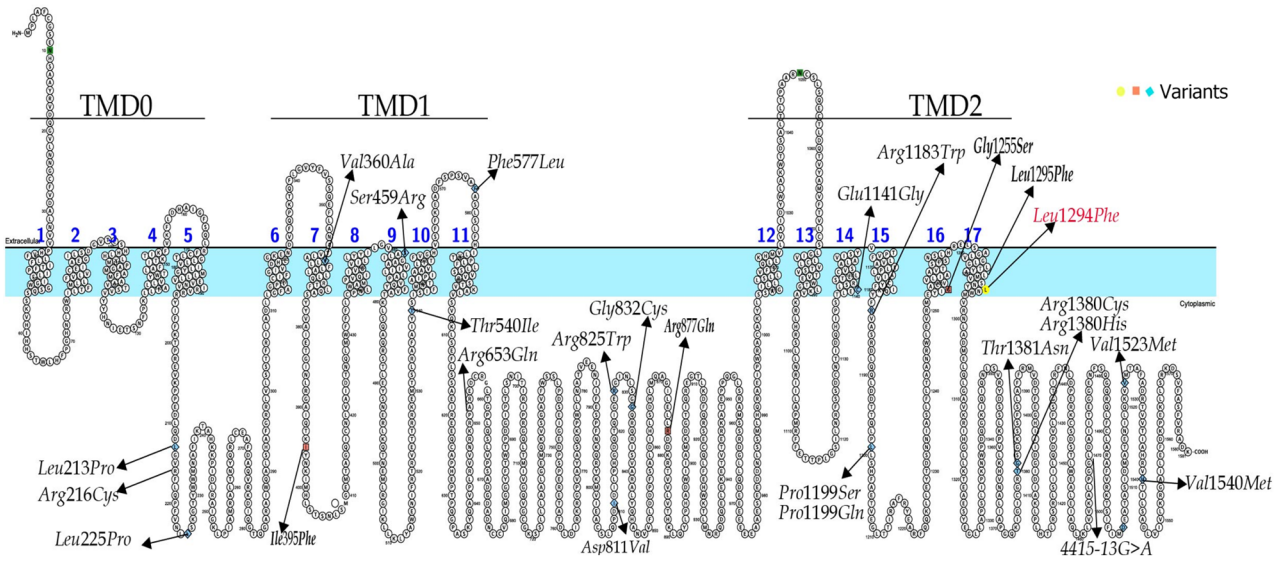

Figure 4 Location of *ABCC8* variants in the sulfonylurea receptor 1 protein reported in patients with transient neonatal diabetes mellitus. Protein visualization was generated using the Protter website (http://wlab.ethz.ch/protter/start/).

patients had ketoacidosis and no family history of diabetes mellitus. During the follow-up period, glyburide proved to be effective and was discontinued after 1 mo and 1 year of oral administration, respectively. No significant side effects of glyburide were observed, suggesting that the age at onset and duration of glyburide treatment differ in patients with the same mutation site. Owing to the ABCC8 variations, patients with TNDM have a high possibility of recurrence in the future, and we will continue to follow-up the children for an extended period.

CONCLUSION

NDM is rarely encountered in clinical practice. In this study, we retrospectively analyzed the clinical phenotypes and *ABCC8* c.3880C>T mutations in 2 infants with TNDM. SU glyburide treatment was effective, and this novel *de novo* variation expands the pathogenic gene mutation spectrum of NDM. All patients diagnosed with diabetes before the 1 year of age should be referred for genetic testing, regardless of their current age, to identify those cases likely to benefit from SU treatment. However, the sample size was limited; therefore, additional evidence and experience should be accumulated over long follow-up periods.

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FOOTNOTES

Author contributions: Shen LH designed and performed the research study and wrote the manuscript; Cui Y and Fu DX designed the research and supervised the manuscript; Wang HZ and Yang HH designed the research and contributed to the analysis; Chen YX provided clinical advice; Wei HH made many suggestions for the revised manuscript; All authors have read and approved the final manuscript.

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LETTER TO THE EDITOR

Enhancing diabetic retinopathy screening: Non-mydriatic fundus photography-assisted telemedicine for improved clinical management

Kira J Szulborski, David J Ramsey

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Abstract

The utilization of non-mydriatic fundus photography-assisted telemedicine to screen patients with diabetes mellitus for diabetic retinopathy provides an accurate, efficient, and cost-effective method to improve early detection of disease. It has also been shown to correlate with increased participation of patients in other aspects of diabetes care. In particular, patients who undergo teleretinal imaging are more likely to meet Comprehensive Diabetes Care Healthcare Effectiveness Data and Information Set metrics, which are linked to preservation of quality-adjusted life years and additional downstream healthcare savings.

Key Words: Diabetes; Diabetic retinopathy; Telemedicine; Tele-ophthalmology; Non-mydriatic fundus photography-assisted telemedicine; Vision screening; Preventative health services; Health policy

Core Tip: Point-of-care screening for diabetic retinopathy by means of teleretinal imaging provides an effective and economical method to improve early detection of eye disease in at-risk populations. Non-mydriatic fundus photography (NMFP)-assisted telemedicine allows critical ophthalmic examinations to reach individuals when resources for eye care are limited or difficult to access. As technology continues to evolve, further research efforts should focus on refining NMFP-assisted telemedicine protocols and evaluating its long-term impact on patient outcomes across the spectrum of healthcare settings.

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TO THE EDITOR

Telemedicine screening for eye disease is key to making eye care more available to the growing numbers of individuals with diabetes mellitus (DM) worldwide[1,2]. We read with great interest the paper by Zhou *et al*[3], which validated the effectiveness of screening patients with DM for diabetic retinopathy (DR) at a major university-based medical center located in Hefei, Anhui Province, China. The authors compared non-mydriatic fundus photography (NMFP)-assisted telemedicine screening with fundus fluorescein angiography as the gold standard for diagnosis and staging of DR. The authors present compelling evidence for the efficacy of NMFP-assisted telemedicine in detecting and staging DR, finding a high degree of consistency between the two modalities. This study has significant implications for improving the early detection of DR, thereby allowing for earlier referrals to ophthalmologists for interventions capable of improving patient outcomes.

We agree with the authors that point-of-care screening for DR incorporating teleretinal imaging (TRI) offers an accurate and cost-effective means for improving early detection of disease in at-risk populations[4-7]. It also has the advantage of increasing patient participation in the care of their DM[8-12] and allows optimal resource allocation within healthcare systems that are at risk of becoming overextended[13-16]. For a comprehensive review of the diagnostic accuracy of telemedicine screening protocols for the detection of DR in various settings see the meta-analysis by Mehraban Far *et al*[17]. However, there is an equal, if not a greater, value derived from the use of TRI to screen lower-risk populations. In this context, its introduction could also reduce the costs associated with diabetic eye examinations, especially for patients without prior evidence of DR or who have completed a comprehensive eye examination within the prior year[18,19]. We particularly want to commend the authors for including in their discussion due attention to how the use of a hand-held NMFP allows for increased accessibility for certain patients who would otherwise be unable to avail themselves of traditional fixed ocular imaging or fundus examining devices. This flexibility, combined with their generally lower acquisition costs and portability, is one of the most compelling reasons for advocating for the expanded use of hand-held fundus cameras.

Though the authors highlighted several significant advantages of NMFP-assisted telemedicine screening, we feel that further discussion is warranted encompassing a broader view of the full extent of their potential benefits for comprehensive diabetes care. Our own recent study examined the factors associated with the utilization of point-of-care TRI for DR screening in a primary care setting in the United States[20]. The patients screened in our study were well-insured, suburban patients. They included both sexes, spanned a broad range of ages, types of health insurance, and, to a lesser extent, races. In this population, we found that participation in TRI was closely associated with the completion of other Comprehensive Diabetes Care Healthcare Effectiveness Data and Information Set (HEDIS) metrics. In particular, those patients screened were more likely to have had their hemoglobin A1c and microalbumin levels measured[21]. This combination of earlier and more regular DR screening, as well as the completion of HEDIS metrics, has the potential to improve more than just the ocular health of these patients. Furthermore, the detection of DR and other diabetes-associated complications may allow for the timely initiation of treatments that may preserve or prevent loss of quality-adjusted life years[22]. Finally, a more complete analysis of the cost-effectiveness of these technologies ought to take into consideration additional indirect economic benefits brought about by the earlier detection and treatment of diabetes-associated complications, as well as the costs linked to downstream healthcare service utilization[23].

One of the features of the manuscript by Zhou *et al*[3] that may cause difficulties for some readers is the use of non-standard abbreviations and nomenclature for certain aspects of diabetic eye disease. For example, the use of the acronym "NDR" to describe patients with "no" DR is very similar to the clinical acronym in common use for nonproliferative diabetic retinopathy, NPDR, and may potentially cause confusion. There is also use of some less common terminology for features associated with DR. For example, in place of the term "microhemangioma" in the context of DR, we recommend using more established terminology to describe small vascular abnormalities such as "microaneurysms" or "intraretinal microvascular abnormalities". The study also does not utilize the most current definition of DR staging. The authors mention relying on the International Federation of Ophthalmological Societies' guidelines for DR screening and staging established in 2002. We urge consultation of more up-to-date standards on diagnosis, management, and treatment of diabetic eye disease such as those published by the American Academy of Ophthalmology and American Diabetes Association[1,2]. Finally, the discussion would have benefited from a more comprehensive examination of the potential

limitations and challenges associated with NMFP-assisted telemedicine. These issues include acknowledgement of the barriers related to patient and provider acceptance of TRI technology, the need to put in place robust data security to protect patient privacy, and the requirement for an adequate referral system to ophthalmologists and retina specialists to assure timely monitoring and treatment of any patients identified to have DR. By addressing these needs, researchers can refine NMFP-assisted telemedicine protocols and increase their implementation in clinical practice.

These issues aside, we want to emphasize that the authors deserve to be recognized for their forward-thinking approach to the introduction of TRI in an underserved population in a major urban center in a Chinese province that has undergone rapid development. Teleophthalmology has been successfully employed in China for many years[24,25]. Elsewhere in Asia, Singapore's Integrated Diabetic Retinopathy Program has also demonstrated significant cost savings, without sacrificing health outcomes in an urban population[4]. Importantly, countries that have established universal DR screening programs supported by registries, such as in the United Kingdom, have achieved considerably higher screening rates among eligible patients with DM and have successfully reduced the incidence of blindness associated with DR[22].

Our own research, as well as many others, focused on the use of TRI in primary medical care settings[21]. It is exciting to see this technology extended for use in an endocrinology department by Zhou *et al*[3]. Endocrinologists see a larger proportion of patients with DM that is often more challenging to control or of longer duration[26]. Many of the patients in this study by Zhou *et al*[3] would, therefore, not likely be considered low-risk for DR. Including additional demographic details such as age and sex distribution, duration of DM, and socioeconomic status would help readers better assess the generalizability of their findings to other populations. Nevertheless, this setting may explain the very high rate of DR encountered in their study population, which is well above the rate expected in the overall diabetic population, including in China[24,25]. In the United States, most of those patients would not generally be monitored by means of NMFP-assisted telemedicine to screen for vision-threatening eye disease.

In conclusion, the study by Zhou *et al*[3] contributes significantly to the literature on DR screening, and it underscores the potential of NMFP-assisted telemedicine in improving early detection and management of DR. As technology continues to evolve, further research efforts should focus on refining NMFP-assisted telemedicine protocols, integrating them into current healthcare practice standards, and evaluating their long-term impact on patient outcomes.

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FOOTNOTES

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LETTER TO THE EDITOR

Vitamin D and selenium for type 2 diabetes mellitus with Hashimoto's thyroiditis: Dosage and duration insights

Yun-Feng Yu, Xue-Li Shangguan, Dan-Ni Tan, Li-Na Qin, Rong Yu

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Abstract

This letter discusses the publication by Feng *et al*. Iodine, selenium, and vitamin D are closely associated with thyroid hormone production in humans; however, the efficacy of selenium and vitamin D supplementation for type 2 diabetes mellitus (T2DM) patients with Hashimoto's thyroiditis (HT) remains controversial. In the retrospective study we discuss herein, the authors highlighted significant improvements in thyroid function, thyroid antibodies, blood glucose, and blood lipid in T2DM patients with HT following addition of vitamin D and selenium to their antidiabetic regimens, underscoring the value of these supplements. Our team is currently engaged in research exploring the relationship between micronutrients and HT, and we have obtained invaluable insights from the aforementioned study. Based on this research and current literature, we recommend a regimen of 4000 IU/day of vitamin D and 100-200 μg/day of selenium for over three months to six months for patients with HT, particularly for those with concurrent T2DM.

Key Words: Vitamin D; Selenium; Type 2 diabetes mellitus; Hashimoto's thyroiditis; Dosage; Duration

Core Tip: Supplementation with vitamin D and selenium shows promise in improving the prognosis of type 2 diabetes mellitus (T2DM) and Hashimoto's thyroiditis (HT). Despite controversies surrounding their efficacy, a retrospective study discussed in this letter reveals significant benefits from adding these supplements to antidiabetic regimens. Based on this research and current literature, we recommend a regimen of 4000 IU/day of vitamin D and 100-200 μg/day of selenium for over three to six months for patients with HT, particularly for those with concurrent T2DM.

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TO THE EDITOR

We are fortunate to have read the article, which was written by Feng *et al*[1] and published in the *World Journal of Diabetes*. We extend our felicitations to the authors for completing the aforementioned retrospective study and providing new insights into treating type 2 diabetes mellitus (T2DM) patients with Hashimoto's thyroiditis (HT). The study by Feng *et al* [1] revealed that, compared with antidiabetic drugs alone, the supplementation of 4000 IU vitamin D and 200 μg selenium daily for three to six months, in combination with standard antidiabetic drugs, significantly improved thyroid function, thyroid antibodies, blood glucose, and blood lipid in T2DM patients with HT. In this letter, therefore, we discuss the ideal dosage and duration of vitamin D and selenium administration in treatment of HT, taking into account the study by Feng *et al*[1] as well as previous literature.

DOSE AND DURATION OF VITAMIN D

The optimal dosage and duration of vitamin D supplementation in HT patients is controversial. An early randomized controlled trial from Türkiye revealed that vitamin D supplementation of 1000 IU per day for one month significantly reduced thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TGAb) in patients with autoimmune thyroid disease[2]. A prospective study from Greece indicated that vitamin D supplementation of 1200-4000 IU per day for four months significantly reduced TPOAb in HT patients, although they observed no significant effects on TGAb or thyroid-stimulating hormone (TSH)[3]. Additionally, a study from Iran found that a vitamin D supplementation of 50000 IU per week for three months significantly reduced TGAb and TSH levels in female patients, compared to those who received a placebo, with no significant effect on TPOAb[4]. Although these studies reported different results on the benefits of vitamin D supplementation, they are all in concordance that vitamin D supplementation is beneficial for HT patients. Taken in combination, the results of these studies suggest that an average daily vitamin D supplementation ranging from 1000 to 7143 IU is effective for treating HT. Of note, the study by Feng *et al*[1] demonstrated that, in addition to thyroid function and thyroid antibodies, 4000 IU of vitamin D per day improved blood glucose and lipid levels in T2DM patients with HT, suggesting that high-dosage vitamin D may have the additional benefit of modulating glucose and lipid metabolism in such patients. In light of this additional evidence, therefore, we recommend supplementation with 4000 IU of vitamin D daily for HT patients, particularly those HT patients with T2DM.

With regard to the appropriate duration of vitamin D supplementation, a clinical study from Turkey discovered that a one-month duration allowed for reduction of TPOAb and TGAb levels in patients with autoimmune thyroid disease[2], which is the lowest effective duration reported to date. A subsequent meta-analysis indicated that vitamin D supplementation for more than three months reduced TPOAb and TGAb levels in HT patients, whereas treatment for three months or less reduced only TGAb levels[5]. A recent meta-analysis further indicated that vitamin D supplementation for longer than three months not only reduced TPOAb and TGAb, but also increased free triiodothyronine (FT3) and free thyroxine (FT4) in HT patients[6]. In contrast, treatments lasting three months only resulted in a reduction of TGAb[6]. These findings, therefore, demonstrate that vitamin D supplementation for longer than three months provided additional benefits for HT patients. Of note, the study by Feng *et al*[1] revealed that vitamin D supplementation for six months was more effective in improving FT3, FT4, TSH, TPOAb, and TGAb in T2DM patients with HT compared with a three-month regimen. With regard to treatment duration, therefore, we recommend that HT patients take vitamin D for 3-6 months or more.

DOSE AND DURATION OF SELENIUM

As with vitamin D, the optimal dosage and duration of selenium supplementation in the treatment for HT remain unclear. With regard to dosage, a recent meta-analysis of 35 studies revealed that a daily selenium supplementation of 100 μg or more reduced TSH and TPOAb in HT patients[7]. Unfortunately, it did not compare the benefits of various selenium dosages. In fact, most previous clinical studies set the daily intake of selenium at 200 μg, regardless of the

selenium type, emphasizing that 200 μg/day of selenium improved the prognoses of HT patients[8,9]. Similarly, the study by Feng *et al*[1] demonstrated that a daily supplementation of 200 μg selenium, in addition to the standard anti-diabetic drugs and vitamin D, effectively improved thyroid function and antibodies in T2DM patients with HT. However, a high dosage of selenium may be associated with potential adverse events. It has been reported that selenium dosages exceeding 400 μg/day can lead to acute toxic symptoms in the gastrointestinal and nervous systems[10], and supplementation of 200 μg/day selenium may increase the risk of developing T2DM[11]. Therefore, we recommend that the daily selenium supplementation for HT patients be maintained between 100 and 200 μg.

With regard to the duration of selenium treatment, an early meta-analysis indicated that a three-month duration of selenium supplementation significantly reduced TPOAb and TGAb levels in patients with autoimmune thyroiditis, while supplementation for six or twelve months did not have a significant effect on TPOAb or TGAb[12]. Subsequently, another meta-analysis found that selenium supplementation led to reduced levels of TPOAb and TGAb after three months, while only TGAb was reduced after six months, and no reduction in thyroid antibodies was seen after twelve months[13]. In contrast, Feng *et al*[1] demonstrated that selenium supplementation for both three and six months reduced TPOAb and TGAb levels. We speculate that this difference may be attributable to the synergistic effect of supplemental selenium and vitamin D in HT patients, as the selenium supplementation by Feng *et al*[1] was based on concurrent vitamin D supplementation. As it has been shown that selenium treatment for three or six months is beneficial, despite concerns about its long-term effects, we recommend that the duration of selenium supplementation should be set at 3-6 months. In summary, combined with research by Feng *et al*[1] and the current literature, we recommend a regimen of 4000 IU/day of vitamin D and 100-200 μg/day of selenium for over three months to six months for patients with HT, particularly for those with concurrent T2DM.

SYNERGISTIC EFFECT OF VITAMIN D AND SELENIUM

In addition to the optimal dosage and duration, the existence of synergy between vitamin D and selenium remains unclear, as does its mechanism. Evidence suggests that selenium enhances the effects of vitamin D in the treatment of HT. Krysiak *et al*[14] included 47 female patients with HT who had normal thyroid function and vitamin D deficiency. Of these, 23 had been treated with selenomethionine for 12 months prior to treatment, while the other 24 had not received selenium treatment. After six months of vitamin D treatment, the levels of TPOAb and TGAb in patients with HT significantly decreased, and these changes were more pronounced in patients who had received selenomethionine[14]. This indicates that selenium supplementation may enhance the therapeutic effects of vitamin D on HT. Although a nutritional study indicated that vitamin D promotes selenium absorption, it is unclear whether vitamin D enhances the efficacy of selenium in the treatment of HT[15]. In summary, there may be a synergistic effect between vitamin D and selenium; however, its strength and formation mechanisms require further exploration.

LIMITATIONS AND PROSPECTS

Although the study by Feng *et al*[1] provided clinical evidence for a supplementation regimen of vitamin D combined with selenium in the treatment of T2DM with HT, it did have some limitations. First, it was a retrospective case-control study with a lack of randomization and blinding of patients, leading to potential selection and implementation biases. Second, as there was no treatment group with selenium-only supplementation, it is unclear whether selenium and vitamin D play a synergistic role in the treatment of T2DM with HT. Third, it is unclear whether the long-term effects of vitamin D combined with selenium are observed in the treatment of T2DM with HT, as the study by Feng *et al*[1] only assessed these effects at three- and six-month timepoints. Considering these limitations, it is too early to conclude that a com-bination of vitamin D and selenium has a significant clinical effect in the treatment of T2DM with HT. In the future, multicenter, randomized controlled, double-blind clinical trials should be conducted to further evaluate the short- and long-term effects of vitamin D alone, selenium alone, and a combination of vitamin D and selenium in the treatment of T2DM with HT.

Finally, we wish to express our appreciation to Feng *et al*[1] for sharing their research and novel finding addressing the clinical effects of vitamin D and selenium in specific HT patients. In addition to increasing confidence in our practice, the aforementioned study provides new insights and evidence for clinicians to gain a deeper understanding of the combination of vitamin D and selenium, ultimately enriching our treatment of T2DM with HT.

CONCLUSION

In conclusion, this study highlights the potential benefits of vitamin D and selenium supplementation in improving thyroid function, reducing thyroid antibody levels, and optimizing blood glucose and lipid levels in patients with T2DM and HT. Based on current research, we recommend a daily regimen of 4000 IU of vitamin D and 100-200 μg of selenium for three to six months. Although our findings are promising, they require validation in larger, randomized controlled trials to confirm their efficacy and safety. Future research should focus on the potential synergistic effects of these supplements and elucidate their long-term effects in patients with concurrent T2DM and HT.

FOOTNOTES

Author contributions: Yu YF, Shangguan XL, Tan DN, Qin LN, Yu R Yu YF and Shangguan XL analyzed the literature and wrote the letter; Tan DN and Qin LN performed the research mentioned in the letter; Yu R proposed the idea and revised the letter; All authors have read and approved the final manuscript. Yu YF and Shangguan XL have made equal contributions to this work as co-first authors for two reasons. Firstly, Yu YF and Shangguan XL made contributions of equal significance throughout the research process. The choice of these researchers as co-first authors acknowledges and respects this equal contribution. Secondly, the research was a collaborative effort, and co-first authorship accurately reflects the distribution of responsibilities and the substantial time and effort invested in completing the study and the resulting paper. In summary, designating Yu YF and Shangguan XL as co-first authors are appropriate for our manuscript as it faithfully reflects our team's collaborative ethos, equal contributions, and diversity.

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