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EDITORIAL

Assessing glycemic and weight-lowering potential of oral semaglutide in type 2 diabetes compared to other GLP-1 receptor agonists in Indian context

Awadhesh Kumar Singh[1,2,3] · **Rajeev Chawla**[4]

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Glucagon-like peptide-1 receptor agonists (GLP-1RAs) have shown a significant reduction in glycated hemoglobin (HbA1c) and body weight in people with type 2 diabetes (T2D). In addition, some of the injectable GLP-1RAs such as liraglutide, semaglutide, and dulaglutide have shown a significant reduction in major adverse cardiovascular events (MACE). Among the injected GLP-1RAs, semaglutide deserves special mention at least for two reasons. A higher strength of injectable semaglutide (2.4 mg) is also approved for obesity. Secondly, injectable semaglutide (1.0 mg) has shown superior HbA1C and weight lowering in T2D over several active comparators in the Phase 3 Clinical Development Programme named SUSTAIN (Semaglutide Unabated Sustainability In Treatment of Type 2 Diabetes). This includes the superiority of injectable semaglutide 1.0 mg in both HbA1c and weight lowering over sitagliptin 100 mg (SUSTAIN 2, Global), canagliflozin 300 mg (SUSTAIN 8, Global), liraglutide 1.2 mg (SUSTAIN 10, European), dulaglutide 1.5 mg (SUSTAIN 7, Global), exenatide extended-release 2.0 (SUSTAIN 3, Global), and basal insulin glargine (SUSTAIN 4, Global) [1–6]. However, injectable semaglutide is not yet available in India. Currently, only two injectable GLP-1RAs such as liraglutide and dulaglutide are available in India. Among these two GLP-1RAs, liraglutide has shown a small but significantly higher weight loss compared to dulaglutide despite similar HbA1c control in AWARD 6 (Assessment of Weekly AdministRation of LY2189265 [dulaglutide] in Diabetes-6) [7].

Orally administered semaglutide is the first oral GLP-1RA approved in 2020 by the United States (US) Food and Drug Administration, the European Medicines Agency, and the Drug Controller General of India (DCGI) for the treatment of T2D, based on extensive Phase 3 Clinical Development Programme named PIONEER (Peptide InnOvatioN for Early diabEtes tReatment). Interestingly, like injectable semaglutide (1.0 mg), oral semaglutide (14 mg) daily has also shown superior HbA1c and weight lowering compared to several active comparators that include empagliflozin 25 mg (PIONEER 2, Global), sitagliptin 100 mg (PIONEER 3 and PIONEER 7, Global), injectable liraglutide 0.9 mg (PIONEER 9, Japanese) and 1.8 mg (PIONEER 4, Global), and injectable dulaglutide 0.75 mg (PIONEER 10, Japanese) [8–13]. These findings suggest that both formulations of semaglutide are seemingly effective HbA1c and weight-lowering agents in people with T2D. Interestingly, there are no Phase 3 head-to-head randomized controlled trials (RCTs) that compared these two formulations of semaglutide in people with T2D. However, an indirect comparison of PIONEER 1 and SUSTAIN 1 trial with oral *vs.* injectable semaglutide, respectively, (against placebo) showed similar proportions of patients with T2D achieved ≥ 5% (44% *vs.* 45%, respectively) and ≥ 10% (14% *vs.* 13%) weight loss despite a higher baseline mean body weight in SUSTAIN 1 (96.8 kg) compared with PIONEER 1 (88.1 kg) [14]. These observations concur with the findings from a study that showed a similar circulating level of semaglutide exposure with two different formulations of oral (14 mg) *vs.* injectable semaglutide (1.0 mg) [15].

Recently, a few observational studies have compared the safety and efficacy of oral *vs.* injectable semaglutide in people with T2D. A retrospective, single-center study (n = 103) from the UK [16] studied the comparative effectiveness of oral (n = 53) *vs.* injectable semaglutide (n =

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Table 1 Head-to-head randomized controlled trials of GLP-1RAs in people with type 2 diabetes

Trial eponym	Comparator arms, *N*	Duration (weeks)	Mean HbA1c (%) changes at EOS	Δ HbA1c (%) at EOS, *p*-value	Mean weight (kg) changes at EOS	Δ Weight (kg) at EOS, *p*-value	Adverse events	Drug discontinuation due to any cause
PIONEER 4 [12]	SEMA 14, 285	52	−1.2	−0.3, $p = 0.0002$	−4.3	−1.3, $p = 0.002$	Nausea: 20%	11%
	LIRA 1.8, 284		−0.9		−3.0		Nausea: 18%	9%
PIONEER 9 [11]	SEMA 14, 48	52	−1.5	−0.3, $p = 0.1$	−2.6	−2.7, $p < 0.0001$	Nausea: 8%	4%
	LIRA 0.9, 48		−1.2		0		Nausea: 0%	0%
PIONEER 10 [13]	SEMA 14, 130	52	−1.7	−0.3, $p = 0.02$	−1.6	−2.6, $p < 0.0001$	Nausea: 9%	6%
	DULA 0.75, 65		−1.4		+1.0		Nausea: 9%	3%
AWARD 6 [7]	DULA 1.5, 299	26	−1.42	−0.06, $p > 0.05$	−2.90	0.71, $p = 0.01$	GI: 36%	6%
	LIRA 1.8, 300		−1.36		−3.61		GI: 36%	6%
SUSTAIN 3 [5]	SEMA 1.0, 404	56	−1.5	−0.62, $p < 0.0001$	−5.6	−3.78, $p <$ 0.0001	Nausea: 22%	9%
	EXE ER 2.0, 405		−0.9		−1.9		Nausea: 12%	7%
SUSTAIN 7 [4]	SEMA 1.0, 300	40	−1.8	−0.41, $p < 0.0001$	−6.5	−3.55, $p <$ 0.0001	GI: 44%	10%
	DULA 1.5, 299		−1.4		−3.0		GI: 48%	7%
SUSTAIN 10 [3]	SEMA 1.0, 290	30	−1.7	−0.69, $p < 0.0001$	−5.8	−3.83, $p <$ 0.0001	GI: 44%	11%
	LIRA 1.2, 287		−1.0		−1.9		GI: 38%	7%

PIONEER Peptide InnOvatioN for Early diabEtes tReatment, *AWARD* Assessment of Weekly AdministRation of LY2189265 [dulaglutide] in Diabetes, *SUSTAIN*, Semaglutide Unabated Sustainability In Treatment of Type 2 Diabetes, *HbA1c* glycated hemoglobin, *EOS* end of study, *GI* gastrointestinal, *SEMA* semaglutide, *LIRA* liraglutide, *DULA* dulaglutide, *EXE ER* exenatide extended-release

50) in T2D. There was no significant difference in mean HbA1c (−1.77% *vs.* −1.90%), mean body weight (−9.0 kg *vs.* −7.2 kg), and mean body mass index (BMI −3.3 kg/m^2 *vs.* −2.5 kg/m^2) lowering with oral *vs.* injectable semaglutide, respectively, at 6-month (p = not significant, for all parameters). Concerning adverse events, gastrointestinal (GI) side effects were similar with both formulations (47% *vs.* 52% with oral *vs.* injectable semaglutide, respectively), and 17% of oral and 10% of injectable semaglutide receivers discontinued the treatment for various reasons. Another

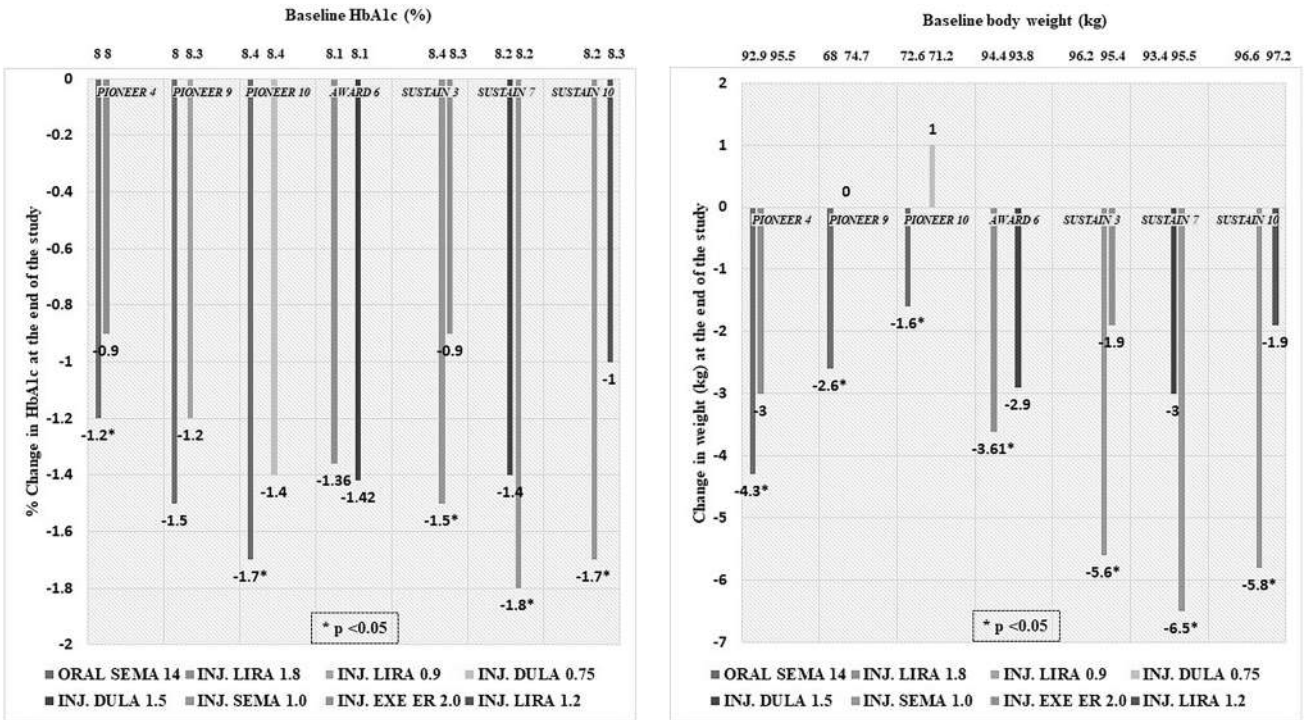

Fig. 1 HbA1c and body weight lowering with GLP-1RAs (head-to-head) in people with type 2 diabetes in RCTs

Table 2 Comparative retrospective studies with oral vs. injectable semaglutide in type 2 diabetes

First author, country	Drug arm, *n*	Duration of study	Male (%)	Mean age, (Yrs.)	Mean weight, (Kg)	Mean BMI (Kg/m^2)	Mean HbA1c (%)	eCVD (%)	Mean HbA1c changes at EOS (%)	Mean weight changes at EOS, (kg)	A1c < 6.5% achieved	≥ 5% weight loss	≥ 10% weight loss	Adverse events	Drug discontinued
Chowdhury et al. [16], UK	Oral, *n* = 53	6 M	49	58.5	109.7	39.3	9.28	11	−1.77	−9.0	NR	NR	NR	GI: 47%.	17%
	Inj., *n* = 50		46	56	114.0	40.0	9.48	14	−1.90	−7.2	NR	NR	NR	GI: 52%	10%
Fadini et al. [18], Italy	Oral, *n* = 107*	18 M	63.6	64.5	83.3	29.3	7.8	14	−0.9	−3.3	34.6	36 %	NR	NR	60% Continued
	Inj., *n* = 107*		63.6	64	84.6	29.7	7.7	10.8	−0.9	−3.7	34.6	52.1 %	NR	NR	70% Continued
Klobucar et al. [17], Croatia	Oral, *n* = 53	6 M	56.6	59!	97.3!	32.9!	8.8!	17	−1.40!	−5.9!	NR	NR	15.1 %	Nausea: 20%	0%
	Inj., *n* = 53		56.6	63!	102!	34.7!	8.0!	22.6	−1.10!	−6.5!	NR	NR	20.7%	Nausea: 22%	0%

Yrs years, *M* months, *Inj.* injectable, *UK* United Kingdom, *BMI* body mass index, *eCVD* established cardiovascular disease, *EOS* end of the study, *Kg* kilograms, *GI* gastrointestinal, *NR* not reported/ retrievable

*Propensity-matched

!Median values

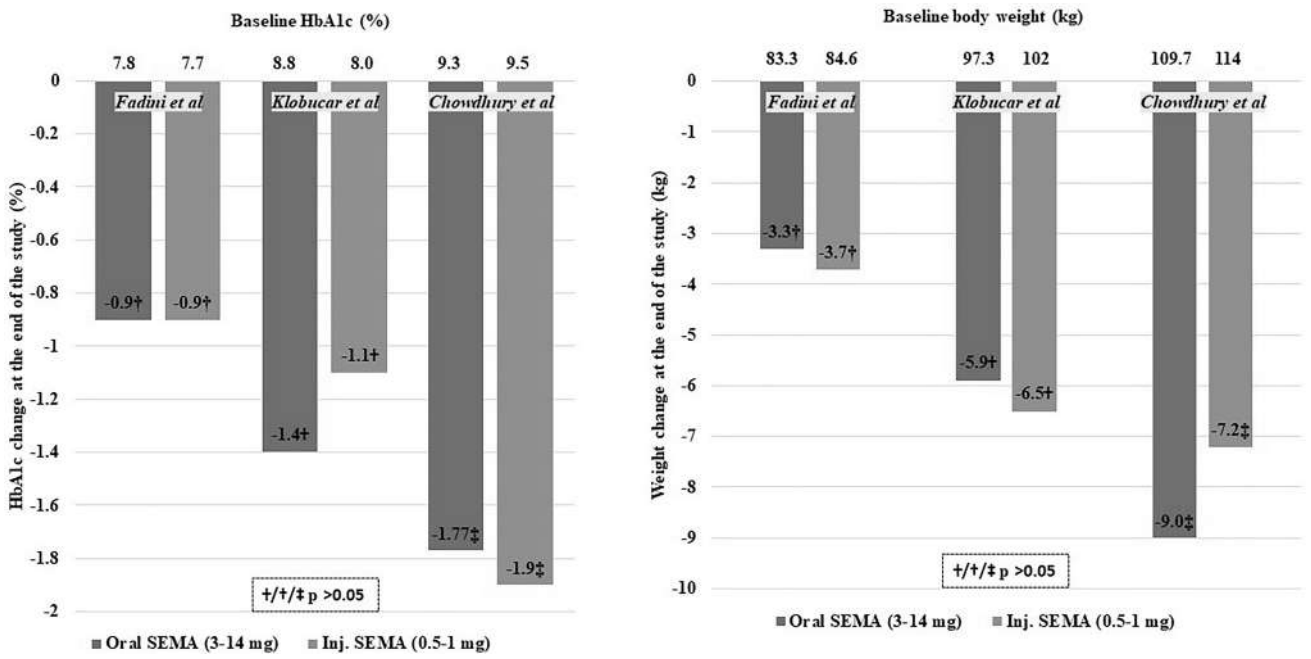

Fig. 2 HbA1c and body weight lowering with oral vs. injectable semaglutide in people with type 2 diabetes in real-world studies

single-center retrospective study ($n = 106$) from Croatia [17] compared the effectiveness of oral *vs.* injectable semaglutide in people with T2D, who are naïve to GLP-1RA. This study showed no significant difference in median HbA1c (−1.4% *vs.* −1.1%, $p = 0.13$) and median body weight (−5.9 kg *vs.* −6.5 kg, $p = 0.71$)) reduction between oral *vs.* injectable semaglutide, respectively, at 6 months. Notably, while baseline median HbA1c was significantly higher in the oral semaglutide arm (8.8% *vs.* 8.0%, oral *vs.* injectable semaglutide, respectively, $p = 0.04$), body weight was insignificantly higher in the injectable semaglutide arm (97.3 kg *vs.* 102 kg, oral *vs.* injectable semaglutide, respectively, $p = 0.08$). A weight loss of > 10% was achieved in similar proportions of patients with T2D on both formulations (15.1% *vs.* 20.7%, oral *vs.* injectable semaglutide, respectively, $p > 0.05$). Concerning safety, nausea, the most common GI side effect was seen in similar proportions of patients with both formulations (20% *vs.* 22%, oral *vs.* injectable semaglutide, respectively) and none discontinued the treatment in both arms. Similarly, a propensity-matched, retrospective study ($n = 214$) from Italy [18] comparatively assessed the effectiveness of oral ($n = 107$) *vs.* injectable ($n = 107$) semaglutide in people with T2D for 18 months. Both formulations of semaglutide reduced HbA1c (−0.9% in each arm) and body weight (−3.3 kg *vs.* −3.7 kg, oral *vs.* semaglutide, respectively) similarly at 18 months. However, a higher proportion of patients have ≥ 5% weight loss (52% *vs.* 36%) and persistently continued (70% *vs.* 60%) on injectable *vs.* oral semaglutide, respectively.

Collectively, the HbA1c and weight-lowering potential of oral semaglutide (7–14 mg) appear to be nearly similar to injectable semaglutide (0.5–1.0 mg) and larger than other GLP-1RAs, currently approved in people with T2D. Table 1 summarizes the findings of results from the head-to-head RCTs of GLP-1RAs conducted to date and Figure 1 graphically represents the efficacy outcome. Table 2 summarizes the baseline characteristics and findings of results from the observational studies conducted to date that compared oral *vs.* injectable semaglutide, and Figure 2 graphically represents the efficacy outcome. Notwithstanding, unlike injectable semaglutide which has shown a significant reduction in MACE (SUSTAIN 6) and has an additional label for cardiovascular (CV) risk reduction, oral semaglutide is yet to show CV superiority over placebo (PIONEER 6) [19, 20]. Since PIONEER 6 was not powered to assess the CV superiority of oral semaglutide over placebo, the SOUL (Semaglutide cardiOvascular oUtcomes triaL, NCT03914326) has been specifically designed for this purpose and is estimated to be complete by July 2024 [21].

Author contribution All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship and take responsibility for the integrity of the work.

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REVIEW ARTICLE

RSSDI endorses the IDF Position Statement on 1 h post load plasma glucose for diagnosis of intermediate hyperglycemia and type 2 diabetes

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Abstract
The Oral Glucose Tolerance Test (OGTT) remains a gold standard for diagnosis of diabetes and prediabetes all over the world and also in India. The original OGTT was a five sample test which included fasting, 30, 60, 90, and 120 min. Later, the test was modified in the US to two sample test 0 and 120 min, i.e., fasting and 2 h after 75 g glucose and this has been in practice all over the world. Traditional diabetologists continue to measure some of the intermediate samples, particularly the 60 min or 1 h value which identifies individuals even before the fasting or 2 h value becomes abnormal. Thus, even before the stage of prediabetes when one has a normal fasting and 2 h value, a raised 1 h value above 155 mg/dl has been shown to predict who will progress to diabetes. A group of 22 international experts recently got together and the IDF Position Statement on the 1 h value was published which shows why the 1 h value in the OGTT should be reintroduced in the routine lab testing of OGTT. This article is an endorsement of the IDF Position Statement on the 1 h value. Introducing the 1 h value in the OGTT is particularly relevant to India which has one of the fastest conversions of prediabetes to diabetes and also a very rapid loss of beta cell function. Identifying early stages of intermediate hyperglycemia can help to prevent diabetes and also reverse the condition.

Keywords Prediabetes · Type 2 diabetes · Position Statement · IDF · RSSDI · 1 hour value · OGTT

Introcuction

The Oral Glucose Tolerance Test (OGTT) first introduced in 1923 by Jerome Conn [1] continues to be the "gold standard" for diagnosis of diabetes and prediabetes in most parts of the world [2]. There is a concerted move in the United States and more recently in the UK and some parts of Europe to dispense with the OGTT altogether and to replace it with the fasting plasma glucose (FPG) and HbA1c test. However, many organizations like the World Health Organization (WHO) and the International Diabetes Federation (IDF) continue to recommend the OGTT for diagnosis of diabetes and prediabetes. Over the years, the diagnostic cut points for diagnosis of diabetes and prediabetes using an OGTT have changed. The glucose load used also used to differ between different countries with 50 g being used in the UK and 100 g used in the US, a few decades ago. All these changed in 1979 when the National Diabetes Data Group (NDDG) standardized the OGTT and recommended using 75 g as the glucose load as a compromise and the diagnostic criteria for diabetes and prediabetes were laid down [3].

The original OGTT was a five sample test which had fasting, 30, 60, 90, and 120 min samples. However, the NDDG simplified the OGTT and three of the intermediate values, i.e., 30, 60, and 90 min, were removed, leaving only the fasting and the 2 h value. The cut points for fasting were later modified and the fasting of 126 mg/dl (7 mmol/l) and 2 h value of 200 mg/dl (11.1 mmol/l) became the gold standard cut points used for diagnosis of diabetes. There was some disagreement with regard to the diagnostic values for prediabetes, particularly impaired fasting glucose (IFG), as the Americans used a cut point of ≥ 100 and ≤ 125 mg/dl for diagnosis of IFG while the WHO and the IDF recommended the use of ≥ 110 and < 125 mg/dl to diagnose IFG. For the

Extended author information available on the last page of the article

diagnosis of impaired glucose tolerance (IGT), there was no controversy, as values between 140 and 199 mg/dl were used for diagnosis of IGT worldwide. The Research Society for Study of Diabetes in India (RSSDI) has also supported the IDF and WHO with respect to these cut points [4].

Review of Literature

Despite dropping the intermediate values in the OGTT, conventional diabetologists in many countries including India continued to do a 3 sample OGTT including the 1 h (or 60 min) value also in the OGTT. Several studies including the San Antonio Heart Study [5], Botnia Study, and the Malmo Preventive Project (MPP) [6] showed that if the 1 h value exceeded 155 mg/dl or more, even in those with normal fasting and 2 h values, it predicted progression to prediabetes or diabetes in the future [7, 8]. Indian data also supported the exact same cut point of 155 mg/dl [7–10]. In a commentary published in Lancet Diabetes and Endocrinology [11], the case for retaining or reintroducing the 1 h value in the OGTT was made. Recently, the IDF has published a Position Statement on the need for reintroducing the 1 h value in the OGTT and presents all the evidence for this [12]. The basic argument is that in people who have normal fasting and 2 h values in the OGTT (which means that technically they do not have diabetes or prediabetes), an elevated 1 h in the OGTT (> 155 mg/dl) could serve as an earlier marker in for development of diabetes or prediabetes in the future. Loss of beta cell function occurs very early in the natural history of diabetes and even by the stage of prediabetes, a considerable amount of beta cell function is lost [13, 14]. It therefore makes sense to identify people earlier in the natural history of diabetes. This will help to start preventive lifestyle measures earlier and this may help to slow down or postpone the loss of pancreatic beta cell function, although admittedly, we need RCTs to prove this.

The IDF Position Statement lists several other advantages of the 1 h OGTT test and these are summarized in Table 1.

Reproduced from the IDF Position Statement [12]

The IDF Position Statement also recommends a screening algorithm for diagnosis of prediabetes (intermediate hyperglycemia) in type 2 diabetes using a diabetes risk score. To identify the high-risk population in India, the Madras Diabetes Research Foundation – Indian Diabetes Risk Score (MDRF – IDRS) which has been extensively validated can be used for this purpose [15–20]; all those who are at high risk can then undergo the three sample OGTT, fasting, 1 h and 2 h samples; the IDF Position Statement says that those who have a 1 h value of more than 209 mg/dl can then be called back for a second OGTT using the 2 h values. While this has some advantages in terms of restricting the tests to 1 h and thereby saving time in a busy clinic and also waiting time for the person being screened, it would mean that the individual has to come to the clinic twice which is impractical especially in lower- and middle-income countries including India. Therefore, it may be more practical to do only one OGTT adding the 1 h sample while doing the OGTT. This will have several advantages. Firstly, the present diagnostic fasting and 2 h cut points for diagnosis of diabetes and prediabetes can remain the same as before, but additionally we will have the 1 h cut point also. Those whose 1 h value is > 155 mg/dl but have normal fasting and 2 h can be considered to have an earlier stage of intermediate hyperglycemia and can be prescribed lifestyle intervention and referred to a diabetes prevention program [12].

A recent review of the global prevalence of prediabetes showed that while in most parts of the world IGT is more common, in South Asia and South East Asia, impaired fasting glucose (IFG) is more common [21, 22]. The recent

Table 1 Advantages of using 1 h value in OGTT [12]

1. Defines high risk of T2D in adults, children, and youth
2. Associated with worsened metabolic and atherogenic profiles
3. Identifies risk for micro- and macrovascular complications and mortality
4. Identifies risk for OSA, CFRD, MASLD, and severity of hepatic fibrosis
5. Occurs *before* the onset of IGT
6. Merits identification *before* IGT occurs
7. Cost-effective for high-risk screening
8. Provides opportunity for earlier detection and intervention in high-risk populations identified with primary screening tools (FINDRISC, ADA)
9. May benefit from lifestyle and pharmacologic interventions to reduce progression to T2D
10. Reduces diagnostic complexity and confusion with current diagnostic criteria for IH
11. Shortens OGTT from 2 to 1 h making it more practical and clinically acceptable
12. Threshold ≥ 209 mg/dl (11.6 mmol/L) defines T2D

OSA Obstructive sleep apnoea, *CFRD* Cystic fibrosis-related diabetes mellitus, *MASLD* Metabolic dysfunction-associated steatotic liver disease

ICMR – INDIAB Study showed that there are 101 million people with diabetes and 136 million people with prediabetes and moreover the majority of those with prediabetes in India have impaired fasting glucose [23]. This means that the 2 h value in OGTT is often normal. Therefore, the 1 h value in the OGTT becomes even more important in India. There is also evidence that progression from prediabetes to diabetes occurs faster in South Asians living in the UK [24] as well as Indians in India [25].

Conclusions

The subtypes of type 2 diabetes in Indians are also slightly different from that reported in Caucasians by Alquist et al. [26]. In India, the severe insulin-deficient diabetes variety (SIDD) appears to be common and diabetes occurs at much lower BMI [27]. Lean type 2 diabetes is also common in India [28–31]. Given these variations in the phenotype of type 2 diabetes and the rapid progression to type 2 diabetes in Indians, the move by the IDF introducing the 1 h value in the OGTT for earlier identification of individuals is to be welcomed and the Research Society for the Study of Diabetes in India (RSSDI) supports and endorses the Position Statement of the IDF on the 1 h post load plasma glucose for the diagnosis of intermediate hyperglycemia and type 2 diabetes [12].

Declarations

Consent to participate Not relevant as it is a review article.

Conflict of interest The authors declare no competing interests.

Research involving human participants and/or animals Not relevant.

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REVIEW ARTICLE

The association between enterovirus (EV) infection and the risk of type 1 diabetes: a meta-analysis

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Abstract

Objective The present systematic review was aimed to explore the possible relationship between enterovirus (EV) infection and type 1 diabetes (T1D) as an autoimmune disease.

Methods The major international databases including PubMed, ISI Web of Science, Scopus, and Embase were searched for human and animal studies published until November 2020.

Results After comprehensive search of databases, 29 eligible studies were included in the meta-analysis. We found that all studies used at least one of the autoimmune antibodies including ICA, GADA, IA2A, and IAA to identify the pre-diabetic subjects. According to the results, the estimated odds ratio of the risk of T1D associated with EV was 1.19 (95% CI, 1.10–1.29, $I^2 = 80.5$). Our finding also revealed that there was a positive association between EV positivity and patients with a higher risk of T1D (OR = 1.35, 95% CI = 1.29 to 1.41).

Conclusion Our findings suggest that EV infections can be a major risk factor for T1D and EV positivity is importantly associated with β-cell autoimmunity and a higher risk of T1D.

Keywords Diabetes · Type 1 diabetes (T1D) · Enteroviruses (EVs) · Autoimmune antibody

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing pancreatic β-cells and failure in insulin synthesis. Etiologically, T1D resulted from cross-talk between genetic markers, the immune system, and environmental factors [1]. T1D is divided into two subclasses; type 1A results from autoimmune degradation of β-cells and type 1B is idiopathic or non-autoimmune diabetes [2, 3]. Based on the epidemiological studies, the prevalence of T1D in the United States is 1 in 300 children and its annual incidence is 2–5% worldwide [4]. Clinically, the progression of T1D can be described by three distinct stages. In the first stage, the patient has no clinical symptoms; however, environmental factors along with genetic susceptibility markers promote triggering β-cell autoimmunity which is characterized by the presence of autoantibodies against insulin (INS), glutamate decarboxylase 65 (GAD65), islet antigen 2 (IA-2), and zinc transporter 8 (ZNT8) [5, 6]. Stage 2 featured abnormal glucose tolerance and mild hyperglycemia due to decreasing in the function and mass of β-cell [7]. Eventually, in stage 3, β-cells are almost destroyed and clinical symptoms such as severe hyperglycemia, polydipsia, polyuria, and polyphagia become apparent [8]. Among the genetic markers, encoding loci of major histocompatibility complex (MHC) are involved in nearly 40–50% of the familial clustering of T1D [9, 10]. Accordingly, variants of class II human leukocyte antigen (HLA) genes such as encoding DQ, DR, and

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DP are participating in the progression of T1D [11]. High-throughput omics analysis models, including genome-wide association, metabolomics, transcriptomics, proteomics, and microbiome analyses, have shown to be a remarkably effective way for discovery of features that are associated with islet autoimmunity [12]. Recently, it has been reported that microbial and viral infections may be instrumental in the development of autoimmune diseases. Microorganisms, through various mechanisms, not limited to molecular mimicry, cross-reactivity, epitope spreading, and polyclonal activation, can trigger autoimmunity [13, 14]. For example, accumulating evidence suggests that alteration in gut microbiota may potentially be associated with immune-mediated diseases including T1D. Several studies in animal models indicate that alterations in the intestinal microbiota are associated with the development of autoimmune diabetes [15, 16]. In this regard, study on viral infections suggested that several viruses including enteroviruses (EVs) may set off the autoimmune response and trigger several immune processes which overwhelm the immune regulatory mechanisms [17].

EVs are known as a group of small, single-stranded, positive-sense RNA viruses belonging to the Picornaviridae family [18]. Previous studies showed that infection with EVs is one of the main environmental agents significantly associated with islet autoimmunity and could potentially trigger T1D [19, 20]. Human pancreatic β-cells are susceptible to infection with EVs, especially by the members of the Coxsackievirus B family. The pancreatic β-cells tropism to EVs is based on two points: first, these cells can internalize the virus through the expression of its specific receptors, and second, pancreatic β-cells have a host factor that facilitates entrance, replication, and persistence of the virus and, therefore, infection with a virus [21]. In this regard, EVs are suspected to contribute to insulin-producing β-cell loss and consequently induced hyperglycemia and diabetes. Dotta et al. described that infection of β-cells with non-cytopathic enteroviral under T1D condition is associated with the failure in glucose metabolism and might potentially result in diabetes [22]. Furthermore, Chehadeh et al. have suggested that infection with enterovirus leads to the secretion of IFNα by β-cells which is followed by up-regulation of class I MHC and activation of the innate immune system [23]. Several studies have revealed that there is an association between several autoantibody including ICA, GADA, IA2A, and IAA positivity and enterovirus infection [24–27]. Altogether, in genetically susceptible subjects, infection with enterovirus along with a decrease in tolerance to β-cells antigens finally resulted in β-cell damage and triggered insulin-dependent diabetes. The association between enterovirus infection and T1D was investigated in a systematic review and meta-analysis study by Yeung et al. previously. But, according to the further studies from 2011 to yet, especially by attention to the HLA genotyping in one hand and controversy in results by previous studies; on the other, in this meta-analysis, we determined the association between enteroviruses and type 1 diabetes.

Methods

Strategy of search

A comprehensive search for experimental studies was conducted by 2 researchers independently. In this regard, researchers have searched PubMed, Science Direct, Scopus, Embase, and Google Scholar databases for papers published from 2011 to 2020 with the English language. The keywords used for the search were as follows: "diabetes mellitus," "type 1 diabetes," "autoimmune antibody," "β-cell autoimmunity," "coxsackievirus," "enterovirus," and "ECHOvirus." Studies that used molecular methods for RNA detection such as RT-PCR and in situ hybridization or the ELISA method for detection of viral capsid protein in the blood, stool, and tissue of T1D and pre-diabetic patients, reported the source and number of samples results were eligible for this meta-analysis, while the exclusion criteria were case reports, meta-analyses, review articles, studies presented in conferences, and/or seminars and letter to editors.

Extraction and statistical analysis of data

Data including author name, year, country, sample size, sample source, enterovirus strains, and detection methods were extracted from included studies. Meta-analysis was performed with at least three studies. Cochrane's Q statistic and the I^2 test were used for the assessment of heterogeneity and considered significant if $I^2 > 50\%$ or p-value < 0.1. Due to the heterogeneity among the results of our studies, the random effects model was used to combine the study findings. Furthermore, in order to evaluate the publication bias, funnel plots and Begger's and Egger's tests were applied.

Results

In this study, a total of 757 articles were returned by searching in described databases to November 2020. Afterward, 390 duplicate articles were removed by Endnote software. By review of the title and abstract, 312 unrelated articles were excluded. Thus, 55 full-text articles were selected, of which 26 articles without expected data for extraction (including autoimmune antibody results, results of approval test for diabetes, and results for detection of virus) did not meet the criteria for inclusion in the meta-analysis and were excluded. Finally, 29 eligible studies were used in the present analysis (Fig. 1).

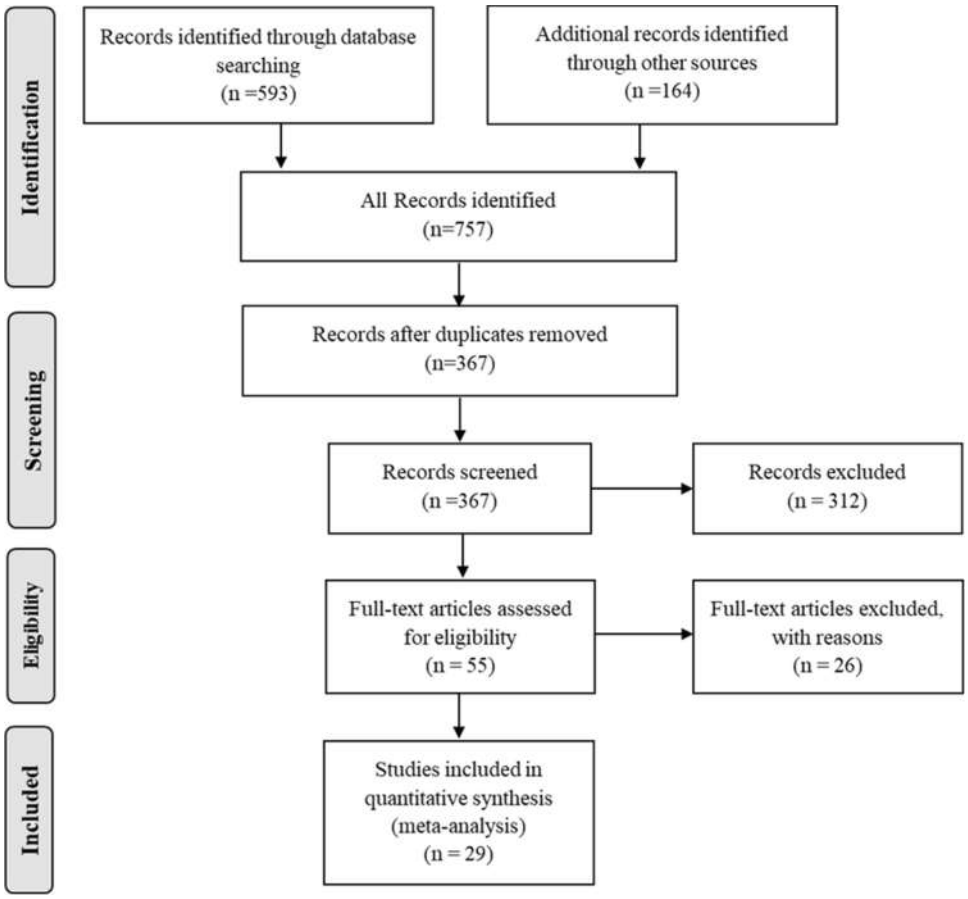

Fig. 1 Diagram of the search strategy

Characteristics of included studies

Pre-diabetic

Characteristics of studies selected by our inclusion criteria are presented in Table 1. As demonstrated in Table 1, eleven studies have used PCR to detect the enterovirus and coxsackievirus B in collected samples from pre-diabetic subjects while three performed the ELISA method to detect the enterovirus and coxsackievirus B (CVB) in study groups. All studies have used at least one of the autoimmune antibodies including ICA, GADA, IA2A, and IAA to identify the pre-diabetic subjects.

Table 1 Characteristics and all parameters included in the selected studies for pre-diabetic subjects

Country	Year	Study	Number		Virus detection method		Strain		Autoantibody	RR	Sample
			Case	Control	PCR	ELISA	EV	CV			
Sweden	2012	Sabina Resˇ ic´ Lindehammer et al	223	592	Yes	Yes	Yes		GADA, IA2A, IAA	1.29	Blood
Finland	2011	Sami Oikarinen et al	38	140	Yes		Yes		ICA, GADA, IA2A, IAA	1.12	Blood
Finland	2014	Olli H. Laitinen et al	183	366	Yes			Yes	ICA, GADA, IA2A, IAA	1.28	Blood
Finland	2017	Hanna Honkanen et al	97	221	Yes			Yes	ICA, IA2A, IAA	1.24	Stool
Cuba	2011	Ileana Cubas-Duen˜as et al	58	224	Yes		Yes		ICA, GADA, IA2A,	2.72	Blood
Norway	2014	Ondrej Cinek et al	133	226	Yes			Yes	IA2A	0.81	Blood
Australia	2012	Wing-Chi G et al	27	40	Yes			Yes	IA2A, IAA	1.18	Blood
Prague	2011	German Tapia et al	339	692	Yes			Yes	IA2A	0.95	Stool
Iran	2018	Omid Zargari Samani et al	35	35		Yes		Yes	GADA	2.17	Blood
Finland	2018	Amir-Babak Sioofy-Khojine et al	169	332	Yes			Yes	GADA, IAA	1.71	Blood
Finland	2011	Lempainen et al	28	202		Yes		Yes	ICA, GADA, IA2A, IAA	3.26	Blood
Finland	2017	Hanna Viskari et al	64	251	Yes			Yes	ICA, GADA, IA2A, IAA	0.82	Stool
Cuba	2012	Luis Sarmiento et al	20	40	Yes			Yes	GADA, IA2A	3.31	Blood

Diabetic

Characteristics of the studies included in our study for diabetic patients are presented in Table 2. Twelve studies have used blood samples to detect the virus while other studies have used tissue samples including the pancreas and small intestine as well as fecal samples. Although the PCR technique has been performed in fourteen studies, two studies used the ISH method as well as three studies have performed the ELISA technique to find out the presence of enterovirus in collected samples.

Meta-analysis of enterovirus and type 1 diabetes high-risk subjects

As demonstrated in Fig. 2, the overall odds ratio (95%, CI) of type 1 diabetes was 1.19 (1.10, 1.29) which indicates that subjects with autoimmune antibodies exhibit a higher risk (1.19-fold) of developing type 1 diabetes. Our analysis showed a significant heterogeneity across the studies ($I^2 = 80.5$, p-value < 0.0001).

Begg and Egger's tests were applied to assess publication bias among studies. Analysis showed no significant publication bias among studies ($p = 0.069$ and $p = 0.18$, respectively). Moreover, the symmetric shape of the funnel plot also approves no evidence of publication bias (Fig. 3).

Meta-analysis of enterovirus and type 1 diabetes

The individual and summary odds ratios of selected studies have shown in Fig. 4. Our analysis revealed that all studies except one had an odds ratio over 1 for enterovirus infection in patients with type 1 diabetes. Meta-analysis revealed that there was a positive association between enterovirus positivity and type 1 diabetes (OR = 1.35, 95% CI = 1.29 to 1.41). The results showed significant heterogeneity among the studies ($I^2 = 85.4\%$, p-value < 0.0001).

Begg and Egger's tests were applied to assess publication bias among studies on enterovirus and type 1 diabetes. There was a significant publication bias among studies by the Begg and Egger tests ($p = 0.046$ and $p < 0.001$, respectively). Moreover, the asymmetric shape of the funnel plot also approves evidence of publication bias (Fig. 5).

Quality of the studies

According to the Newcastle–Ottawa scores, all selected studies had high quality and there were no studies with low quality.

Discussion

The present systematic review and meta-analysis aimed to assess the association between enterovirus infection and type 1 diabetes. Type 1 diabetes results from the interplay between genetic predisposition, the immune system, and environmental factors. Epidemiological and experimental data strongly suggest a role for enterovirus in T1D triggering, but a lot of controversies and unanswered questions remained**.** In this systematic review, a total of 757

Table 2 Characteristics and all parameters included in the selected studies for diabetic subjects

Country	Year	Study	Number		Virus detection method			Strain	Sample
			Case	Control	PCR	ISH	ELISA		
Finland	2012	Maarit Oikarinen	39	41	Yes	Yes		ev	Tissue
Finland	2014	Sami Oikarinen1	249	249			Yes	cvb1, cvb2, cvb3	Blood
Egypt	2018	Adel Abdel-Moneim et al	382	100	Yes			ev	Blood
Turkey	2018	Murat Karaoglan et al	40	30	Yes			cvb1, cav7	Blood
florida	2016	Xiaofang Bian et al	42	42	Yes		Yes	ebv, cvb	Blood
Finland	2012	Hanna Viskari et al	171	316	Yes			CBV4 or EV	Blood
Egypt	2017	Waled M. El-Senousy et al	382	100	Yes			ev	Blood
Italy	2018	Giovanni Federico et al	82	117	Yes			ev	Blood
Tunisia	2017	Imen Boussaid et al	95	141	Yes			ev	Blood
Norway	2016	M Hodik et al	27	24	Yes			cbv	Pancreatic tissue
Egypt	2017	M. Abdel-Latif et al	382	100	Yes			ev, cv	Blood
Italy	2013	Salvatoni A et al	24	61	Yes				Blood
Australia	2019	Ki Wook Kim et al	35	26	Yes				Fecal sample
Italy	2012	Mercalli V et al	25	21	Yes	Yes		ev	Small intestine
Australia	2018	Anne-Louise Ponsonby et al	333	660	Yes			ev	Blood
France	2018	Magloire Pandoua Nekoua et al	15	8			Yes	CV-B4	Blood

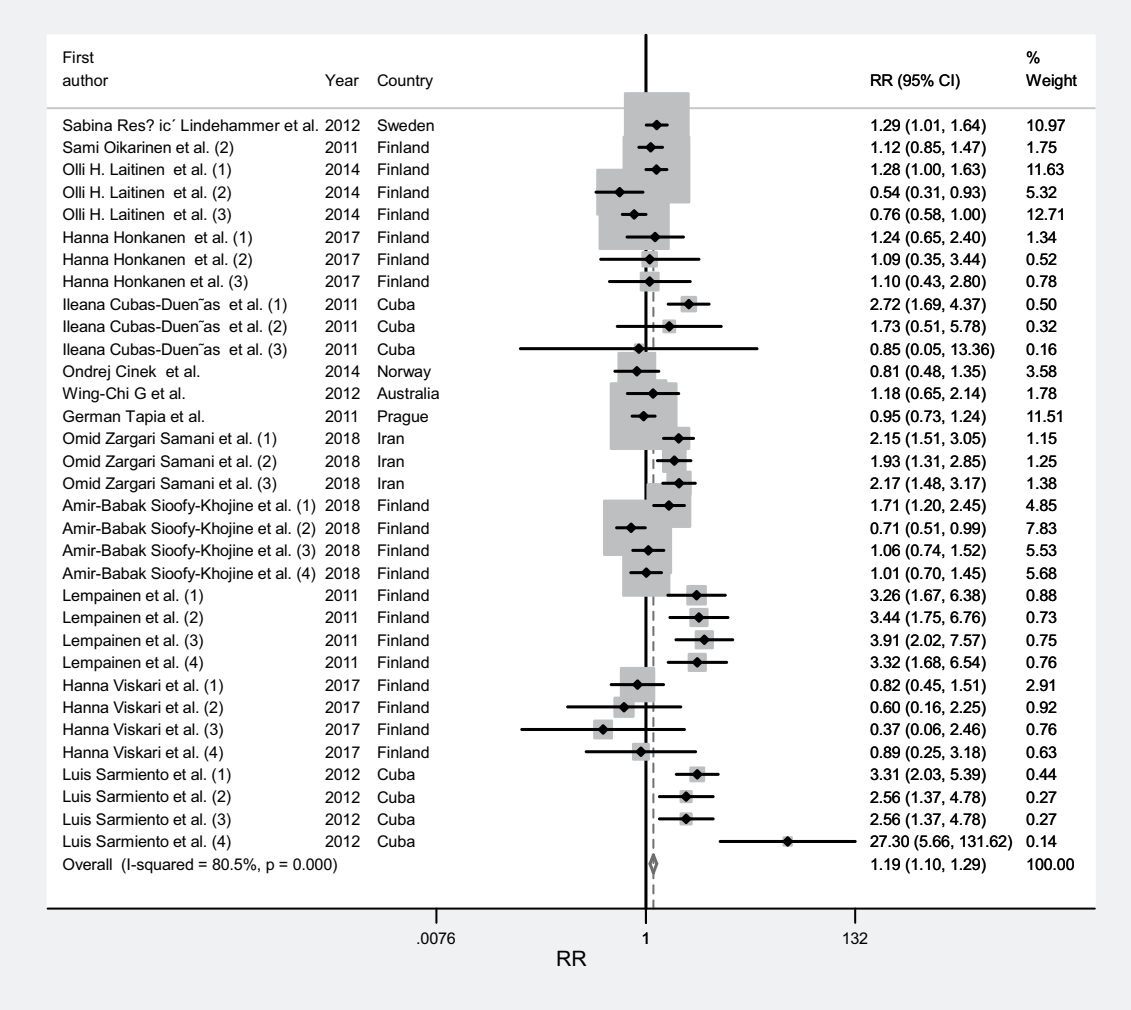

Fig. 2 Forest Plot of association between enteroviruses and type 1 diabetes high-risk subjects

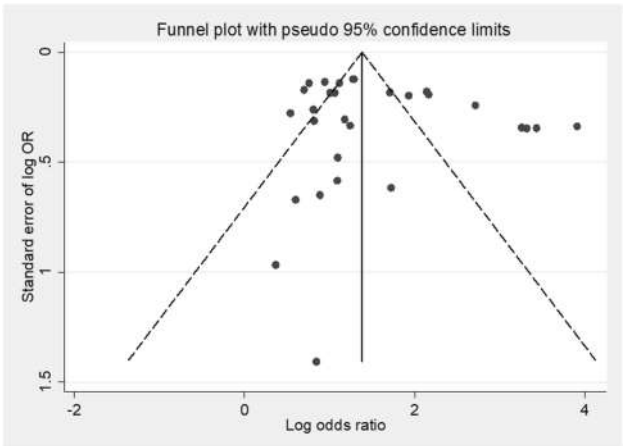

Fig. 3 Funnel plot of the association between enterovirus and type 1 diabetes high-risk subjects

articles were returned by searching in described databases from 2011 to 2020, and 29 eligible studies were used in the present analysis. The individual odds ratio and the overall estimation of meta-analysis revealed a positive association between enterovirus infection and type 1 diabetes**.**

An increasing incidence rate of type 1 diabetes has been observed for the last few decades, especially in young individuals (less than five years old) [28]. T1D is a multifactorial autoimmune disease resulting from the loss of functional insulin-producing pancreatic islet b cells in genetically susceptible individuals, but it is not entirely clear how b cells are damaged or destroyed [29]. The rapid spread of T1D disease in the world cannot be explained solely by genetic predisposition but also by environmental factors including viral infections [30]. In line with our findings, previous meta-analyses have shown a clinically significant association between enterovirus infection and islet autoimmunity or type 1

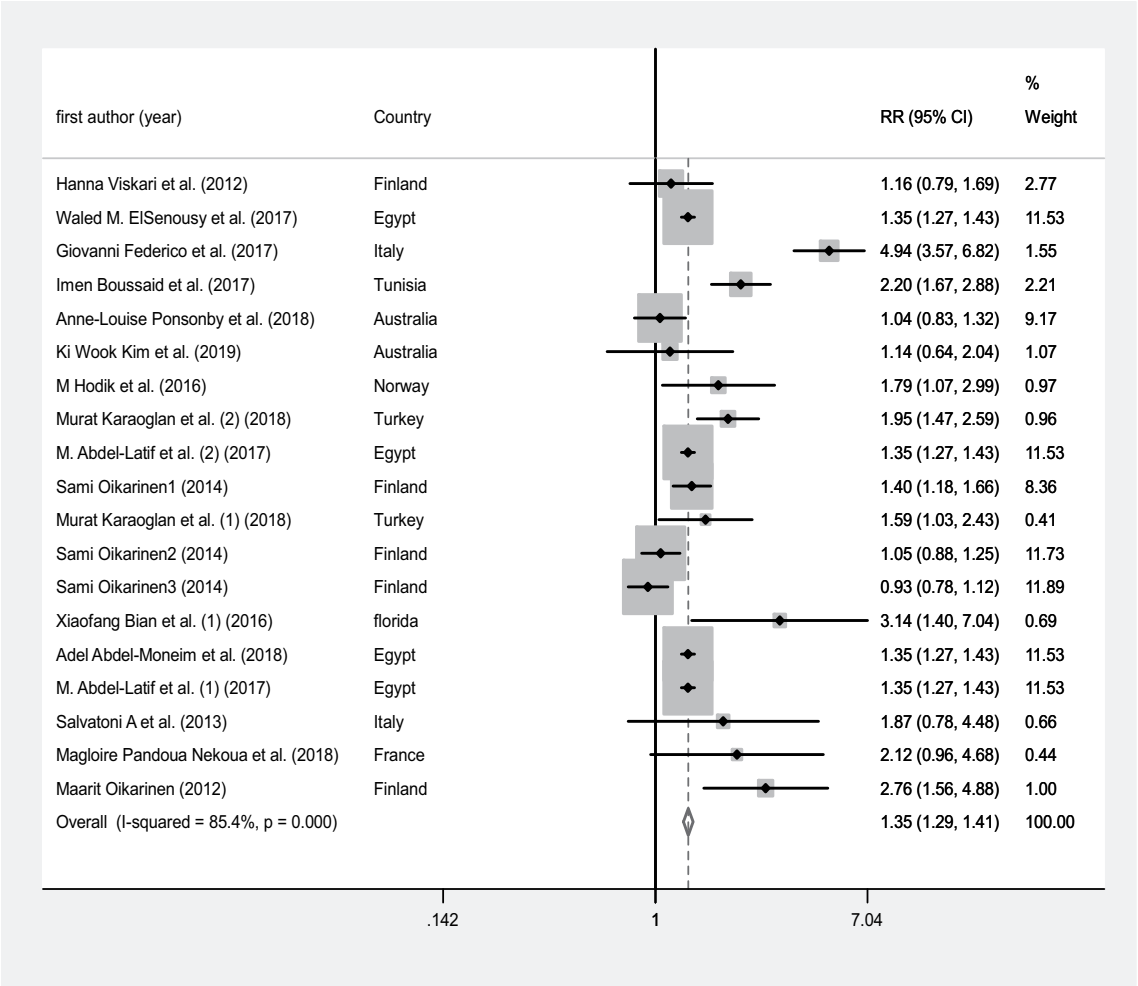

Fig. 4 Forest Plot of association between enteroviruses and type 1 diabetic subjects

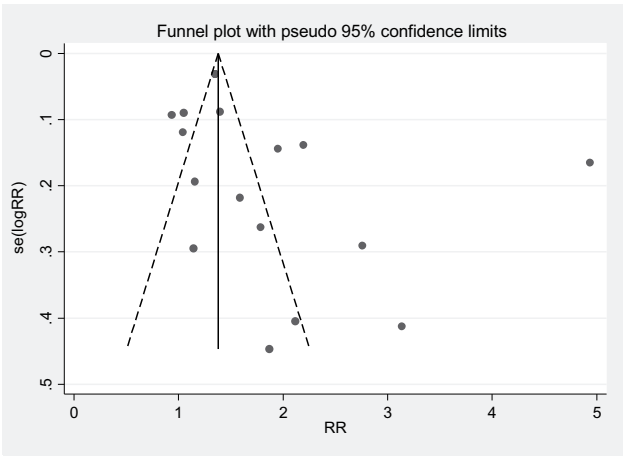

Fig. 5 Funnel plot of the association between enteroviruses and type 1 diabetic subjects

diabetes onset [31]. EV and particularly the serotypes of coxsackievirus B are the most likely viruses to be linked with the triggering of T1D [32]. These viruses are found to play a major role in the induction of the disease based on three possible mechanisms [33]. The first is the invasion of pancreatic cells by viruses through cell surface receptors and replication followed by the induction of innate immunity against the cells leading to destruction [34]. The second is altered immunity as a result of viral infection leading to autoimmunity [35]. The third is the induction of cross-reactive immunity between EV and pancreatic β-cell antigens as a result of antigen mimicry [36]. Previously, evaluation of the gut virome, metaproteome, and microbiota in young children uncovered a striking association between viral infection and trigger of islet autoimmunity and promote T1D [16]. On the other hands, enterovirus infection may exert a dramatic change and functional remodeling in microbiome and provoke autoimmunity and autoantibody production [37]. In this

regard, Gavin et al. have noted that multi-omic analyses of the viruses and the gut microbiome in childhood revealed a relationship between the microbiome, metaproteome, and virome diversity in the stool of children collected before and at the onset of islet autoimmunity [17].

Previous studies have also demonstrated that autoantibody production including ICA, GADA, IAA, and ZnT-8 (pancreaticβ-cell–specific zinc transporter) [38] as well as cytokine production including IL-1β, TNF–α, IFN-γ, IL-12, and IL–17 were found to be highly characteristic for T1D patients [39]. In this regard, Yeung et al. performed a meta-analysis on enterovirus and pre-diabetic individuals with positive at least one autoimmune antibody, including ICA, GADA, IA2A, and IAA, and reported a relationship between enterovirus infection and autoimmune antibody positivity [31]. In agreement with the reported data, we found that pre-diabetic individuals with positive at least one autoimmune antibody, including ICA, GADA, IA2A, and IAA, are significantly associated with enterovirus infection. It has been reported that all four islet autoantibodies—ICAs, IA-2 antibodies, GAD antibodies (GADAs), and insulin autoantibodies—are commonly found in childhood type 1 diabetes, as well as in non-diabetic relatives of type 1 diabetic patients, risk of type 1 diabetes in future is directly proportional to the positivity with the number of autoantibodies [40].

Enterovirus as a major environmental agent which is contributing to type 1 diabetes has been discovered in type 1 childhood diabetic patients with positive at least one autoimmune antibody including ICA, GADA, IA2A, and IAA [41]. Moreover, some studies showed a relationship between EV infection and several autoantibody including ICA, GADA, IA2A, and IAA positivity in patients [24–27]. The role of the enterovirus in autoimmunity is complex, and therefore, a better understanding of the autoimmunity of these viruses in T1D help design new strategies to treat and prevent disease. Although the results of this meta-analysis of observational studies cannot prove that infection with enterovirus plays a causal role in the pathogenesis of type 1 diabetes, our findings provide additional support to the studies with reports of enterovirus RNA positivity in pancreatic tissue of type 1 diabetic patients.

Conclusion

In conclusion, our meta-analysis results suggest that enterovirus infections are associated with type 1 diabetes. Data analysis has also revealed a positive association between enterovirus infections and β-cell autoimmunity. Our findings are in agreement with the idea that the infection with an enterovirus may contribute to damage to the β-cells and possibly trigger type 1 diabetes.

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

Declarations

Conflict of interest The authors declare no competing interests.

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REVIEW ARTICLE

Genetic screening for pathogenic variants in type 2 diabetes of the Arab Gulf population: A systematic review and meta-analysis

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Abstract

Objective The Arab Gulf is highly vulnerable to T2DM and its serious consequences. The manner in which these populations respond to such alterations in their surroundings may largely be governed by their genetic makeup. This review aimed to screen the genetic loci candidates that are associated with T2DM to assess the most prominent one in the early diagnosis of this chronic dysfunction.

Methods Variable pieces of literature were searched to assess the association between pathogenic single-nucleotide polymorphisms (SNPs) and the onset of T2DM in the Arab Gulf countries. The effects of odd ratio (OR), sample sizes, and collaborations of the captured genes of the eligible studies were analyzed. The protocol was registered in National Institute for Health Research.

Results Twenty-seven pathogenic SNPs were identified in 16 genes that were reported in 31 articles encompassing 15,982 patients and 11,976 controls. The highest numbers of conducted research were localized in Iraq and Saudi Arabia with 39% and 32%, respectively. *HNF4A* and *TCF7L2* genes represent the most extensively studied pathogenic genes in terms of the number of individuals included and the number of T2DM-related loci, respectively. Intron SNPs exhibited the highest percentages of pathogenic loci associated with T2DM with 61%. Moderate association between the pathogenic SNPs and disease outcome was observed, but strengths and weights of association vary across studies.

Conclusion For a better understanding of the molecular etiology of T2DM, finding SNPs, and establishing a meaningful genotype-phenotype connection for complicated diabetic disorders, the cumulative relevance of identified pathogenic SNPs in Arab Gulf was shown.

Keywords Arab Gulf · Association · Etiology · Polymorphism · Risky SNPs

Introduction

Diabetes mellitus is among the most popular research trends in modern medicine, in which genetic variables play a critical role in its onset and development. Diabetes leads to major health issues and complications such as retinopathy, neuropathy, exhaustion, weight loss, microvascular and macrovascular abnormalities, nephropathy, and numerous types of cardiovascular illnesses like hypertension and atherosclerosis; diabetes affects patients' quality of life. Type 2 diabetes mellitus (T2DM), the most common type of diabetes, has a significant negative impact on patient's quality of life and places a significant financial burden on the country's healthcare system [1].

For the time being, diabetes affects more than 500 million individuals globally, accounting for more than 10.5% of the adult population [2]. The last two decades have

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seen a significant increase in the prevalence of T2DM, which is attributed to its steady expansion, as evidenced by numerous studies [3]. The rate of T2DM prevalence has been estimated to exhibit a dangerous elevation as shown in the majority of reports that revealed that the number of T2DM patients having diabetes is estimated to be 640 million patients by the onset of 2040 [4]. Data from the WHO show that diabetes-related mortality has substantially expanded, and in the next 20 years, the number of people with diabetes is predicted to more than double. The Arab Gulf has the highest percentage (24.5%) of diabetes-related deaths in people of working age. The tenth edition of the IDF indicated that the global prevalence of diabetes has reached pandemic levels in this region. Though the association between genetic polymorphism and T2DM has been highlighted in the Arab world [5, 6], noticeable gaps of knowledge still exist in the genetic polymorphic variations that might be associated with the progression of the disease in the Arab Gulf region. Accordingly, genetic association studies of T2DM are beneficial in providing additional data that might aid in the early diagnosis and management of complications surrounding T2DM [7]. Despite the involvement of several genetic loci in the onset of T2DM, the present epidemic of this chronic complication cannot be only explained by one or a few loci [8–10]. Furthermore, the intensity of the possible association of these genetic loci may exhibit vast biological differences among populations worldwide.

The prevalence of T2DM is alarmingly high in the Arab Gulf, with some countries experiencing elevating rates of this disease. While lifestyle factors such as diet, physical activity, and obesity are known to contribute to the disease's risk [1], genetics is also believed to play a crucial role. Identifying the genetic loci candidates associated with T2DM in the Arab Gulf population is therefore essential to understanding the disease's underlying mechanisms and developing effective prevention and treatment strategies tailored to this population. Thus, it is necessary to pick up candidate genes implicated in molecular pathogenesis to find out the pathophysiological mechanisms of T2DM and to enhance its prognosis using the relevant biological markers [11].

This review aims to present a systematic review and meta-analysis of the genetic candidates most commonly associated with T2DM in the Arab Gulf region. By highlighting the potential of systems genetics, the review also seeks to identify these genes and identify the underlying mechanisms that contribute to the onset and progression of T2DM in this region. Various criteria were employed in this review to exclude the non-standardized publications in this area of expertise. This work provides a deeper understanding of the factors affecting T2DM as per their investigations in the Arab Gulf populations.

Materials and methods

Search strategy

Prior to commencing the review, we registered the International Perspective Register of Systematic Reviews (PROSPERO) protocol of the National Institute of Health (NIH) under the ID number 427938 (https://www.crd.york.ac.uk/PROSPERO/) [12]. Our study adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. To conduct this systematic review, we conducted an exhaustive literature search across four electronic databases: Google Scholar, PubMed, Web of Science, and Scopus. This search encompassed articles published from the inception of each database up to February 24, 2023. In order to include as many relevant studies as possible for our current meta-analysis, we broadened our search criteria to include the following terms: "DM," "Type 2 diabetes," "genetics," and "SNPs," in conjunction with the names of the Arab Gulf countries (Bahrain, Iraq, Saudi Arabia, Oman, Emirates, Kuwait, and Qatar). The study started with the identification of potential studies, which were then screened based on their titles and abstracts. Eligibility criteria were applied, and the selected studies were included. Once extracted, they were synthesized and analyzed, and the results were reported.

Study selection

This review focused on prospective studies that investigated the relationship between single-nucleotide polymorphisms (SNPs) within genes associated with the pathogenesis of type 2 diabetes mellitus (T2DM). The definitions of the disease were derived from the International Classification of Diseases [14]. The inclusion and exclusion criteria for studies were as follows: included studies were those that examined T2DM and its association with specific SNPs. Excluded studies were those that (1) focused on type 1 diabetes mellitus (T1DM), (2) investigated gestational diabetes mellitus (GDM), (3) examined other types of diabetes mellitus, (4) were not written in English, (5) were published in non-peer-reviewed journals, and (6) were conducted outside the geographical boundaries of the Arab Gulf region.

Eligibility criteria

The full-text of published articles that fell within one or more of the following criteria was excluded from downstream analyses: (1) association analyses performed on a small population (less than 60 people in total), (2) the data of the controls that were not compatible with Hardy–Weinberg

equilibrium (HWE), (3) analyses revealed the presence of a non-pathogenic or protective association of a SNP with T2DM, (4) analyses that revealed non-significant or a borderline association with T2DM, and (5) SNPs that are not fully identified or not deposited in the dbSNP.

Data extraction

Data extraction for genes, details of the SNPs, number of patients and controls, genotyping method, *P*-value and odd ratio (OR) of significance, and the location of the conducted investigation were shown in a separate table for each considered genotype-phenotype association analysis. The extracted data were generated based on the alphabetic name of the gene. The following information was extracted from the T2DM-associated studies: the name of the gene, SNP ID, type/position of the pathogenic SNP, number of patients and controls, the genotyping method used, study area, and publication cited.

Statistical analysis

Standard meta-analysis was conducted using the R programming software environment, and their presentation was enhanced via advanced Microsoft Excel modules using the NumXL 1.68, Camel (NUMXL, Shigaco IL 60604, USA). The association between the pathogenic SNPs and T2DM in the Arab Gulf populations was conducted using pooled ORs and 95% confidence intervals (CIs). The distribution, magnitude of the effect sizes across studies, and consistency of the results were assessed by means of a bubble chart. In a bubble chart, each study was represented by a bubble, with the size of the bubble representing the weight or sample size of the study, and the position of the bubble indicating the effect size of the study. The potential cooperation of the identified genes was figured out using String-10 [15]. The String-10 data were retrieved and annotated using the Cytoscape software [16]. The Panther software was used to annotate the molecular functions of the synthesized genes and the pathways they involved [17].

Results

Our results demonstrate the importance of carefully selecting SNPs for analysis in genetic association studies. By excluding non-pathogenic and protective SNPs, our analysis was focused on those genetic variants that are more likely to contribute to disease susceptibility. It has been demonstrated that these SNPs exhibited significant association with the risk of T2DM with varying degrees of intensity.

Utilizing Google Scholar, PubMed, Web of Science, and Scopus resources ensured a thorough and extensive search for relevant studies ($n = 2340$). By searching across multiple databases, the duplicated research of the identified data was removed ($n = 520$). By conducting a comprehensive literature search on the residual articles ($n = 1820$), a diverse set of T2DM-related studies was gathered, ensuring a more representative and comprehensive body of evidence for the meta-analysis. The rigorous screening was identified as a crucial step in the review process for determining the relevance of studies for inclusion. The titles and abstracts of research articles were typically assessed to identify studies that had the potential to meet the criteria defined for the meta-analysis. Accordingly, studies that are concerned with T1DM ($n = 198$), GDM ($n = 212$), and other types of DM (68); not written in English ($n = 324$); not published in peer-reviewed journals ($n = 262$); or reported outside the border of the Arab Gulf ($n = 638$) were eliminated from further consideration. After omitting these researches, the residual number of the studies that were found within the inclusion criteria was only 116. A further layer of rigorous elimination was applied to provide as accurate as eligible data to reflect the actual association between pathogenic SNPs and T2DM in the Arab Gulf world. This elimination was represented by omitting studies conducted on small populations of less than 60 individuals ($n = 16$), their controls did not fall within HWE ($n = 13$), articles that found a protective association with T2DM ($n = 28$), articles reported non-significance association with T2DM ($n = 23$), or articles described non-fully identified SNPs due to their non-demonstrated positions or the absence of any mention for the rs number in the dbSNP ($n = 10$). Accordingly, the residual number of eligible studies that were included in the meta-analyses was found to constitute only 26 studies. The entire process is illustrated in Fig. 1, which depicts each step and the number of studies included or excluded.

Based on the results obtained from the 26 eligible articles, a total of 27 pathogenic SNPs were identified in 16 genes that were located in variable positions in the human genomes (Table 1). The potential pathogenicity of these SNPs has been indicated due to their significant association with T2DM in the Arab Gulf region. The functional potential of these SNPs has been elucidated by their involvement in diabetes development and progression through influencing several critical mechanisms, such as gene expression, protein stability, and cellular function. While ongoing research is investigating specific pathogenic SNPs linked to diabetes [18], studies in the Arab Gulf region have pinpointed 27 SNPs that could contribute to the disease's pathogenesis. These SNPs could play a role in the development of T2DM in this region. Among these SNPs, the *TCF7L2* gene holds the highest count (10 SNPs), constituting the majority of the T2DM-associated loci in the Arab Gulf. Additionally, four T2DM-related SNPs are located in the *VDR* gene, while each one of the *ADIPOQ*, *FTO*, and *KCNJII* genes encompasses three T2DM-related SNPs.

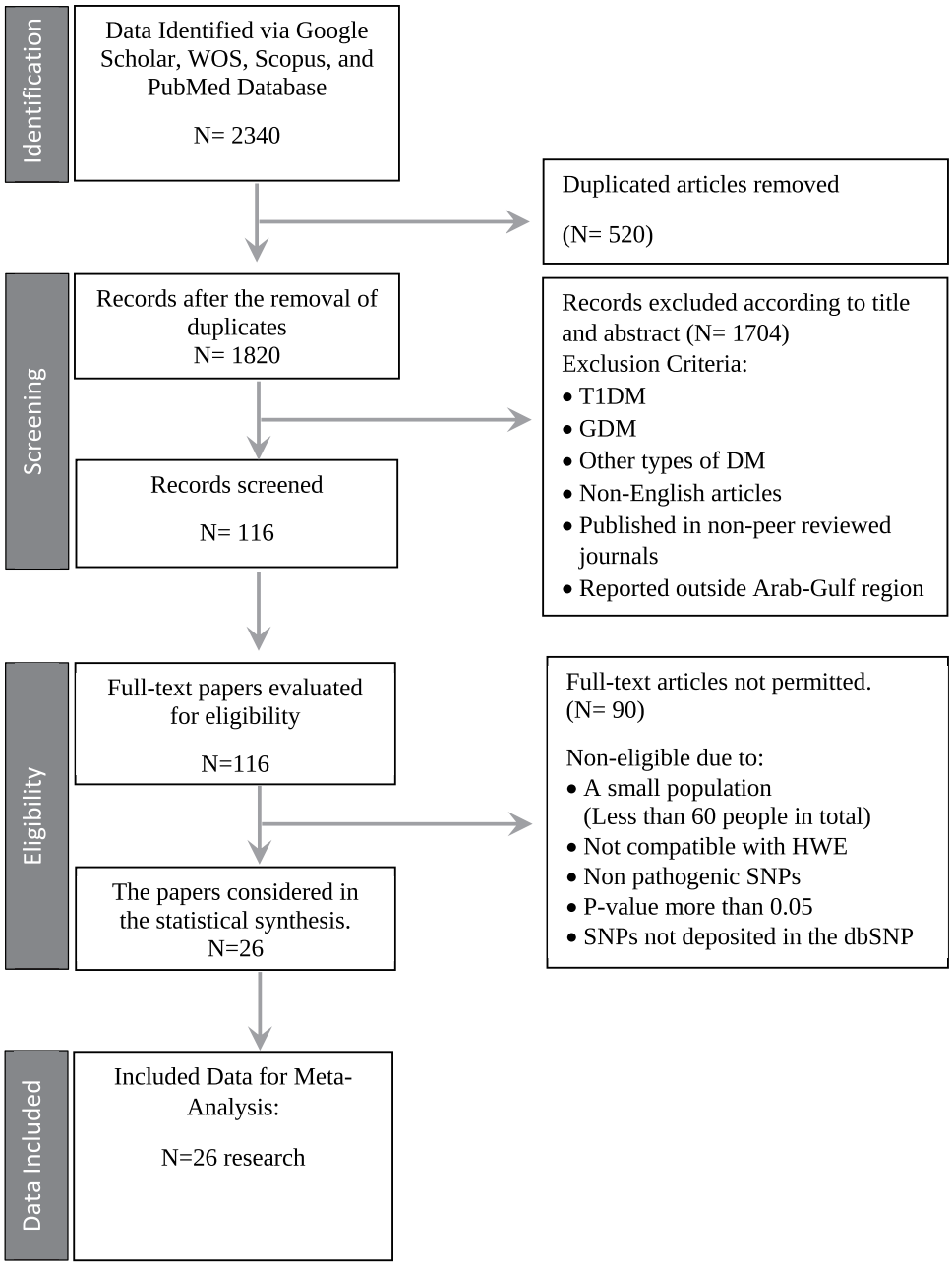

Fig. 1 PRISMA diagram for the systematic review

In contrast, the remaining 22 genes display a less frequent distribution of the rest of these SNPs. The categorization of these SNPs as pathogenic entails a complex process involving genetic analysis and an evaluation of how they may impact an individual's susceptibility to the disease. The classification of T2DM-related SNPs as pathogenic SNPs has been carried out using various rigorous and multifaceted methods aimed at understanding the potential influence of these SNPs on traits associated with T2DM. These classifications play a pivotal role in comprehending the genetic foundations of T2DM and can provide insights for personalized medicine strategies, risk assessments, and interventions for individuals at risk of developing the condition. The initial step in identifying T2DM-related SNPs in the Arab Gulf region has primarily entailed the utilization of various traditional genotyping techniques, often subsequently verified through sequencing. Additionally, computational approaches that predict the impact of SNPs on the structural and functional aspects of genes have been employed to assess how these genetic variations may affect the disease [19]. Pathogenic SNPs linked to T2DM have predominantly been identified within intron sequences, whereas other types of pathogenic SNPs have been found in various coding and noncoding sequences. The classification of SNPs as pathogenic can also help elucidate disparities in susceptibility to T2DM among the Arab Gulf population. These findings were based on a comprehensive analysis of

Table 1 The details of genes showed significant associations with diabetes mellitus type II in Arab Gulf populations

No.	Gene	SNP	Type of SNP	Patients	Controls	Genotype method	*p*-value	OR	Country	Reference
1.	*C5AR2*	rs149572881	Missense	376	175	PCR-RFLP	.009	3.7	Saudi Arabia	[20]
2.	*ACE*	rs1799752	INDEL	117	75	Allele-specific PCR	.018	3.0	Iraq	[21]
3.	*JAZF1*	rs864745	Intron variant	400	400	PCR-RFLP	.02 to .002	1.2–3.9	Saudi Arabia	[22]
4.	*ADIPOQ*	rs2241766	Silent	314	257	PCR-RFLP	.0001	5.4	Iraq	[23]
	ADIPOQ	rs17300539	Promoter	400	400	PCR-RFLP	.04 to .004	1.62-3.19	Iraq	[24]
	ADIPOQ	rs266729	Promoter	135	135	PCR-RFLP	.01	3.67	Iraq	[25]
5.	*HNF4A*	rs4812829	Intron variant	1166	1235	Allele-specific PCR	.00068	1.27	Saudi Arabia	[26]
6.	*VDR*	rs1544410	Intron variant	400	400	PCR-RFLP	.001	2.17	Iraq	[27]
	VDR	rs1544410	Intron variant	368	259	TaqMan real-time PCR	.001	2.08	Saudi Arabia	[28]
	VDR	rs731236	Silent	368	259	TaqMan real-time PCR	.030	1.43	Saudi Arabia	[28]
	VDR	rs2228570	Missense	400	400	PCR-RFLP	.024	1.30	Iraq	[29]
7.	*FTO*	rs9939609	Intron variant	120	60	ARMS-PCR	.040 to .031	Not shown	Iraq	[30]
	FTO	rs9939609	Intron variant	400	400	PCR-RFLP	.0000	1.87-5.64	Iraq	[31]
	FTO	rs17817449	Intron variant	400	400	PCR-RFLP	.0000	1.65-3.04	Iraq	[31]
8.	*SLC30A8*	rs13266634	Intron variant	89	96	Sanger sequencing	.04	7.42	Saudi Arabia	[32]
9.	*MC4R*	rs2229616	Missense	415	323	TaqMan real-time PCR	.001	1.82	Saudi Arabia	[33]
	MC4R	rs6567160	Intron variant	464	415	Genome-wide association	.0093	1.70	Emirates	[34]
10.	*TCF7L2*	rs12255372	Intron variant	351	351	Allele-specific PCR	.0213	1.34	Saudi Arabia	[35]
	TCF7L2	rs12255372	Intron variant	188	180	TaqMan real-time PCR	.03	1.47	Emirates	[36]
	TCF7L2	rs12255372	Intron variant	890	686	TaqMan real-time PCR	0.042	1.16	Emirates	[37]
	TCF7L2	rs7903146	Intron variant	106	106	Tetra ARMS-PCR	.001	2.53	Iraq	[38]
	TCF7L2	rs7903146	Intron variant	264	153	TaqMan real-time PCR	.0063	1.80	Emirates	[39]
	TCF7L2	rs7903146	Intron variant	464	415	Genome-wide association	.0056	1.73	Emirates	[34]
	TCF7L2	rs7903146	Intron variant	1124	590	TaqMan real-time PCR	.0029	1.36	Qatar	[40]
	TCF7L2	rs4506565	Intron variant	1124	590	TaqMan real-time PCR	.0037	1.33	Qatar	[40]
	TCF7L2	rs4506565	Intron variant	351	351	Allele-specific PCR	.0070	1.39	Saudi Arabia	[35]
	TCF7L2	rs10885409	Intron variant	272	216	TaqMan real-time PCR	.035	1.49	Emirates	[41]
11.	*CDKN2*A/B	rs10811661	Intron variant	400	400	PCR-RFLP	.004	1.47–4.24	Iraq	[42]
	*CDKN2*A/B	rs10811661	Intron variant	992	294	TaqMan real-time PCR	.02	1.40	Oman	[43]
12.	*LEP*	rs11761556	5′-UTR	120	100	PCR-SSCP	.01	4.58	Iraq	[44]
	LEP	rs12706832	Intron variant	120	100	PCR-SSCP	.01	2.38	Iraq	[44]
13.	*MCP-1*	rs1024611	Intron variant	135	100	Tetra ARMS-PCR	.01	3.35	Iraq	[45]
14.	*KCNJII*	rs5219	Stop-gained	40	20	PCR-RFLP	.001	7.0	Iraq	[46]
	KCNJII	rs5219	Stop-gained	992	294	TaqMan real-time PCR	.00005	1.74	Oman	[43]
	KCNJII	rs5219	Stop-gained	550	335	Real-time PCR	.0001	1.7	Saudi Arabia	[47]
15.	*SLMAP*	rs17058639	5′-UTR	238	104	TaqMan real-time PCR	.018	3.23	Qatar	[48]
16.	*IRS1*	rs1801278	Missense	376	380	PCR-RFLP	.040	1.78	Saudi Arabia	[49]
	IRS1	rs2943641	Missense	376	380	PCR-RFLP	.001	1.48	Saudi Arabia	[49]

27,958 individuals in Arab Gulf countries, which collectively encompassed 15,982 patients and 11,976 controls.

In addition to the traditional meta-analysis conducted on the relationship between SNPs and TD2M, we utilized a minor allele frequency (MAF) analysis as a quality control threshold. This was done to highlight SNPs with low MAFs, which could potentially affect the reliability of results [50]. The analysis of the data presented in Table 2 has unveiled that the majority of minor alleles in the scrutinized SNPs manifest varying levels of significant impacts on T2DM within the Arab Gulf region. Table 2 shows that more than two-thirds of the investigated SNPs are associated with these significant effects on the disease. This data underscores that the substantial effects of the studied SNPs are not confined solely to major alleles but also encompass minor alleles. Furthermore, this observation may extend to the genotypes, suggesting that multiple genotypic forms may influence T2DM, rather than just one specific genotypic form.

The *HNF4A* gene, with 1166 patients and 1235 controls investigated, had the highest number of individuals studied. However, based on the collected data, it was deduced that the *TCF7L2* gene was the most extensively screened in the Arab Gulf. Within this gene, four intron SNPs were found to exhibit significant pathogenic associations with T2DM in four countries using variable genotyping techniques. *TCF7L2*:rs7903146 showed the highest number of significant associations with T2DM in Arab Gulf countries. This is due to the presence of four different studies that referred to this association in Iraq [38], Emirates [34, 39], and Qatar [40]. *TCF7L2*:rs12255372 showed a significant association with T2DM in Saudi Arabia [35], and Emirates [36, 51]. *TCF7L2*:rs4506565 was also associated with T2DM in two studies conducted in Saudi Arabia and Emirates [41, 52]. *TCF7L2*:rs10885409 exerted a significant association with T2DM in Emirati subjects [41]. Following the extensively investigated SNPs *TCF7L2* gene, three SNPs in *ADIPOQ* and *VDR* genes exerted pathogenic association with T2DM in variable Iraqi and Saudi populations [23–25, 28, 29]. However, other listed genes showed a lower number of significantly associated SNPs with T2DM.

All over the instigated Arab Gulf countries, the highest number of studies on diabetic-related genes were found in Iraq with 39%, which was followed by Saudi Arabia with 32% (Fig. 2a). Out of sixteen screened genes that were investigated in the included researches, the highest number of investigated individuals was found in the *HNF4A* (hepatocyte nuclear factor 4 alpha) gene with 1166 patients and 1235 controls (Fig. 2b). The generated pie chart also showed that the most frequently used technique in the genotyping of these genes was polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with 34%, which was followed by TaqMan-real-time PCR with 32% (Fig. 2c), whereas each of the other genotyping techniques of PCR-single strand conformation polymorphism (PCR-SSCP) [53], genome-wide association studies (GWAS) [54], and tetra-amplification refractory mutation system (ARMS)-PCR [55] did not exceed more than 5% of T2DM assessment in Arab Gulf populations. Due to their higher frequencies of polymorphism over exon SNPs [56], intron SNPs were the most intensively investigated in terms of their association with T2DM in Arab Gulf countries with 61% (Fig. 2d), whereas SNPs in the other locations were found to exhibit lower ratios of associations with 13% of missense SNPs, 8% of stop-gained SNP, 5% of 5′UTR and silent SNPs, and 3% of INDEL SNP.

A direct comparison between OR and sample sizes was displayed in a bubble chart (Fig. 3). This comparison demonstrated the strength of the association between the two variables and indicated the level of significance of that association as determined by the *p*-value. The majority of the ORs were located between values 1 and 2, indicating a moderate association between the genetic variant and the disease outcome. Two studies have particularly high ORs [32, 46], indicating a stronger association. It is interesting to note that despite the moderate association between the genetic variant and the disease outcome in the majority of the studies, there is significant variation in the p-values. This suggested that some studies exhibited stronger statistical significance than others did, even though their ORs were found to be similar. The values of the risky ORs ranged from 1.16 in *TCF7L2*;rs12255372 [37] to 7.42 in *SLC30A8*;rs13266634 [32]. It was inferred from the bubble chart that three studies were found to exert larger effect sizes than other studies included in the analysis. These studies showed the highest weight values with OR values of 1.27 [27], 1.33 [40], and 1.16 [37], respectively. Due to these studies, the presence of moderate associations with T2DM is expected.

The *in silico* analyses of the identified T2DM-related genes indicated the presence of variable intensities of network interaction among the captured genes, which suggests the presence of numerous levels of collaboration among them. However, *KNCJ2*, *SLMAP*, and *C5AR2* genes did not exert any sort of collaboration with the presented network (Fig. 4a). The pie representations of these T2DM-related genes indicated that 36% of them were involved in binding activities. Other activities were found in fewer percentages with 18% of molecular transducer activity, 14% of catalytic and molecular function activities, and 9% of transporter and transcription regulator activities (Fig. 4b). More detailed information on the pathways these genes are involved was explained. Gonadotropin-releasing hormone receptor and p53-related pathways were found to exhibit the highest percentage with 15%. The majority of the other pathways were identified in 7%, including cadherin, Wnt, CCRK, cytokine, interleukin signaling, angiogenesis, and Alzheimer disease-presenilin pathways (Fig. 4c). Among the other T2DM-related genes, two of the insulin/IGF pathways were also identified in 7%.

Table 2 The details of minor allele frequencies of diabetes mellitus type II-related SNPs in Arab Gulf populations

No.	Gene	SNP	Variation	MAF in patients	MAF in controls	*p* value	OR	Country	Reference
1.	*C5AR2*	rs149572881	C > T	0.039	0.0133	0.0411	2.9920	Saudi Arabia	[20]
2.	*ACE*	rs1799752	D/I	0.239	0.373	0.0395	0.6410	Iraq	[21]
3.	*JAZF1*	rs864745	T > C	0.425	0.321	0.0038	1.3230	Saudi Arabia	[22]
4.	*ADIPOQ*	rs2241766	T > G	0.22	0.1175	0.0001	0.4371	Iraq	[23]
	ADIPOQ	rs17300539	G > A	0.226	0.135	0.0001	1.6759	Iraq	[24]
	ADIPOQ	rs266729	C > G	0.277	0.14	0.0017	1.9737	Iraq	[25]
5.	*HNF4A*	rs4812829	G > A	0.0428	0.024	0.001	1.7364	Saudi Arabia	[26]
6.	*VDR*	rs1544410	C > T	0.52	0.35	0.0001	1.4893	Iraq	[27]
	VDR	rs1544410	C > T	0.442	0.377	0.024	1.13	Saudi Arabia	[28]
	VDR	rs731236	A > G	0.441	0.413	0.5262	1.0689	Saudi Arabia	[28]
	VDR	rs2228570	A > C	0.71	0.43	0.0001	1.6512	Iraq	[29]
7.	*FTO*	rs9939609	T > A	0.333	0.466	0.1679	0.7143	Iraq	[30]
	FTO	rs9939609	T > A	0.2	0.3125	0.0052	0.6400	Iraq	[31]
	FTO	rs17817449	G > T	0.3625	0.2675	0.0032	1.3551	Iraq	[31]
8.	*SLC30A8*	rs13266634	C > T	0.185	0.1458	0.3866	1.2713	Saudi Arabia	[32]
9.	*MC4R*	rs2229616	C > T	0.0087	0.125	0.0001	0.0401	Saudi Arabia	[33]
	MC4R	rs6567160	G > A	0.33	0.278	0.019	1.70	Emirates	[34]
10.	*TCF7L2*	rs12255372	G > T	0.437	0.339	0.0117	1.2899	Saudi Arabia	[35]
	TCF7L2	rs12255372	G > T	0.338	0.384	0.5429	1.1103	Emirates	[36]
	TCF7L2	rs12255372	G > T	0.394	0.358	0.3290	1.0998	Emirates	[37]
	TCF7L2	rs7903146	C > T	0.406	0.292	0.014	1.65	Iraq	[38]
	TCF7L2	rs7903146	C > T	0.3725	0.4223	0.0063	1.80	Emirates	[39]
	TCF7L2	rs7903146	C > T	0.41	0.33	0.0056	1.73	Emirates	[34]
	TCF7L2	rs7903146	C > T	0.0809	0.0711	0.5838	1.1109	Qatar	[40]
	TCF7L2	rs4506565	A > G	0.0151	0.0101	0.4059	1.4872	Qatar	[40]
	TCF7L2	rs4506565	A > G	0.4094	0.4909	0.0072	1.3909	Saudi Arabia	[35]
	TCF7L2	rs10885409	T > C	0.474	0.446	0.5979	0.9413	Emirates	[41]
11.	*CDKN2*A/B	rs10811661	T > C	0.22	0.32	0.0001	1.69	Iraq	[42]
	*CDKN2*A/B	rs10811661	T > C	0.164	0.201	0.020	1.40	Oman	[43]
12.	*LEP*	rs11761556	C > A	0.625	0.37	0.008	1.93	Iraq	[44]
	LEP	rs12706832	A > G	0.383	0.61	0.006	1.75	Iraq	[44]
13.	*MCP-1*	rs1024611	A > G	0.51	0.38	0.06	1.6	Iraq	[45]
14.	*KCNJII*	rs5219	T > C	0.15	0.06	0.0004	5.00	Iraq	[46]
	KCNJII	rs5219	T > C	0.320	0.222	0.00005	1.74	Oman	[43]
	KCNJII	rs5219	C > T	0.415	0.405	0.675	1.04	Saudi Arabia	[47]
15.	*SLMAP*	rs17058639	C > T	0.341	0.403	0.009	1.76	Qatar	[48]
16.	*IRS1*	rs1801278	C > T	0.045	0.026	0.0588	1.7181	Saudi Arabia	[49]
	IRS1	rs2943641	C > T	0.299	0.223	0.0107	1.3376	Saudi Arabia	[49]

Discussion

The Arab Gulf states are a collection of Arab nations that share a Gulf as a boundary. Iraq, Kuwait, Bahrain, Saudi Arabia, Oman, the United Arab Emirates, and Qatar are the seven Arab League members in the area that share this Gulf [57]. According to the IDF database, three of the Arabian Gulf countries have occupied the highest prevalence of T2DM in 2021 with 25.5%, 17.7%, and 16.4% for Kuwait, Saudi Arabia, and Qatar respectively. Due to the significant role of the genetic factor in the disease's onset and progression, the identification of genetic variants associated with T2DM is crucial for understanding the underlying mechanisms of the disease and developing effective prevention strategies.

Our conducted meta-analyses suggest that there is a moderate association between the genetic variant and the disease outcome, but the strength of this association varies across studies. The studies with higher ORs may be particularly important for further investigation, as they

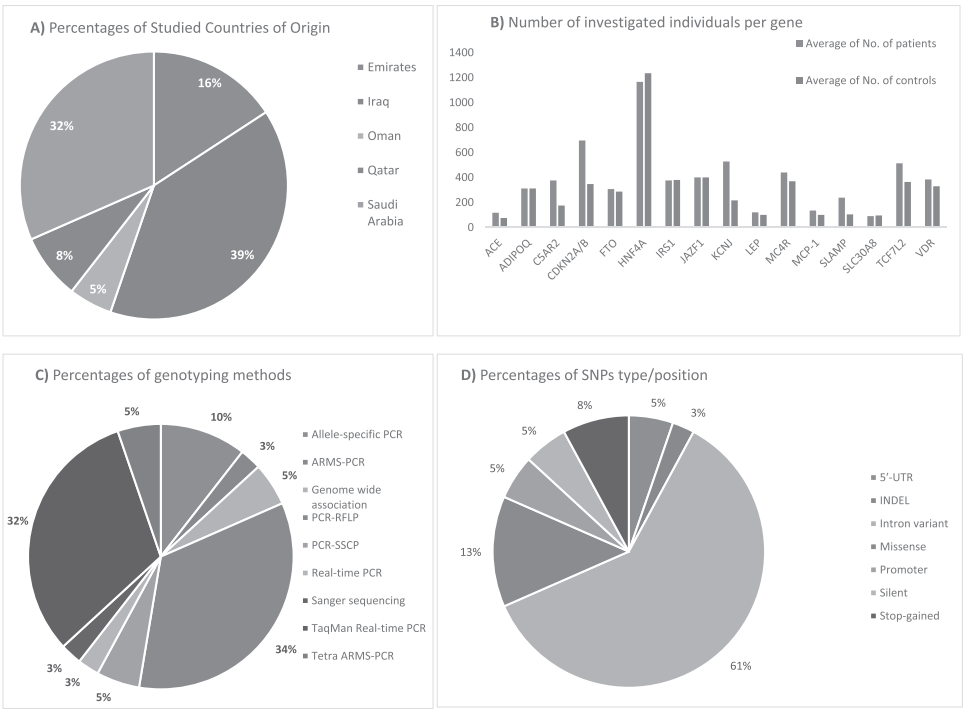

Fig. 2 The main probability measures of the study, in which studied countries of origin, the studied individuals (patients and controls) per gene, genotyping protocols, and SNP description are shown in **A, B**, **C**, and **D**, branches, respectively

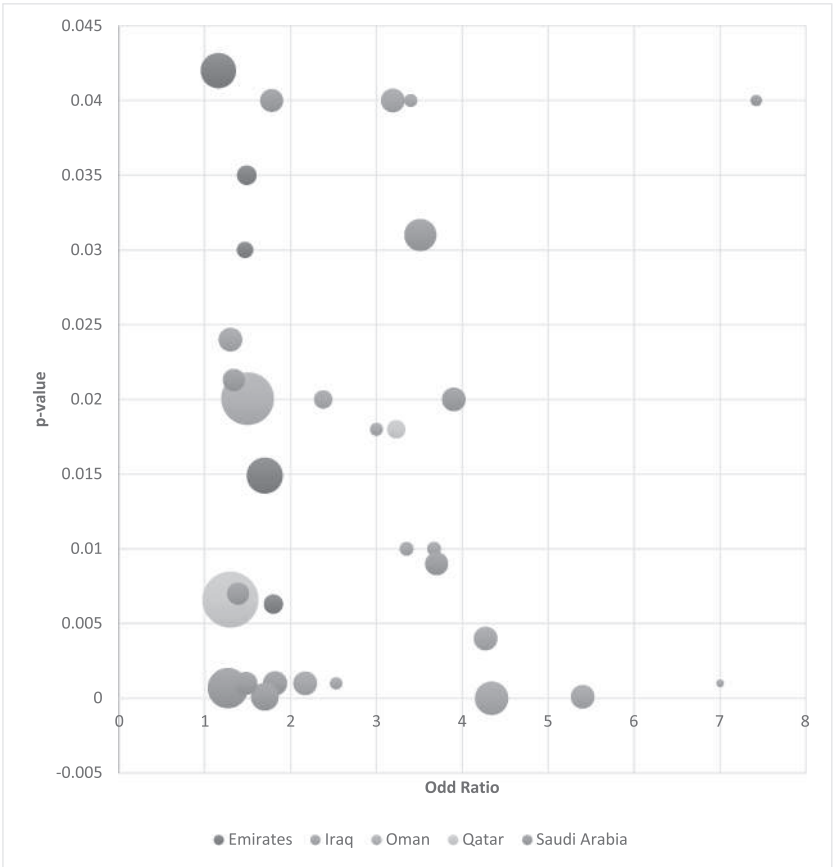

Fig. 3 Bubble chart of odd ratios and *p*-value for T2DM in Arab Gulf Population

suggest a stronger association between the genetic variant and the disease outcome. It is important to note that the studies with ORs between 1 and 2 also tend to have higher sample sizes compared to those with higher odd ratios. Specifically, the studies with the highest ORs (between 7 and 8) have the lowest reported sample sizes. This suggests that the associations observed in these studies may be more prone to bias or chance findings due to smaller sample sizes. In contrast, the associations observed in the studies with odd ratios between 1 and 2 may be more reliable due to their larger sample sizes, despite the range of p-values reported. Therefore, while the ORs provide some indication of the strength of the association between the genetic variant and disease, it is also important to consider the sample sizes of the studies when interpreting the results.

The data of this study indicated that the *TCF7L2* gene represents the most pathogenic gene in terms of the number of identified T2DM-related SNPs. Within this gene, rs7903146, rs12255372, rs4506565, and rs10885409 showed the highest level of pathogenicity in terms of their association with the developments of T2DM in the majority of Arab Gulf countries, whereas the other pathogenic genes did not exhibit such accumulated association with the onset of T2DM. The *TCF7L2* SNPs showed variable degrees of significant associations with T2DM in Saudi, Emirati, Iraqi, and Qatari populations [34–36, 38–41, 51, 52]. However, all the observed associations are located in intron sequences and no other signification association was identified within the sequences of the *TCF7L2* gene. In addition to the findings of the intron SNPs of *TCF7L2* gene, one intronic SNP of the *HNF4A* gene (rs4812829) has given noticeable outcomes in the genotype-phenotype association in this region. Due to the highest number of incorporated populations, it can be stated that this SNP is the most extensively investigated in the Arab Gulf. The polymorphism of HNF4:rs4812829 SNP was found to be significant in Saudi patients with T2DM yielding a considerable risk for patients with T2DM [26]. HNF4 is also known to be involved in insulin-signaling pathways [58]. This suggestion is supported by the generated Panther pathways, which may explain a possible role for this pathogenic SNP in altering and affecting this scheduled role of metabolism. Several other examples showed the high availability of intronic variation in relation to T2DM in Arab Gulf countries. One of these examples is represented in the *MCP-1* gene, which encodes for monocyte chemoattractant protein-1 gene that plays a principal role in the inflammatory process [59, 60]. Within the *MCP-1* gene, the intronic SNP rs1024611 was significantly associated with increased susceptibility to diabetic foot ulcers in Iraqi T2DM Patients [45]. The *FTO* gene polymorphism of the intron rs9939609 SNP showed a significant association with BMI and HDL-C levels in obese diabetic Iraqi males but did not affect other tested biochemical parameters [30]. The polymorphisms of this SNP and rs17817449 of the *FTO* gene may participate in the development of insulin resistance and hence the occurrence of T2DM in obese patients in Iraqi people [31]. On the contrary, non-significant results were detected between the rs9939609 SNP and T2DM in the Western Saudi population and Emirates [33, 39]. Furthermore, another study conducted on Saudi subjects indicated the absence of any significant association between this SNP and T2DM [32]. Based on the GWAS data, the intronic *MC4R*:rs6567160 SNP also showed a significant association with T2DM in the Emirati population [34]. Two intronic variants of the *CDKN2A/B* gene were also found to exhibit significant associations with T2DM in Arab Gulf countries. *CDKN2A/B* gene rs10811661 SNP was implicated in T2DM pathogenesis, whereas the other intronic variant (rs2383208 SNP) did not impact the disease in the Iraqi population [42]. Further confirmation of the role of the intron rs10811661 SNP in the susceptibility to T2DM was also suggested in a wider spectrum of samples in the Omani subjects [43]. On the other hand, other pathogenic SNPs have been found to exhibit a different

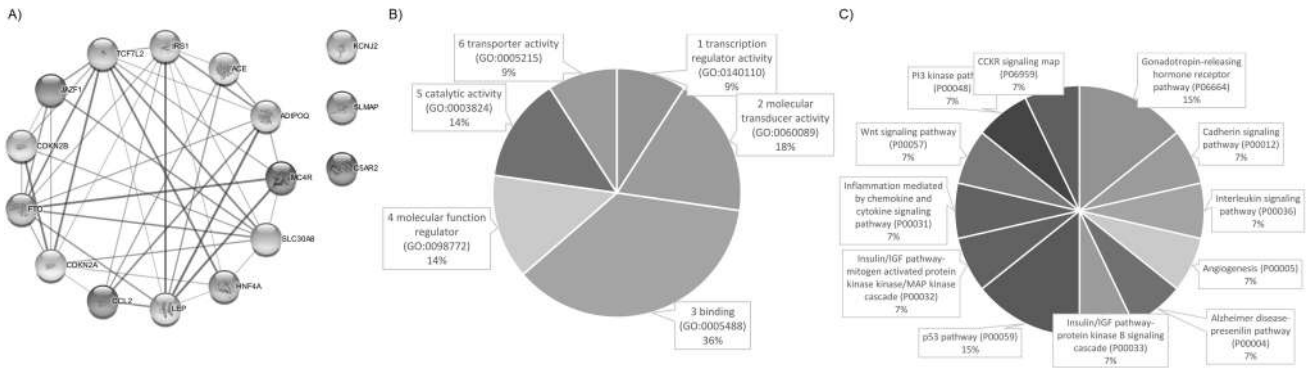

Fig. 4 Bioinformatics analyses of the T2DM-related genes captured in this study. **A** Predicted protein-protein interaction of the captured genes. **B** Predicted molecular functions ratio among the encoded products. **C** Predicted the cellular pathways in which the captured T2DM-related genes are involved

mechanism in relation to T2DM. This can be exemplified in the significant relationship between *C5AR2*; rs149572881, which induced a missense effect of p.233Pro>Leu on the protein, with T2DM in the Saudi subjects [20]. Due to this amino acid substitution, a direct effect of this SNP on the encoded G-protein coupled receptor 1 would be expected [61]. Another example of the effect of the non-synonymous SNP on the development of T2DM in the Arab Gulf is found in the *IRS1* gene, or insulin receptor substrate-1 gene that is implicated in the risk of T2DM [62, 63]. Mutations in this gene have been linked to T2DM and insulin resistance [64, 65]. The genetic polymorphisms of two missense SNPs of *IRS*-1 gene (rs1801278 and rs2943641) have been shown to significantly impair IRS1 function and its subsequent association with T2DM in Saudi subjects [49], whereas *KCNJ2* gene followed another mechanism of association with T2DM by directly truncating its encoded protein by one stop-gained (rs5219) SNP. This SNP showed a significant association with the susceptibility of T2DM in the Iraqi subjects [46], which has also been confirmed by two large-scale investigations performed in Omani and Saudi subjects. Both studies indicated the significant association of this SNP with the risk of T2DM [43, 47]. However, no direct association between rs5219 and T2DM was detected in Emirati subjects [39].

Among all the identified T2DM-related SNP, only one INDEL SNP was found in the *ACE* gene. Recent data indicated an evident association between genetic polymorphism of *ACE*:rs1799752 and T2DM in Iraqi subjects [21, 66]. In agreement with the predicted network of T2DM-related genes, many disorders, including renal disease, stroke, and Alzheimer's disease, have been linked to the polymorphism of the *ACE* gene [67]. *ADIPOQ* gene exhibited two T2DM SNPs, in which one SNP is located in the coding sequences with silent effects and two SNPs located in the promoter sequences. The distribution of *ADIPOQ*:rs266729 SNP in T2DM patients was significantly associated with T2DM in Iraqi subjects [25]. Within the promoter region of the *ADIPOQ* gene, the polymorphism of the rs17300539 SNP was also implicated in the development of T2DM, which caused variable metabolic changes in diabetic Iraqi patients [24]. In addition to intron variation, the *LEP* gene also exerted its effect by a 5′-UTR-SNP (rs11761556). This data is in line with its reported association with insulin resistance and the emergence of T2DM [68, 69]. Another example of the effect of the UTR-SNP on T2DM in the Arab Gulf is found in the *SLMAP* gene. *SLMAP*:rs17058639 SNP has also been suggested to act as a risk factor for the susceptibility to diabetic retinopathy in Qatari patients with T2DM [48]. On the other hand, the *VDR* gene, which affects a range of metabolic pathways [70], showed variable types of pathogenic SNPs associated with the T2DM in the Arab Gulf countries that ranged from intronic (rs1544410 and rs1544410), to silent (rs731236), and missense (rs2228570).

Whatever the mechanism through which each identified SNP exerts its significant association with the onset of T2DM in the Arab Gulf, this meta-analysis has highlighted the genetic loci candidates associated with T2DM in the Arab Gulf population and assessed their potential for early diagnosis of the disease. It shed light on the extent of the association between genetic loci candidates and T2DM in the Arab Gulf population.

Conclusions

The study identified 27 pathogenic SNPs in 16 genes that were located in variable positions in the human genome in the Arab Gulf countries. The *HNF4A* gene was found to exhibit the most common association with T2DM based on the large sample sizes included. The *TCF7L2* gene was found to be the most extensively screened gene in Arab Gulf, with four intron SNPs exhibiting significant pathogenic associations with T2DM in four countries using variable genotyping techniques. The conducted meta-analyses suggest a moderate association between the captured genes and T2DM. However, it is important to note that the studies with higher ORs tend to have smaller sample sizes, which may make their results more prone to bias or chance findings. In contrast, the associations observed in studies with ORs between 1 and 2 may be more reliable due to their larger sample sizes, despite the range of p-values reported. The *in silico* analyses of the identified T2DM-related genes indicated the presence of variable intensities of network interaction among the 13 genes, which suggests the presence of numerous levels of collaboration among them. The results suggest that intronic variation may play a significant role in the development of T2DM in Arab Gulf countries. The variability of genes and SNPs associated with T2DM among different Arab Gulf populations emphasizes the need for further research and personalized approaches to manage and prevent T2DM. The study of genetic variants holds promise for improving the early detection of T2DM in the Arab Gulf population, ultimately leading to better health outcomes and quality of life for those affected by this chronic dysfunction.

Author contribution K. N. J. M. designed the study and conducted the literature search. M. R. conducted the data extraction and analyzed the statistical data. M. B. S. A. conducted the in silico analyses and wrote the manuscript. All the authors revised the subsequent drafts for important intellectual content, read, and approved the final version of the manuscript. Both K. N. J. M. and M. R. contributed equally to this work.

Data Availability The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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REVIEW ARTICLE

Effects of exercises and manual therapy on nerve conduction studies of lower limb in patients with diabetes and diabetic peripheral neuropathy: A systematic review

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Abstract

Background Diabetes and related peripheral neuropathy result in various sensory and motor complications. Such changes are documented early and more precisely in nerve conduction studies than in clinical evaluation and quantitative sensory testing. Different exercises and mobilization also affect the same differently.

Objective This review aimed to compile the current evidence on the effectiveness of exercises and manual therapy on nerve conduction studies of lower limbs in patients with diabetes and diabetic peripheral neuropathy and to evaluate the underlying mechanisms.

Methods Studies that examined the effects of different exercises and manual therapy on nerve conduction studies of lower limbs in patients with diabetes mellitus and diabetic peripheral neuropathy were searched on available databases. The PRISMA statement was followed. Quality check was done using the Pedro scale.

Results Thirteen studies matched the inclusion criteria. Interventions included moderate-intensity aerobic exercises, resistance exercises, tai chi exercises, sensorimotor and gait training, neurodynamic mobilization, and a combination of aerobics and resistance training.

Conclusion The present systematic review suggests that 8 to 12 weeks of physical exercise improves nerve conduction velocity of the motor tibial, peroneal nerve, and sensory sural nerve in diabetes with or without peripheral neuropathy.

Keywords Diabetes · Diabetic neuropathy · Manual therapy · Exercises · Nerve conduction studies

Abbreviations

CMAP	Compound muscle action potential
DM	Diabetes mellitus
DPN	Diabetic peripheral neuropathy
IENFD	Intra epidermal nerve fiber density
NAPA	Nerve action potential amplitude
NCS	Nerve conduction studies
NCV	Nerve conduction velocity
SNAP	Sensory nerve action potential
PEDRO	Physiotherapy evidence database
PRISMA	Preferred reporting items for systematic reviews and meta-analysis
PICOS	Population, intervention, comparison, outcomes, study design
TENS	Transcutaneous electrical nerve stimulation
ADA	American diabetes association
HbA1c	Hemoglobin A1c
BMI	Body mass index
QST	Quantitative sensory testing

Introduction

Diabetic peripheral neuropathy (DPN) is the most common debilitating complication of diabetes. The prevalence of DPN is 21.3 to 34.5% [1], and it increases with age and duration of diabetes [2]. During the course of the disease, 40–59% of patients develop neuropathic symptoms due to the involvement of sensory and motor peripheral nerves [3–5]. Symptoms present as electric, burning, stabbing,

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shooting, sharp aching pain, and dysesthesias that occur mostly at night and disturb sleep [6–8]. Loss of innervation of motor axons results in reduced muscle strength and atrophy in lower limb musculature [9–11]. These nerve function changes result from hypoxia induced by microvascular changes and impaired nerve perfusion [12–14]. If not diagnosed in the early phase of the disease can impose the risk of falls [15–17], lower limb amputations [18], impaired quality of life [19], anxiety, and depression [20, 21]. Thus, glycemic control, lifestyle modification, exercises, and early diagnosis are the keys to preventing disease progression [22, 23]. NCS are one such diagnostic tool that is considered the gold standard for the diagnosis of DPN [24, 25]. Various parameters of nerve conduction like distal and proximal latency [26], NCV [27], sensory nerve action potentials [28], and amplitude of motor/sensory response [29] all shows variations in DPN patients in comparison to healthy population. Since nerve functions are sensitive to changes in diabetic patients with or without clinical neuropathy [30], electrophysiological abnormalities are also noticed among asymptomatic diabetic patients [31].

Different systematic reviews and meta-analysis showed lifestyle modification along with exercises have been found to be effective on various clinical outcome measures in the diabetes and DPN. Similar studies are available describing the positive impact of changes in lifestyle and various types of exercise on lower limb nerve conduction measures in such individuals. But no systematic review or meta-analysis has examined the efficacy of such exercise trials on nerve conduction parameters of lower limb in population of concern. Therefore, purpose of this study was to review the current evidence on effectiveness of exercises and manual therapy on NCS of lower limb nerves in diabetes and DPN.

Methods

Search and information sources, Google Scholar, PubMed, and Cochrane library, were searched. Studies published between January 2005 and December 2022 were included. Studies with different exercise interventions and have seen its effects on nerve conduction of lower limb nerves in DM and DPN were considered. Strategy used to search related articles was done by using population, intervention, comparison, outcome measures, and study design (PICOS) method; population of diabetes with or without peripheral neuropathy; interventions such as physical exercises, balance, whole body vibration, tai chi, manual therapy, and its comparison with control; and placebo, no treatment, other type of exercises, electrotherapy, or pharmacotherapy. Outcome measures searched were NCS, nerve functions, and NCV. Search was not limited to any specific study type. Reference list of all the selected articles and related systematic review was also searched. Search strategy is listed in Table 1.

Inclusion criteria

1) Diagnosis of DM with and without peripheral neuropathy.
2) Exercises, balance, manual therapy as main intervention compared with controls, no intervention, electrotherapy or usual care.
3) NCS of sural, peroneal, and tibial nerve (one or all) as outcome measures (NCV, distal latency, proximal latency, nerve action potential amplitude).
4) Studies enrolling participants of any age or gender.
5) Studies published in English language.
6) Human studies.
7) Studies with quantitative results.

Exclusion criteria

Studies were excluded with diagnosis of neuropathy other than diabetic neuropathy. Studies published before 2005.

Selection process

Abstract and title of retrieved articles was screened by two independent authors. All the full text articles that fulfilled eligibility criteria were included for analysis. After selection

Table 1 Search strategy

Database	Search strategy (Mesh work)
PubMed	"Diabetes Mellitus" [Mesh] AND ("Diabetes Complications" [Mesh] OR "Peripheral Nervous System Diseases" [Mesh] OR "Diabetic Neuropathies" [Mesh]) AND ("Musculoskeletal Manipulations" [Mesh] OR "Therapy, Soft Tissue" [Mesh] OR "Manual therapy" OR "Physical Therapy" OR "Resistance Training" [Mesh] OR "Exercise Therapy" [Mesh])
Cochrane Library	"Diabetes Mellitus" OR "Diabetes complications" OR "Type 2 Diabetes" OR "Diabetic neuropathy" AND "Neural conduction" OR "Nerve conduction studies" OR "Nerve functions" OR "Nerve conduction velocity" AND "Musculoskeletal manipulations" OR "Manual therapy" OR "physical therapy" OR "Resistance training" OR "Aerobic exercises" OR "Physical exercises" OR "Balance exercises" OR "Tai chi"
Google Scholar	diabetes mellitus OR type 2 diabetes OR diabetic neuropathy AND nerve conduction studies OR nerve functions OR nerve conduction velocity AND physical exercises OR manual therapy OR balance exercises OR aerobic exercises OR tai chi

of studies, data was extracted about author and year of publication, study design, participant characteristics, inclusion criteria, exclusion criteria, interventions, outcomes, and results. Two independent authors used the PEDRO scale to rate the methodological quality of included articles (Table 2). Third author was consulted in case of confusion between first two authors. Internal validity score was also calculated for selected studies which was calculated using sum of 7 items (2, 3, and 5 through 9). Methodological quality of studies was further classified on the basis of Internal validity Score as limited (0–3 IV score), moderate (4–5 IV score), and high quality (6–7 IV score).

Results

Selection of studies: A total of 1616 studies were found on effects of different exercises/physiotherapy on nerve functions in DM or DPN after a detailed search of mentioned databases. After removal of duplicates, titles of 1578 studies were screened. After removal of irrelevant studies and studies other than DM or DPN, 115 studies were further considered. Eighty-eight studies plus two studies (using snowballing references), total 90 studies were screened for abstract reading after removal of systematic/ narrative reviews. Seventy-five studies were removed through PICOS method and eligibility criteria; 15 studies were considered for full text review. Of these, 13 studies were selected for analysis (PRISMA flow chart, Fig. 1). After rating the PEDRO score through two independent authors, inter-rater agreement between the two reviewers was found 10/13, which suggests a percentage of 76.923%.

Study characteristics

Out of 13 selected studies, seven studies [32–38] were randomized controlled. One study [29] was parallel group comparative study. One study [39] was prospective cohort study, and four studies [40–43] were single group prepost study design. Total sample of 641 was offered by all studies. Average age of participants ranges from 40 to 70 years in most of studies covered for review. Research population selected was of type 2 DM in nine publications [29, 33–35, 37–41]. Two studies [32, 36] included participants of both types 1 and 2 DM, and two studies did not specified the type of DM [42, 43]. Two studies [32, 39] included patients of diabetes without any signs and symptoms of neuropathy. Eleven studies [29, 33–38, 40–43] included population of diabetes with peripheral neuropathy. Different authors used different methods for initial screening of DPN. Most of studies [29, 32, 34, 35, 37, 43] used NCS to confirm presence of diabetic neuropathy. Others [32, 35, 36, 38] used Michigan neuropathy screening instrument, physical examination by specialist [41], Michigan diabetic neuropathic score [33, 35, 38], neuropathy scale score and Utah early neuropathic scale [39], and pin prick sensation on sole of foot [42]. Study characteristics are mentioned in Table 3.

Interventions

All the studies included in the review have seen effects of different kind of exercises on diabetes and diabetic neuropathy. Most of interventions used were supervised moderate intensity aerobic exercises on treadmill or stationary bicycle [32–35, 37, 41, 43], manual therapy (tibial nerve mobilization) [42], resistance training [37, 38], combined aerobics

Table 2 Scores for PEDro criteria

Author. year	1	2	3	4	5	6	7	8	9	10	11	QS/10	IVS	Variability
Dixit et al. 2014	Yes	1	1	0	0	0	1	0	0	1	1	5	Limited	3
Singleton et al. 2014	Yes	1	0	1	0	1	1	1	1	1	1	8	Moderate	5
Hung et al. 2009	Yes	0	0	1	0	0	1	1	0	1	1	5	Limited	2
Serry et al. 2016	Yes	1	0	1	0	0	0	1	1	1	1	6	Limited	3
Balducci et al. 2006	Yes	1	0	1	0	0	0	1	1	1	1	6	Limited	3
Gholami et al. 2018	Yes	1	1	1	0	0	1	1	0	1	1	7	Moderate	4
Gholami et al. 2021	Yes	1	1	1	0	0	1	1	0	1	1	7	Moderate	4
Ahmad et al. 2020	Yes	1	1	1	0	0	1	1	0	1	1	7	Moderate	4
Stubbs et al. 2019	Yes	1	1	1	0	0	1	0	0	1	1	6	Limited	3
Alsubiheen et al. 2017	Yes	0	0	0	0	0	0	0	0	0	1	1	-	0
Azizi et al. 2019	Yes	0	0	0	0	0	1	1	0	0	1	3	Limited	2
Kluding et al. 2012	Yes	0	0	0	0	0	0	0	0	0	1	1	-	0
Doshi and Singarvalen. 2019	Yes	0	0	0	0	0	0	1	1	0	1	3	Limited	2

QS Overall quality score; *IVS* internal validity score

*Criteria 1 score is not included in the overall PEDro rating

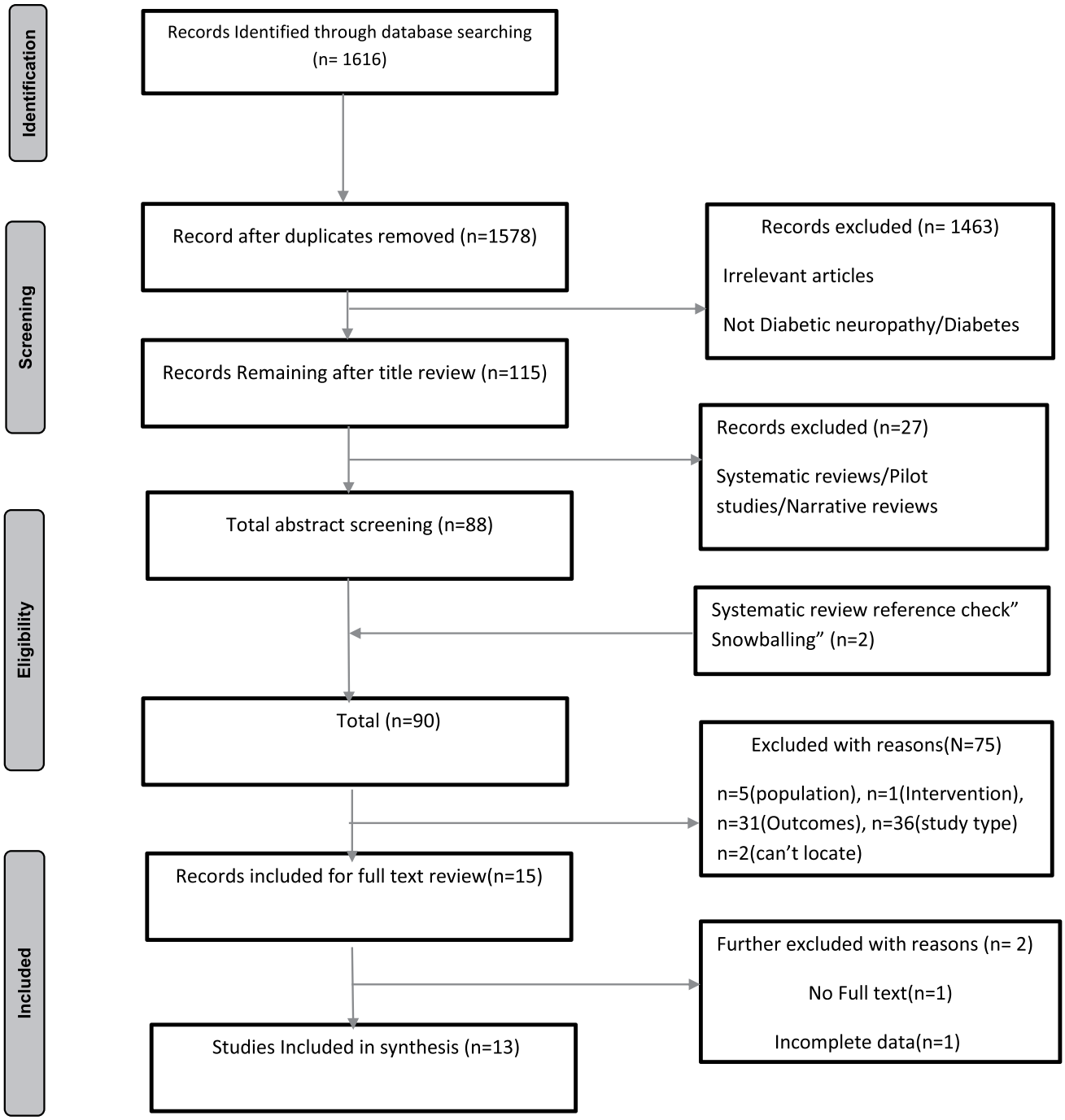

Fig. 1 PRISMA flowchart

and resistance exercises [37, 39, 43], sensorimotor exercises and gait training [36], stretching and breathing exercises along with tai chi exercises, mental imaginary exercises [40], and with Cheng's tai chi exercises [29]. Study duration of aforesaid studies varied from 3 weeks [42], 8 weeks [33, 34, 36, 40, 41] to 12 weeks [29, 35, 37, 38]. Few studies were of even longer duration of 1 year [39] and 4 years [32]. Frequency of exercises varied from 30 to 90 min per week [39], 2 times a week [40, 43], 3 sessions per week [29, 32, 34–38, 41], 5 sessions a week [42], and 3 to 6 sessions a week [33].

Comparators

Other than four studies [40–43] which were single group prepost study design, intervention group was compared with

Table 3 Study characteristics

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
Balducci et al. 2006 [32]	$N=78$	Type 2/type 1 diabetes	CNS (central nervous system) dysfunction	G1: sedentary patients	Peroneal motor NCV, NAPA, DL
	Male = 39	No signs/symptoms of DPN	MS (musculoskeletal deformity) that prevents participation,	G2: Treadmill brisk walk on 50% to 85% of the heart rate reserve	Sensory sural NCV, NAPA, DL
	Females = 39	Able to walk 1.6 km distance without/with assistance	L/L (lower limb) arthritis/pain that limits exercise	4-sessions per week for 4 years	VPT at malleolus/Hallux
	G1: control, $n=47$ diabetics, with sedentary lifestyle		H/o severe CV diseases that contraindicate the exercise	Study duration: 4 years	
	Age: 52.9 ± 13.4		vestibular dysfunction, H/o of angina, postural hypotension, Plantar skin pressure ulcers		
	G2: Supervised exercise group, $n=31$		MNSI (Michigan Neuropathy Screening Instrument scores) ≥ 2.5		
	Age: 49 ± 15.5 years		Sural nerve amplitude < 6 µs, distal latency < 3 ms		
	4-year prospective randomized intervention study.		Peroneal nerve amplitude of < 2mv, distal latency of < 6.2mv		
Hung et al. 2009 [29]	$N=65$	Type 2 DM,	Contraindications to moderate exercise	Both groups practice	Fasting blood sugar
	G1: control, $n=28$, healthy participants	On oral hypoglycemic agents receiving metformin, sulfonylurea, or both	H/O of cardiovascular, pulmonary/neurologic disorders other than DM	Cheng's TCC, for 3 sessions a week, 60 min a session	Mean insulin resistance
	Age: (56.6 ± 13.3) years		Previously practiced TCC	10-min warm-up (including stretching and balancing exercises), 40 min TCC exercise (includes 37 movements), 10-min cool-down	NCV, distal latency, proximal amplitude of tibial, peroneal, sural, median, ulnar nerves bilaterally
	G2: intervention, $n=32$, with DM			Study duration: 12 weeks	
	Age: (58.1 ± 13.4) years				
	Parallel group comparative study with a pre- and post-design				
Dixit et al. 2014 [33]	$N=87$, both gender	Type 2 DM	Vitamin B12 deficiency	G1: Standard medical care, foot care, diet	Latency, amplitude, duration, and NCV of motor peroneal and sensory sural nerve

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	G1: control group: $n = 40$	MDNS >7 (Michigan Diabetic Neuropathy Score)	Postural hypotension	G2: Exercise training in the range of 40–60% of heart rate reserve	MDNS (Michigan Diabetic Neuropathy Score)
	Age: 59.45 ± 1.16		Foot ulcers	Sessions: 3–6 days of the week	
	G2: experimental group: $n = 47$		Walking with assistive devices	moderate intensity treadmill exercises, minimum of 150 min/week to a maximum of 360 min/week of work out	
	Age: 54.40 ± 1.24		Foot amputation	Study duration: 8 weeks	
	Parallel group randomized controlled trial		Peripheral arterial disease		
			Vision impairments		
			Neurological/ musculoskeletal impairments (acute sciatica or vestibular dysfunction)		
			Cognitive impairments		
			Score of ≥ 30 on MDNS		
			H/O active retinal hemorrhage, recent laser therapy, cardiac risks, revascularization of CABG		
			Seeking any other therapy for DPN		
			Age > 70 years		
Singleton et al. 2014 [39]	$N = 100$	Age 30–70 years	Utah early neuropathy scale score > 4	G1: Quarterly counseling on diet and moderate home exercise	IENFD (ankle/proximal thigh)
	G1: counseling, $n = 40$, age: 58.4 ± 6.7	Type 2 diabetes	symptoms of distal L/L, Sensory loss, numbness/neuropathic pain consistent with peripheral neuropathy,	G2: supervised exercise for 30–90 min weekly. Aerobic and resistance training (leg press, biceps curls)	Six-minute walk distance
	G2: Intervention group $n = 60$, Age: 56.4 ± 6.9, supervised exercises		On coumadin,	Baseline fitness for 1 year	Metabolic parameters

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	Prospective, single-blinded cohort study		Pregnant women	Progression in aerobic exercises according to RPE and resistance according to maximum weight lifted for one repetition	Sural, radial sensory response
			Significant CV (cardiovascular) disease	Study duration:12 months	Tibial, peroneal motor response
					F responses, proximal conduction velocity
Serry et al. 2016 [34]	$N=60$	Type 2 DM ≥ 10 years	BMI ≥ 30 kg/m 2	G1: TENS at 15 Hz, pulse width 250 µs both L/L 3 times a week for 8 weeks, with regular pharmacological therapy	Medial plantar NCV
	Age 40–60 years	DPN ≥ 5 years	H/o renal failure myocardial infarction, heart failure	G2: Aerobic exercise on a stationary bicycle,3 times a week for 8 weeks, regular pharmacological therapy	VAS
	Males: 28	BMI:18.5 to 29.9 kg/m	Sensory manifestations due to any other diseases (lumbar disc prolapsed)	G3: Nerve growth stimulant; vitamin B complex and oral hypoglycemics	
	Females: 32	HbA1c < 6.5%	Circulatory problems such as intermittent claudication		
	G1: TENS group, $n=20$	Ambulant/independent patient	Skin diseases/ foot ulcers;	Study duration:8 week	
	Age: 51.6 ± 4.75	MMT (manual muscle testing) L/L ≥ grade 4			
	G2: exercise group, $n=20$				
	Age: 51.7 ± 4.44				
	G3: pharmacological group, $n=20$				
	Age: 51.95 ± 4.38				
	Randomized controlled trial				
Gholami et al. 2018 [35]	$N=31$	Type 2 diabetes and peripheral neuropathy	Patient on insulin therapy	G1: maintain habitual physical activity level	NCV/NAPA of sural, peroneal, tibial nerves

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	All male patients	Diabetes > 5 years,	DM < 5 years,	G2: 3-familiarization sessions: 10 min warm up, 15 min treadmill walk,10 min cool down followed by aerobic exercise program for 3 months (walking, jogging. or running on treadmill, 50–70% of heart rate reserve)	EST (exercise stress test)
	G1: control group, $n = 15$	HbA1c: Between 6.6% and 12%, -Diagnosed DPN	No neuropathy, medical history, exercise restriction	Three sessions per week	Glycemic control/body composition
	Mean age: 42 ± 4.6 years, mean weight: 89.3 ± 11.9 kg	MNSI score ≥ 3	Patient on regular exercises	Study duration:12 weeks	
	G2: Experimental group, $n = 16$ Mean age: 43 ± 6.4 years, mean weight: 86.5 ± 15.3 kg	Moderate neuropathy according to MDNS			
	Randomized controlled study	Absent amplitude in nerve conduction studies			
		No contraindication to exercise,			
		Inactive patients as per rapid assessment of physical activity (RAPA) questionnaire			
Gholami et al. 2021 [38]	$N = 34$	H/o diabetes > 5 years,	Not permitted to participate in exercise	G1: Control group	NCV and NAPA of sural and peroneal nerve
	Age > 60 years	HbA1c > 6.6%, inactive lifestyle	Orthopedic issues, foot deformity, ulcers, absent nerve action potential amplitude	G2: The resistance exercise program: Thrice a week /12 week ~ 90 min per session	CAVI (cardio-ankle vascular index)
	All males	mild to moderate stage of DSPN		11 exercises for large muscle groups with free weights / machines	MDNS
	G1: control ($n = 14$)			1–3 circuits with 10–15 reps for each exercise at between 50 and 60% of 1RM	MNSI
	Age: 64 ± 3 years				ABI (ankle brachial index)

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	G2: experimental ($n = 15$)				
	Age: 63 ± 3 years				
	Randomized controlled trial			Study duration-12 weeks	
Ahmad et al. 2020 [36]	$N = 44$, Both gender	Age between 45 and 75 years	Neurological impairment	G1: Diabetes foot care education	Proprioception
	G1: control group, $n = 22$	Type 1 DM or 2 ≥ 7 years	Major vascular complication	G2: sensorimotor and gait training thrice a week for 8 weeks (total 24 sessions)	conduction velocity, duration, amplitude of peroneal and tibial nerve
	Age:57.24 ± 8.85	BMI between 18.5 and 29.9 kg/m^2	Severe retinopathy	50–60 min of exercise	Surface EMG of tibialis anterior, medial gastrocnemius, vastus lateralis and multifidus
	G2: intervention group, $n = 22$	Score > 2/13 in the MNSI questionnaire	Severe nephropathy	Both groups received education regarding foot care and diabetes control once every two weeks for 30 min	
	Age: 60.33 ± 8.48	Scored > 1/10-point scale of MNSI physical examination	Severe musculoskeletal impairment to lower limb	Study duration:8 weeks	
	Two-arm, parallel group randomized controlled trial with single blinding	Impaired vibration perception	Cardiovascular complication		
		Ability to walk independently	Receiving any supervised Physical intervention		
			Plantar ulceration		
			Partial or total amputation		
Stubbs et al. 2019 [37]	$N = 45$	Fasting plasma glucose ≥ 126 mg/dL or 2-h plasma glucose concentration ≥ 200 mg/dL after a 75 g oral glucose tolerance test	Foot ulceration	G1: sedentary control	Latency, NCV, SNAP of sensory sural, median, ulnar nerves
	Males' = 43	Stable levels of (HbA1c) defined as having ≤ 1.5% change in HbA1c levels during the previous 6 months	Unstable heart disease/comorbid conditions limiting exercise	Attends 12-week health education promotion	Latency NCV, CMAP of tibial/peroneal motor nerves
	Females' = 2	Positive or negative distal sensory symptoms and nerve conduction abnormalities in at least two distal nerves	Disorders of the central nervous system causing weakness or sensory loss	G2: aerobic exercises	QST

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	Age: 45–80 years	Positive/negative sensory symptoms	medical conditions associated with neuropathies such as alcoholism, liver disease, kidney disease, toxic exposure, vitamin deficiency	10 min Warm up/30–40 min treadmill walking, 10 min cool down	SF-36 V health survey questionnaire
	G1: sedentary controls ($n=12$)		Autoimmune disorders immunoglobulin abnormalities, cancer or hypothyroidism	G3: isokinetic strengthening:	ENFD (epidermal nerve fiber density)
	Age: 61.0 ± 7.0 years			3 to 6 sets of 10 repetitions each of isokinetic leg extensions	Treadmill endurance
	G2: aerobic ($n=11$)			G4: aerobics + isokinetic exercises	Metabolic parameters
	Age: 61.9 ± 8.3 years			36-sessions treadmill walking + 36 sessions isokinetic strengthening	
	G3: isokinetic strength ($n=11$)			10 min active cool down	
	Age: 64.2 ± 9.5 years			Study duration: 12 weeks	
	G4: combination aerobic–isokinetic strength training ($n=11$)			Follow up at 12 weeks and 24 weeks	
	Age: 63.0 ± 6.6 years				
	Randomized controlled trial				
Azizi et al. 2019 [41]	$N=38$	Type 2 DM	Foot ulcers, vascular, musculoskeletal, neurological disorders	Light stretching, warm-up exercises, treadmill walking with moderate intensity, and cooldown exercises	Distal sensory latency and amplitude for the sural nerve
	Age: 56.9 ± 6.2 years	Distal peripheral neuropathy	Impaired balance or walking,	Exercise 40 to 45 min with the intensity of 70–85% of their maximum HR(heart rate)	Distal motor amplitude, velocity, and F-wave for tibial and deep peroneal nerves
	Males: 14	systolic/diastolic blood pressure < 160/100 mm Hg,	Disabilities such as peripheral arterial disease, postural hypotension, visual defect, vestibular disorders, herniated disc	Study duration:8 weeks	
	Females: 21	HbA 1c < 7%,	DM > 10 years		

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	Single-blind, 1-group, before-and-after clinical trial	Fasting blood glucose < 200 mg/dL	neuropathy > 5 years		
			H/o seeking treatment for peripheral neuropathy		
			Unwilling to follow the exercise program		
Kluding et al. 2012 [43]	$N = 19$	Signs and symptoms of DPN	Serious cardiac pathology—Musculoskeletal problems that would limit exercise ability	Moderate level of aerobic exercises using recumbent stepper, upright cycle and treadmill after light stretches, (50–70% of VO2 reserve) and strengthening exercises of moderate resistance in range (7–8 out of 10). Abdomial curls, bicep curl, chest press, lateral pulldown, leg extensions, seated leg curls, seated rows, shoulder press, squats, triceps press	100 mm VAS scale
	One group	Age group of 40–70 years	Open feet wounds	Study duration:10 weeks	HbA1C
	Age: 58.4 ± 5.98 years		Inability to ambulate independently		MNSI symptom score
	Male—8		Stroke/other CNS pathology		IENF
	Females—9		Stage 2 hypertension		Quantitative sensory testing
	Age—58.4 ± 5.98		H/o lidocaine allergy		MNSI physical exam score
	pre-test post-test design				NCV, amplitude, latency of sural, peroneal and tibial nerve
Doshi and Singarvalen. 2019 [42]	$N = 20$	Diabetic neuropathy with bilateral pinprick sensation over the sole of foot	Diabetic ulcer	Tibial nerve mobilization	Sensory sural nerve conduction velocity
	Males: $n = 9$	Both male and female participants	Comorbid disorders	Five sessions/week for 3 weeks	
	Age: 46.33 ± 4.79 years	Age group of 50–60 years		Study duration: 5 months	
	Females = 11	Ability to understand and co-operate for instructions of the test			

Table 3 (continued)

Author	Subjects (without dropouts) Age ± S.D Study design	Inclusion criteria	Exclusion criteria	Intervention	Outcome measures
	Age: 50.36 ± 7.24 years Experimental prepost study design				
Alsubiheen et al. 2017 [40]	$N = 20$	Type 2 DM (2–20 years)	On medications which can affect balance,	TC (tai chi) exercise combined with mental imaginary 1-h sessions, 2 times a week for 8 weeks	HbA1c
	Age: 63.8 ± 8.1 years	Duration of onset of diabetes was 10.8 ± 5.4 years	H/o of frequent falling, vision problems, orthopedic/ neuromuscular/cardiovascular impairments that restrict exercise	15 min of warm-up exercises, including stretching, loosening the muscles, breathing exercises,	ABC scale (the activities-specific balance confidence)
	Prepost study design	Mean HbA1C 6.8 ± 0.8		10 min of basic walking drills with and without hand techniques,	FRT distance (functional reach test)
		Hb a1c > 6.5,		15 min of TC Yang style technique teaching	OLS time (one leg standing test)
		Fasting blood glucose > 129 mg % before intervention		Study duration: 8 weeks	Soleus H-reflex latency and H/M ratio
		Full blown diabetes			NCV, latency and amplitude of sural and superficial peroneal nerve
		not practiced TC			
		Do not exercise more than once per week			
		BMI between 10 and 35 kg/ m^2			
		Normal/ controlled blood pressure			
		normal ROM			
		Atleast 5/5 muscle power bilaterally			

standard care [33, 34, 36, 38], moderate home exercises [39], and habitual physical activity [35]. Balducci et al. [32] compared the supervised exercise group with diabetic patients of sedentary lifestyle. Hung et al. [29] made comparison between healthy participants and diabetic population using tai chi exercises. Stubbs et al. [37] compared standard care against aerobic exercises, isokinetic exercises, and with combination of aerobics and isokinetic exercises. One study [34] even compared the aerobic exercise group with two other groups, one group was given TENS (transcutaneous electrical nerve stimulation) and another one was on oral hypoglycemic drugs and nerve growth stimulants.

Outcome measures

Sensory nerve functions

Total of eight studies [32, 33, 35, 37, 38, 40–43] included NCS of sural sensory nerve as outcome measure. One study [40] included superficial peroneal nerve along with sural nerve and another study [34] included NCS of medial plantar nerve.

Sural nerve

Balducci et al. [32] showed non-significant increase in experimental group and a significant decrease in the control group in NCV of sensory sural nerve. Difference in distal latency and NAPA of sural nerve was also non-significant in both groups after 4 years of aerobic exercise training on treadmill. After 8 weeks of moderate intensity aerobic exercises on treadmill, Dixit et al. [33] found a significant difference for conduction velocity and a non-significant difference for latency, duration, and amplitude in the two groups. Gholami et al. [35] showed NCV of sural nerve increased significantly in the exercise group and non-significant changes in NAPA after 12 weeks of aerobic exercises on treadmill. Kluding et al. [43] showed non-significant changes in NCS of sensory sural nerve after 10 weeks of moderate intensity aerobic exercises on treadmill, stepper, upright cycle along with strengthening exercises. Alsubiheen et al. [40] observed significant improvement in velocity, amplitude and latency of sural nerve after completing 8 weeks of tai chi exercises. Azizi et al. [41] demonstrated statistically significant increase in sural sensory nerve action potential amplitude and non-significant changes in latency after 8 weeks of aerobic exercise program on treadmill with moderate intensity. Doshi and Singarvelan. [42] showed statistically significant difference in NCV of sural nerve after 3 weeks of tibial nerve mobilization. No changes were seen in electrophysiological parameters of sural nerve by Stubbs et al. [37] irrespective of kind of exercise for 12 weeks. Though, Gholami et al. [38] achieved significant improvements in NCV of sural nerves, without any improvement in NAPA.

Medial plantar nerve

Serry et al. [34] showed that there was no statistically significant differences in sensory conduction velocity of medial plantar nerve in any of three groups after 8 weeks of aerobic exercises on stationary bicycle or TENS treatment when compared to standard medical care.

Superficial peroneal nerve

Significant improvements were noticed by Alsubiheen et al. [40] in velocity, amplitude and latency of superficial peroneal nerve after 8 weeks of tai chi exercises.

Motor nerve functions

Ten studies [29, 32, 33, 35–39, 41, 43] observed effects of different exercise interventions on motor functions of peroneal nerve and eight studies [29, 35–39, 41, 43] assessed such effects on both peroneal and tibial nerves.

Peroneal and tibial nerves Balducci et al. [32] showed that after four years of aerobic exercise training, there was significant increase in NCV of peroneal motor nerve in intervention group whereas control group showed insignificant decrease in conduction velocity. There was no significant difference in DL and NAPA of peroneal nerve between the two groups. Hung et al. [29] showed significant improvements in NCV of motor tibial nerve in DM group after 12 weeks of tai chi exercises. No significant changes were observed in distal latency and proximal/distal amplitudes in DM group post intervention.

Following 8 weeks of moderate intensity aerobic exercises, Dixit et al. [33] found that there was a significant difference in the conduction velocity of the distal segment of the peroneal nerve. However, there was no significant difference for latency and duration. Also, there was significant increase in the peroneal nerve's mean velocity. Singleton et al. [39] observed no significant improvements in tibial F-response latency and Peroneal NCV after 1 year of aerobics and resistance exercises.

According to Gholami et al. [35], the peroneal motor nerve's NCV increased from 39 m/s at week 0 to 40.4 m/s at week 12 ($p = 0.021$). In the motor NCV of the peroneal and tibial nerves, however, the time group interaction was not statistically significant. There were no statistically significant changes in NAPA of tibial or peroneal nerve in any of the groups after 12 weeks of aerobic exercise. Ahmad et al. [36] observed 6.43% increase in the intervention group in

conduction velocity of the peroneal nerve in comparison to 0.6% increase in control group. It was also notable that there was a difference in the peroneal nerve's conduction velocity for both the time effect and the time group interaction. Tibial nerve's conduction velocity likewise shown a significant temporal effect. Intervention group showed 12.46% increase in conduction velocity and control group showed and 8.83 increase in conduction velocity of tibial nerve. There were significant improvements in time × group interaction for tibial nerve latency. Although the distal latency of the tibial nerve decreased in the sensorimotor exercise and gait training group, the latency increased in the control group. With regard to time or group, the peroneal nerve's amplitude and duration did not significantly change.

Kluding et al. [43] did not observed any significant changes in latency, amplitude or conduction velocity of peroneal and tibial nerve after combination of 10 weeks of moderate intensity aerobic exercises and strengthening exercises. Azizi et al. [41] observed significant increase in CMAP amplitude of tibial nerve and significant decrease in NCV and F-wave of tibial nerve. For deep peroneal nerve, there was statistically significant increase in NCV and non-significant changes in CMAP, amplitude and F-wave after 8 weeks of moderate intensity aerobic exercises. Stubbs et al. [37] did not achieved any significant improvements in motor nerve conduction of either nerve in any intervention of 12 weeks. On the contrary, Gholami et al. [38] achieved significant improvements in MNCV of peroneal nerve after 12 weeks of resistance exercises (changes in NCS with different exercises are mentioned in Table 4).

Discussion

This systematic review evaluated the changes in NCS of lower limb sensory as well as motor nerves after different exercises and manual therapy in patients with DM with and without peripheral neuropathy. All the studies that included tai chi exercises, sensorimotor training, neurodynamic mobilization and most of studies with moderate intensity aerobic exercises on treadmill or bicycle have showed improvement in NCVs of motor peroneal, tibial nerves and sensory sural nerve with non-significant effects on that sensory NAPA and latency in DM with or without peripheral neuropathy.

NCS are known to document the severity and changes in neuropathy and the outcomes are reproducible and standardized [44]. Nerve conduction detects neuropathy even before signs develop, thus diagnostic value of NCS is better than clinical examination, Vibration perception threshold or other neuropathy symptom scores as sensory neuropathies are better picked by SNAP than VPT and motor neuropathies are appreciated more in CMAP than clinical examinations [45].

Sensory symptoms presented in diabetic neuropathy patients begins with injury to sensory nerve fibers that results from demyelination which precedes axonal loss as evident in neurophysiological studies [46, 47], Later involving the motor fibers [48]. Conduction velocity and amplitude of sural and peroneal nerves are known to be reduced in diabetics in comparison to healthy individuals [49]. Thus, predicting the changes in nerve conduction can better predict the effects of different exercises on neuropathy than other outcome measures.

Moderate intensity exercises

Amongst the six studies [32–35, 37, 41] that examined effects of moderate intensity aerobic exercises, two studies [33, 35] observed improvements in sensory NCV of sural nerve without any significant changes in NAPA, latency and duration after 8 weeks and 12 weeks of moderate intensity aerobic exercises respectively. Gholami et al. [35] also observed significant reduction in fasting glucose levels, HbA1c, and BMI levels indicating that the exercises has resulted in improved glucose control that facilitated blood flow to peripheral nerves. As has been suggested sensory nerves are more sensitive to hyperglycemia and exercise related adaptations, so gets affected in early course of disease and shows improvements even faster [50, 51].Though Dixit et al. [33] did not find any improvements in metabolic parameters but observed reduction in insulin dosage in experimental group whereas the control group had increased insulin dosage.

Authors suggested reversal of impaired oxygenation brought about by improved nitrous oxide production could have prevented micro- and macrovascular complications that might have reversed the neuropathy [52]. On the contrary Azizi et al. [41] found significant improvements in sural sensory NAPA without any changes in latency. As suggested by Orlando et al. [53], electrical activities of nerves improve by improving neural collateral sprouting brought about by exercises by means of increased blood flow to meet metabolic requirements. Azizi et al. [41] stated that improved action potential amplitude is the result of reduced lower limb edema and distance between the nerve and point of recording electrical activity. Over the course of 12 weeks of intervention, Stubbs et al. [37] found no differences in HbA1c in the group that received moderate intensity aerobic exercises. Even the other two groups with resistance exercises and combined aerobic and resistance training did not showed any changes in nerve conduction or glucose parameters. Although there were minor improvements in sensory nerve functioning ($p = 0.01$) and noticeable improvement in nerve fiber density in the intervention groups, the authors credit this to the enhanced localized production of several neurotrophic (BDNF, NGF, NT-3) and associated factors by

the sensory ganglia. Serry et al. [34] who compared aerobic exercises with pharmacological group and group receiving TENS also did not observed any changes in medial plantar nerve SCV. Though the reason they gave for the same is that medial plantar sensory NCS provided a more sensitive diagnosis of DPN, even in patients with normal range measurements in the sural nerve [54, 55]. On the contrary, Frigeni et al. [56] recommended examination of dorsal sural nerve rather than medial plantar nerve for comfortable and accurate diagnosis. Similarly, Kural et al. [57] concluded that distal nerve NCS, particularly the dorsal sural nerve, has excellent diagnostic power comparable to sural NNT recording in DPN.

Another study that examined effects of moderate intensity aerobic exercises was Balducci et al. [32] who observed non-significant increase in NCV of sural nerve and significant reduction of same in control group without any significant changes in DL and NAPA of sural nerve. Since Balducci et al. [32] enrolled patients of diabetes without neuropathy unlike Dixit et al. [33] and Gholami et al. [35] who included patients with neuropathy symptoms observed that the patients who performed moderate intensity aerobic exercises developed less sensory and motor neuropathy as compared to sedentary patients over 4 years of duration. Though he did not achieve any changes in glucose parameters and BMI and considered the local microvascular changes in peripheral nerves resulted from exercises responsible for the outcomes of study. Improvement in metabolic requirements, endothelial vasodilation [58, 59], and higher vascular growth factor expression resulted from exercises are considered as the factors responsible for the effects [60]. Amongst the studies that investigated effects of moderate intensity aerobic exercises on motor nerve conduction three studies [32, 33, 41] observed significant improvements in NCV of peroneal nerve; however, two studies [35, 37] did not observed any significant improvements in motor peroneal/tibial nerve conduction. Possible explanation for same is differential nerve fiber involvement among DSPN patients as small unmyelinated sensory nerve fibers can regenerate faster than large myelinated motor fibers [48, 61, 62].

Resistance training

Resistance training is known to induce neuroplasticity [63] and enhance nerve regeneration by activating the effects of neurotrophin and increasing expression of brain derived neurotrophic factors [64]. Four studies [37–39, 43] observed effects of resistance training either alone or in combination with other exercises on nerve conduction parameters of lower limb nerves. Amongst these, three studies [37, 39, 43] included moderate intensity aerobic exercises in combination with moderate resistance strengthening exercises but did not observe any significant improvements in sural, tibial, or peroneal nerve conduction parameters; however, there were appreciable changes in IENFD in all three studies. As Singleton et al. [39] observed significant improvements in proximal thigh and distal ankle IENFD, Kluding et al. [43] found increased axonal branching at the proximal biopsy site and Stubbs et al. [37] noticed marked improvement in about 50% patients in ENFD, suggesting regeneration of distal nerves. In a different study that looked at how resistance training alone affected DPN, Gholami et al. [38] found that there were significant improvements in sural sensory NCV both among and between groups, but no significant changes in SNAP. Motor peroneal NCV and NAPA also improved significantly in experimental group and between groups after 12 weeks of training.

Tai Chi

Two studies [29, 40] observed effects of tai chi exercises on type 2 DM patients. Alsubiheen et al. [40] observed significant improvements in NCV, amplitude as well as latency of sural sensory and superficial peroneal nerves after 8 weeks of tai chi exercises 1 h daily with mental imaginary session and TC yang exercises twice daily. They suggested that improved peripheral micro circulation brought about by increased cardiac output was responsible for improved nerve conduction. Hung et al. [29] who examined effects of tai chi exercises of 12 weeks duration observed significant improvements in Motor NCV of tibial nerve without any changes in CMAP. Tai chi is a body-mind exercise that includes combination of weight shifting, postural alignment, coordinated with synchronized deep breathing [65, 66], and concentration on complete movement as complex series of movements which is essential for re learning of damaged nervous system makes it different from other exercises [7, 67]. Tai chi exercises may be related to improve blood sugar control and insulin resistance, along with improved NO release that is responsible for bringing improvement in NCV.

Manual therapy

Doshi and Singarvalem. [42] achieved significant improvements in SNCV of sural nerve after 3 weeks of neurodynamic mobilization of tibial nerve in DPN patients. Neurodynamic mobilization is known to reduce neural edema and concentration of proinflammatory mediators, thereby promoting nerve regeneration and neural plasticity [68, 69]. Neurodynamic mobilization also improves vibration perception thresholds [70], neuropathic pain, and quality of life in DPN patients [71]. Various cadaver [72, 73] and animal studies [74] also shows the effectiveness of manual therapy in DPN.

Table 4 Results

Author. year	Outcome measurement	Results					Conclusion
		Pre control	Post control	Pre intervention	Post intervention	*p*-value	
Balducci et al. 2006 [32]	Peroneal motor						Significant differences in delta in NCV for both peroneal and sural nerve between the intervention and control group. Peroneal motor NCV significantly increased in the intervention group and insignificantly decreased in the control group. No significant increase in sensory sural nerve in the intervention group and significant decrease in the control group. No significant difference in both peroneal and sural DL and NAPA between the two groups.
	NCV (m/s)	46.6 ± 3.2	46 ± 5.36	47 ± 3.27	48.8 ± 2.24*	$p < 0.05$, Delta NCV, $p < 0.001$	
	NAPA (mV)	2.93 ± 1.68	2.70 ± 1.07	3.19 ± 1.9	2.81 ± 0.98		
	DL (m/s)	4.41 ± 0.64	4.40 ± 0.61	4.38 ± 0.83	4.34 ± 0.49		
	Sural sensory						
	NCV (m/s)	47.0 ± 3.5	44.3 ± 7.84*	47.1 ± 4.01	47.5 ± 3.18	$p < 0.05$, Delta NCV, $p < 0.001$	
	NAPA (μV)	20.3 ± 5.02	19.4 ± 4.66	21.5 ± 5.24	21.7 ± 5.44		
	DL (m/s)	3.43 ± 0.54	3.27 ± 0.52	3.40 ± 0.92	3.25 ± 0.50		
Hung et al. 2009 [29]	Right Peroneal motor						Patients with DM improved significantly, both in right NCVs and left NCVs. No significant improvements in the control group. Proximal amplitudes increased in the DM group, did not reach a significant increase (right: $p = 0.077$; left: $p = 0.085$). No significant improvements in the control group. Proximal amplitudes increased in the DM group, did not reach a significant increase (right: $p = 0.077$; left: $p = 0.085$). Distal amplitude of any nerve in the DM group did not significantly change. Non-significant changes in both groups in distal latency after intervention.
	NCV(m/Sec)	48.4 ± 3.9	48.8 ± 4.1	45.8 ± 4.4	45.6 ± 3.3	($p = 0.046$)	
	DL (msec)	3.38 ± 0.56	3.43 ± 0.46	3.62 ± 0.74	3.62 ± 0.70		
	Proximal amplitude(mV)	7.74 ± 3.30	8.29 ± 3.95	6.04 ± 2.25	6.18 ± 2.34		
	Left Peroneal motor.						
	NCV (m/sec)	48.4 ± 3.3	48.4 ± 4.1	45.9 ± 4.9	45.6 ± 3.2	($p = 0.041$)	
	DL (msec)	3.48 ± 0.51	3.44 ± 0.47	3.45 ± 0.50	3.45 ± 0.53		
	Proximal amplitude (mV)	7.54 ± 3.32	8.01 ± 3.89	5.96 ± 2.54	6.30 ± 2.53		
	Right tibial motor						
	NCV (m/sec)	47.8 ± 4.4	47.9 ± 4.0	43.4 ± 5.2	45.5 ± 4.4	$p = 0.027$	
	DL (msec)	3.63 ± 0.53	3.59 ± 0.60	3.89 ± 0.77	3.89 ± 1.0		
	Proximal amplitude (mV)	16.3 ± 5.16	16.8 ± 4.79	11.5 ± 6.41	11.7 ± 7.13		
	Left tibial motor						
	NCV (m/sec)	47.5 ± 4.4	47.4 ± 4.5	44.1 ± 4.9	45.3 ± 4.3	$p = 0.036$	
	DL (msec)	3.73 ± 0.58	3.68 ± 0.52	4.08 ± 0.86	3.86 ± 0.83		
	Proximal amplitude (mV)	17.0 ± 5.78	17.3 ± 5.29	10.3 ± 6.37	10.7 ± 6.52		
Dixit et al. 2014 [33]	Peroneal motor						Distal peroneal NCV: significant difference in two groups at 8 weeks. (p value less than 0.05 was considered significant) Sural sensory nerve at 8 weeks: significant difference in two groups for conduction velocity. No significant differences in latency, amplitude and duration in either nerve.
	NCV (m/s)	38.40 ± 1.36	38.21 ± 1.31	42.48 ± 1.25	45.56 ± 1.24	$p = 0.03$	
	Latency (msec)	3.33 ± 1.78	3.16 ± 1.83	4.04 ± 1.57	4.34 ± 1.25	$p = 0.11$	
	Amplitude (mV)	4.55 ± 2.28	4.75 ± 2.13	6.81 ± 2.07	6.31 ± 2	$p = 0.65$	
	Duration (msec)	10.69 ± 1.27	10.89 ± 1.23	9.99 ± 1.27	10.76 ± 1.23	$p = 0.08$	

Table 4 (continued)

Author. year	Outcome measurement	Results					Conclusion
		Pre control	Post control	Pre intervention	Post intervention	*p*-value	
	Sural Sensory						
	NCV (m/s)	28.23 ± 1.49	28.53 ± 1.49	23.67 ± 1.81	31.39 ± 1.58	P < 0.001	
	Latency (msec)	3.39 ± 1.35	3.39 ± 1.45	3.51 ± 1.50	3.45 ± 1.38	*p* = 0.33	
	Amplitude (mV)	3.23 ± 2.19	3.94 ± 2.23	2.48 ± 2.55	2.14 ± 2.38	*p* = 0.85	
	Duration (msec)	1.49 ± 1.50	1.46 ± 1.90	1.45 ± 1.89	1.86 ± 1.75	*p* = 0.27	
Singleton et al. 2014 [39]	Peroneal Motor						Non-significant changes in neuropathy measures between groups at 12 months. Significant improvements in IENFD in exercise group.
	NCV (m/sec)	44.9 ± 4.0	2.4 ± 8.3	44.7 ± 4.4	0.8 ± 7.7	*p* = 0.36	
	Tibial F-wave						
	F-response Latency (msec)	52.7 ± 6.9	0.7 ± 4.0	53.1 ± 5.7	-0.2 ± 2.9	*p* = 0.28	
Gholami et al. 2021 [38]	Peroneal motor						There was significant difference between groups in sural sensory and peroneal motor NCV. Increase of motor Peroneal APA in the experimental group.
	NCV (m/s)	36.02 ± 9.41	35.90 ± 8.97	33.01 ± 8.88	35.38 ± 8.72	*p* = 0.001	
	NAPA (mV)	2.95 ± 1.33	2.98 ± 1.29	2.92 ± 1.16	3.12 ± 1.15	*p* = 0.034	
	Sural sensory						
	NCV (m/s)	28.56 ± 8.92	28.93 ± 8.69	27.62 ± 8.75	30.06 ± 8.56	*p* = 0.001	
	NAPA (μV)	4.67 ± 2.01	4.64 ± 1.99	4.54 ± 2.06	4.63 ± 2.05	*p* = 0.139	
Kluding et al. 2012 [43]	Peroneal motor	NA	NA				No significant changes in any of the nerve conduction study or quantitative sensory testing. Significant improvements were found in ratings of the worst pain over the past months. IENF branching at the proximal biopsy site (0.16 to 0.27 branch nodes/fiber, *p* = .008).
	NCV (m/s)			39.6 ± 11.5	38.9 ± 10.9	*p* = 0.46	
	Latency (ms)			4.1 ± 1.25	4.1 ± 1.17	*p* = 1.0	
	Amplitude (mV)			4.48 ± 2.5	4.53 ± 2.6	*p* = 0.85	
	Tibial motor	NA	NA				
	NCV (m/s)			35.1 ± 10.8	38.4 ± 10.8	*p* = 0.71	
	Latency (ms)			3.63 ± 1.04	3.69 ± 1.13	*p* = 0.58	
	Amplitude (mV)			6.66 ± 5.3	6.6 ± 5.2	*p* = 0.85	
	Sural sensory	NA	NA				
	Latency (ms)			2.99 ± 1.8	2.69 ± 1.8	*p* = 0.23	
	Amplitude (μV)			6.41 ± 5.9	5.59 ± 5.14	*p* = 0.35	

Table 4 (continued)

Author. year	Outcome measurement	Results						Conclusion
		Pre control	Post control	Pre intervention	Post intervention	*p*-value		
Alsubiheen et al. 2017 [40]	Superficial peroneal	NA	NA					Significant improvements in velocity, amplitude and latency of sural nerve. In the superficial peroneal nerve, significant improvements were observed in velocity and latency, No significant changes in amplitude. Soleus H-reflex amplitude significantly increased ($p=0.02$), no significant changes for latency and H/M ratio.
	NCV (m/s)			28.3 ± 4.8	32.4 ± 5	$p=0.02$		
	Amplitude (μV)			8.4 ± 2.1	8.3 ± 1.9	$p=0.96$		
	Latency (ms)			3.2 ± 0.5	2.8 ± 0.5	$p=0.01$		
	Sural sensory	NA	NA					
	NCV (m/s)			30.9 ± 3.6	33.8 ± 3.9	$p=0.01$		
	Amplitude (μV)			18.2 ± 4.1	7.6 ± 1.2	$p=0.01$		
	Latency (ms)			3.7 ± 0.4	3.4 ± 0.4	$p=0.01$		
Azizi et al. 2019 [41]	Deep peroneal motor	NA	NA					Significant increase in sural sensory nerve action potential amplitude, tibial amplitude of compound muscle action potential, and deep peroneal nerve conduction velocity. Decrease in tibial F-wave and nerve conduction velocity.
	NCV (m/s)			46.5 ± 0.5	48.3 ± 0.6	$p=0.001$		
	NAPA (mV)			2.5 ± 0.7	2.5 ± 0.8	$p=0.552$		
	F-wave (ms)			58.5 ± 2.1	58.2 ± 2.4	$p=0.086$		
	Tibial motor	NA	NA					
	NCV (m/s)			45.0 ± 2.4	43.3 ± 3.1	$p=0.001$		
	NAPA (mv)			2.8 ± 0.9	3.1 ± 1.1	$p=0.001$		
	F-wave (ms)			59.4 ± 1.9	58.9 ± 2.3	$p=0.024$		
	Sensory sural	NA	NA					
	NAPA (μV)			5.9 ± 1.7	7.9 ± 2.5	$p<0.001$		
	Latency (ms)			3.8 ± 0.6	3.9 ± 0.3	$p=0.702$		
Doshi & Singarvalem. 2019 [42]	Sensory NCV	N/A	N/A	21.42 ± 3.08	25.37 ± 4.90	$p<0.05$		Statistically significant difference in the nerve conduction velocity of sural nerve after giving the intervention for three weeks.
		Pre control	Post control	Pre-intervention	Post-intervention	*p*-value (Pre-Post or Group effect)	*p*-value (Time × group)	
Gholami et al. 2018 [35]	Peroneal motor							Sural NCV in the exercise group increased significantly. Changes in peroneal and tibial motor NCV and (NAPA) in all nerves studied were not different between groups ($p>0.05$).
	NCV(m/s)	41.8 ± 4.4	42.0 ± 5.2	39.0 ± 3.6	40.4 ± 4.4	$p=0.021$	$p=0.113$	
	NAPA (μV)	3.0 ± 0.6	3. ± 0.6	3.2 ± 1.7	3.3 ± 1.3	$p=0.418$	$p=0.976$	

Table 4 (continued)

Author. year	Outcome measurement	Results						Conclusion
		Pre control	Post control	Pre intervention	Post intervention	*p*-value		
	Tibial motor							
	NCV(m/s)	40.0 ± 3.9	40.5 ± 4.8	38.3 ± 6.6	40.2 ± 6.1	NA	$p = 0.278$	
	NAPA (μV)	4.7 ± 2.0	4.8 ± 1.8	5.0 ± 1.3	5.4 ± 1.5	$p = 0.684$	$p = 0.366$	
	Sural sensory							
	NCV(m/s)	33.7 ± 2.5	33.0 ± 2.8	35.2 ± 4.3	37.3 ± 6.2	$p = < 0.05$	$p = 0.07$	
	NAPA (μV)	6.7 ± 2.1	6.8 ± 2.1	7.1 ± 2.6	7.4 ± 2.5	$P = 0.364$	$P = 0.654$	
Ahmad et al. 2020 [36]	Peroneal motor							Conduction velocity of the peroneal nerve showed 6.43% increase in the intervention group. No significant changes in latency, amplitude and duration of the peroneal nerve within and between-group comparisons. There was an increase in conduction velocity of tibial nerve in both groups. Decrease in distal latency of tibial nerve in intervention group.
	NCV (m/Sec)	36.37 ± 7.9	36.60 ± 8.47	38.37 ± 6.62	40.84 ± 5.88	$p = 0.183$	$p = 0.022$	
	Latency (msec)	4.12 ± 1.32	4.15 ± 1.2	4.19 ± 1.2	3.70 ± 0.75	$p = 0.579$	$p = 0.061$	
	Amplitude (mV)	4.45 ± 1.85	4.34 ± 1.67	4.44 ± 2.21	4.44 ± 2.05	$p = 0.507$	$p = 0.061$	
	Duration (msec)	10.61 ± 2.8	10.86 ± 2.4	10.32 ± 2.02	10.06 ± 2.03	$p = 0.453$	$p = 0.222$	
	Tibial motor							
	NCV (m/sec)	38.39 ± 11.69	41.78 ± 10.8	37.94 ± 7.35	42.67 ± 8.57	$p = 0.941$	$p = 0.503$	
	Latency (msec)	4.59 ± 0.79	4.91 ± 0.94	4.80 ± 1.13	4.39 ± 0.92	$p = 0.574$	$p = 0.03$	
	Amplitude (mV)	6.12 ± 3.89	6.56 ± 4.3	6.74 ± 3.83	6.72 ± 3.56	$p = 0.758$	$p = 0.279$	
	Duration (msec)	7.92 ± 2.02	8.33 ± 2.03	8.04 ± 1.34	8.03 ± 1.71	$p = 0.872$	$p = 0.346$	
		Group A (TENS) Pre	Group A (TENS) Post	Group B (Exercise) Pre	Group B (Exercise) Post	Group C (Pharma) Pre	Group C (Pharma) Post	
Serry et al. 2016 [34]	Medial Plantar Sensory							No significant effect on medial plantar NCV in any group. No significant difference between three groups in NCV.
	NCV(m/sec)	27.69 ± 3.14	27.74 ± 3.15	28.01 ± 2.67	28.07 ± 2.62	27.89 ± 3.13	27.91 ± 3.07	
		At Baseline (Sedentary group)	At 12 weeks (Sedentary group)	At Baseline (Exercise groups)	At 12 weeks (Exercise groups)	No. of individual patients exhibiting improvement of individual responses		
				Three different Exercise groups: Aerobic, Strength, Combine				

Table 4 (continued)

Author. year	Outcome measurement	Results					Conclusion
		Pre control	Post control	Pre intervention	Post intervention	*p*-value	
Stubbs et al. 2019 [37]	Peroneal & Tibial Motor						No significant difference was observed in tibial and peroneal motor nerve within and across experimental groups (among any of the group exercise), regardless of type of exercise. Qualitative improvement in NAPA of sensory sural nerve that had been absent in prior baseline evaluation.
	NCV	Values NR	Values NR	Values NR	Values NR	> 10% increase	
	Latency	Values NR	Values NR	Values NR	Values NR	> 10% decrease	
	Amplitude	Values NR	Values NR	Values NR	Values NR	> 10% increase	
	Sural Sensory						
	NCV	Values NR	Values NR	Values NR	Values NR	> 10% increase	
	Latency	Values NR	Values NR	Values NR	Values NR	> 10% decrease	
	Amplitude	Values NR	Values NR	Values NR	Values NR	> 10% increase	

DL Distal Latency; *NAPA* Nerve action potential amplitude; *NCV* Nerve conduction velocity; *NA* Not Available; *NR* Not reported; $p < 0.05$ was considered as significant difference

Sensorimotor training

Sensorimotor training is known to improve sensory inputs and muscle activation that aids in preserving joint stability [75]. These techniques regulate the movement through central nervous system and are known to improve balance, proprioception [3, 76], and gait parameters [77] in DPN. Ahmad et al. [36] showed significant improvements in NCV of peroneal and tibial nerve after 8 weeks of sensorimotor and gait training without any significant improvements in latency, duration, and amplitude. This restoration in nerve function mechanism might be due to reversal of chronic hypoxia of nerves brought about by increase in endoneural blood flow, decrease in nitrous oxide that prevented polyol pathway in diabetes and had deleterious effects on Schwann cells and endothelium [52, 60, 78].

Most of the studies included in this review that showed improvements in NCS of lower limb nerves have followed the exercise recommendation of at least every alternate day and minimum 150 min/week of physical activity including aerobic exercises and resistance training for optimal glycemic and health outcomes as suggested by "The American Diabetes Association"(ADA) [79]. Other than two studies [39, 43] that did not show significant improvements in nerve conduction parameters of lower limb nerves have given weekly exercises of less frequency and duration than recommended by ADA. This points towards the importance of duration and frequency of exercises.

Though chronic hyperglycemia and dyslipidemia caused by insulin resistance causes oxidative stress and thus cellular damage resulting in neuropathic symptoms in DPN [80, 81], studies [35, 36, 38, 43] had shown improvements in metabolic parameters along with NCS while others [32, 33, 37, 41] do not. Neuropathy develops even in prediabetic patients and diabetic patients with glycemic control [22] indicating that there can be some other mechanisms other than glucose control through which physical exercises can enhance nerve regeneration [32, 39, 82–84]. Exercise was thought to promote the expression of neurotrophins, including glial-derived neurotrophic factor (GDNF) and brain-derived neurotropic factors (BDNF) [83] and insulin-like growth factor-1 (IGF-1) that modulates axonal plasticity [85]. According to a study done by Asensio-Pinilla et al. [82], mice trained on a treadmill had higher numbers and larger sizes of regenerated axons, more mature myelination, and more pronounced muscular hypertrophy than mice who received no exercise interventions. Wilhelm et al. [84] also observed that exercise training increases BDNF in neurons by which regenerating axons overcome a lack of BDNF expression in cells in the pathway through which they regenerate. In a study, exercise group has shown improvements in the amplitude of the CMAP and shorter distal latency in the exercise group compared to no-exercise indicating better myelination of the regenerating fibers [85].

Limitations of the review

This review has included both RCT's as well as prepost study designs that has compromised the quality of study. Inaccessibility of some databases that was proposed prospectively in PROSPERO registration has further limited the scope of findings. There was heterogeneity in inclusion criteria, diagnostic measures, duration, and frequency of exercises given, so proposed meta-analysis could not be done, and outcomes cannot be generalized. Small sample size and shorter duration of studies can affect the outcomes in NCS.

Strength of study

This is the first study to analyze effects of different exercises on NCS of lower limb in diabetes and diabetic peripheral neuropathy. The study examined the scientific literature on both diabetes and diabetic neuropathy to ensure that the findings can be applicable to a wider population and demonstrate the risk of neuropathy that may arise in diabetic patients even before clinical presentation. This study contributes significant clinical data for a health problem that needs urgent attention both domestically and internationally to the body of existing research and can be used as a reference to incorporate physical exercises and early detection of nerve conduction parameters to halt the disease's progression.

Implications for future studies

Neuropathic symptoms associated with diabetes are result of changes in axons that take a specific time period to be presented in nerve conduction depending on rate of degeneration or regeneration. Thus, to evaluate such changes and effects of different physical exercises on same needs more homogenous studies of longer duration.

Conclusion

Majority of studies included in this review used moderate intensity aerobic exercises, though they varied in methodological quality, dosage, and duration of intervention. This systematic review indicates that moderate intensity aerobic exercises, tai chi exercises, sensorimotor training, and manual therapy modifies neuropathic symptoms caused by hyperglycemia and improves NCV of sensory sural and motor peroneal/tibial nerves. Manual therapy seems to be a promising management in treatment of symptoms associated with DPN, though due to limited number of studies results cannot be generalized.

Declarations

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Association of microalbuminuria with left ventricular diastolic dysfunction in type 2 diabetes mellitus

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Abstract

Background Diabetes mellitus is an important independent risk factor for the development of cardiovascular and renal disease which has been the cause of death in majority of the diabetic population. Albuminuria has been shown to predict cardiovascular morbidity and mortality in diabetics.

Objective The purpose of this study was to correlate microalbuminuria and left ventricular diastolic dysfunction (earliest manifestation of heart disease) in type 2 diabetes mellitus to aid in subclinical diagnosis, risk stratification, and prompt management of cardiovascular disease in diabetic patients.

Methods This study assessed the incidence of left ventricular diastolic dysfunction (LVDD) using echocardiography and its correlation with microalbuminuria and with other parameters like age, gender, BMI, duration of diabetes mellitus, and glycosylated hemoglobin in 90 normotensive, type 2 diabetic patients in a tertiary care hospital in Western India.

Results The prevalence of LVDD in our study is 59% ($n = 53$). The mean age of the study subjects was 60.7 years. Out of 59 subjects with BMI ≥ 25 kg/m^2, diastolic dysfunction was seen in 31 patients. The mean duration of diabetes mellitus in our study is 11.5 years. Good glycemic control, i.e., HbA1c ≤ 7.0, was seen in 13 patients with LVDD. Out of 53 subjects with LVDD, 48 (90.5%) subjects had microalbuminuria.

Conclusion There is a significant correlation between microalbuminuria and LVDD in type 2 diabetes mellitus, independent of other parameters studied (age, sex, BMI, glycemic control, and duration of diabetes).

Keywords Diabetes mellitus · Left ventricular diastolic dysfunction · Echocardiography · Microalbuminuria · HbA1c

Introduction

Diabetes mellitus is one the most common causes of morbidity and mortality in India and around the world [1]. The incidence of diabetes mellitus and its complications is showing an enormous rise in the recent years. It has been identified as an important independent risk factor for the development of cardiovascular and renal disease [2]. Diabetic patients may develop underlying cardiovascular disease without showing signs or symptoms of the same until it is too late or advanced disease sets in.

Left ventricular diastolic dysfunction (LVDD) is the earliest pre-clinical manifestation of heart disease, which precedes systolic dysfunction and symptomatic heart failure. The pathogenesis of LVDD is not clearly understood and has been proposed that many underlying mechanisms such as microvascular disease, autonomic dysfunction, metabolic disorders, and interstitial fibrosis have been suggested as etiological factors [3–6]. It is characterized by delayed relaxation and increased filling time leading

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to decreased contraction velocity and cardiac output [7]. This can lead to activation of renin angiotensin causing myocardial injury and myocardial remodeling. Arrhythmias, pump failure, and death are all possible outcomes. Early diagnosis and treatment of diastolic dysfunction can prevent it from progressing to overt heart failure and thus help reduce morbidity and death. With the emergence of Doppler echocardiography, a non-invasive diagnostic tool, identifying LVDD has become less cumbersome [8].

Microalbuminuria is a sign of endothelial dysfunction and is the earliest manifestation of diabetic nephropathy. It has been shown to predict cardiovascular morbidity and mortality in type 2 diabetes mellitus independent of conventional cardiovascular risk factors like age, hypertension, and dyslipidemia [9, 10]. Although the mechanism of the association of albuminuria with cardiac events is unclear, the vascular changes that lead to renal dysfunction may also be present in the vasculature of the heart and thus contribute to cardiac dysfunction.

Whether albuminuria is associated with LVDD independent of other factors remains unclear. This cross-sectional study is aimed at determining an association between microalbuminuria and LVDD in type 2 diabetes mellitus.

Methods

Study design

The subjects for the study were selected from patients with type 2 diabetes mellitus in out-patient and in-patient department in a tertiary care hospital in Western India. This cross-sectional study was conducted between January 2021 and June 2022. After approval from Scientific Committee and Institutional Ethics Committee, written consent was obtained from all the study participants who satisfied the inclusion criteria.

Inclusion criteria:

1. Duration of diabetes mellitus > 5 years (Longer duration of diabetes increases the risk of microvascular and macrovascular events) [20].
2. Male and female patients > 18 years of age.

Exclusion criteria:

1. Patients with a previous or current history of heart disease.
2. Presence of comorbidities known to influence left ventricular function, i.e., thyroid disease, alcoholism, and hypertension (to avoid possible confounding factors).
3. Abnormal ECG suggestive of ischemic heart disease or bundle branch block etc. or RWMA on echocardiography.
4. Ejection fraction < 50%.
5. Urine albumin-to-creatinine ratio > 300 mg/g creatinine.

Laboratory investigations

Ninety patients with type 2 diabetes mellitus were included in the study. All patients were subjected to detailed history taking and thorough physical examination. Blood sugar levels (fasting and 2 hour post prandial), HbA1c levels, and urine albumin-creatinine ratio (UACR) were performed, and a 12-lead electrocardiogram was obtained.

Normal UACR is < 30mg/g creatinine and microalbuminuria is defined as UACR 30-300 mg/g creatinine. Macroalbuminuria, i.e., UACR > 300 mg/g creatinine, was excluded from the study.

Echocardiographic evaluation

All the patients underwent transthoracic echocardiographic evaluation. Two-dimensional, M-mode and Doppler examination were done. Left ventricular diastolic function was assessed by parameters like diastolic transmitral peak velocities (*E* and *A*), *E*/*A* ratio, *E*′, *E*/*E*′ ratio, deceleration time (DT), systolic/diastolic ratio (*S*/*D* ratio), and left atrial volume.

Left ventricular diastolic dysfunction was diagnosed and classified into grades according to recommendations from American Society of Echocardiography and the European Association of Cardiovascular Imaging as follows [11, 12] (Table 1).

Statistical analysis

Data collected were entered in Microsoft Excel sheet, and analysis of data was done using statistical package for social sciences (SPSS). For comparison of data between two

Table 1 Grades of left ventricular diastolic dysfunction

Parameters	Normal	Grade 1	Grade 2	Grade 3	Grade 4
E/*A*	0.75–1.5	≤ 0.75	0.75–1.5	≥ 1.5	> 1.5
E/*E*′	< 10	< 10	≥ 10	≥ 10	≥ 10
DT	> 140	-	> 140	< 140	< 140
S/*D* ratio	*S* ≥ *D*	*S* > *D*	*S* > *D*	*S* < *D*	*S* < *D*
LV relaxation	Normal	Reduced	Reduced	Reduced	Reduced
LA volume	Normal	> 34	> 34	> 34	> 34

E, early transmitral flow velocity; *A*, atrial flow velocity; *E*′, early diastolic velocity; *DT*, deceleration time; *S*/*D*, systolic/diastolic; *LV*, left ventricle; *LA*, left atrium

Table 2 Characteristics of study population

Characteristics	Category	*N*	%
Age (years)	18–35	0	0
	36–55	29	32.22
	56–75	56	62.22
	≥ 76	5	5.55
Sex	Male	47	52.22
	Female	43	47.78
BMI	Underweight	1	1.11
	Normal	30	33.33
	Overweight	42	46.67
	Obese	17	18.89
Duration of diabetes	≤ 15	75	83.33
	> 15	15	16.67
HbA1c	≤ 7.0	26	28.89
	> 7.0	64	71.11

groups, chi-square test was used. The confidence limit for significance was fixed at 95% level with p value < 0.05. Correlation (Pearson correlation coefficient) testing and multiple regression analysis were also carried out on the data.

Results

Clinical characteristics

A total of 320 patients with type 2 diabetes mellitus were screened, and 90 subjects who satisfied the inclusion criteria were included in this study. Out of these 90 participants, 47 were male, and 43 were female. The mean age was 60.79 ± 11.33 years, and mean BMI was 26.46 ± 3.83 kg/m^2. The characteristics of this study population are shown in the Table 2.

Table 3 Comparison of different parameters in diabetic patients with and without LVDD

Parameter	With LVDD (n = 53)	No LVDD (n = 37)	p value
Age (years)	63.13 ± 9.63	57.43 ± 12.78	0.0179
BMI (kg/m^2)	26.17 ± 3.84	26.87 ± 3.81	0.3956
Duration of diabetes	11.49 ± 4.92	9.89 ± 4.45	0.1182
HbA1c	9.52 ± 2.83	9.11 ± 3.26	0.5270
UACR	104.84 ± 69.65	52.78 ± 59.55	0.0004

Left ventricular diastolic dysfunction

As assessed with echocardiography, the prevalence of LVDD was 59% (n = 53) and is depicted in Fig. 1.

The mean age of diabetic subjects was higher with LVDD (63.13 ± 9.63 years) compared to those without LVDD (57.43 ± 12.78 years). 54.7% were females, and 45.3% were male. Out of 53 subjects, 22 (41.5%) had normal BMI, 20 (37.7%) were overweight, and 11 (20.8%) were obese. 75.5% subjects with LVDD had poor glycemic control, i.e., HbA1c > 7%. There were no significant differences between patients with LVDD and without LVDD for age, sex, BMI, duration of diabetes, and glycemic control as summarized in Table 3.

Association with microalbuminuria

In all the 90 patients with diabetes mellitus, 64 (71%) had microalbuminuria, and out of 53 subjects with LVDD, 48 (90.5%) had microalbuminuria, and 5 (9.5%) had no albuminuria. This was statistically significant with p value 0.0004 (Fig. 2).

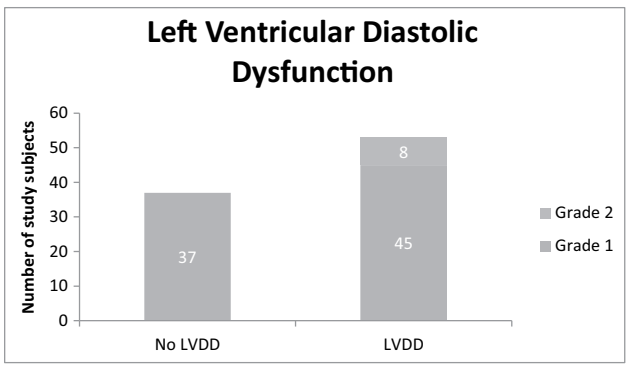

Fig. 1 Prevalence of LVDD

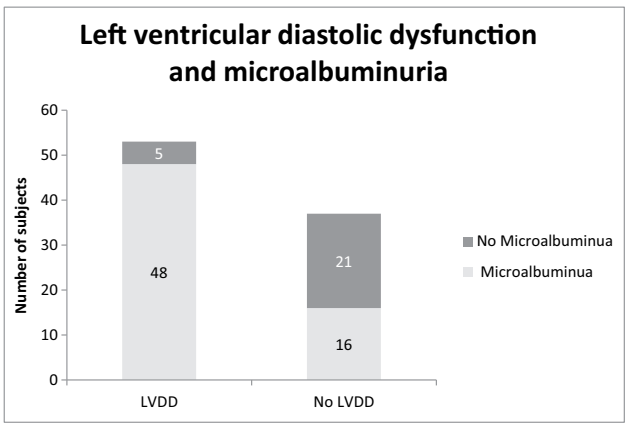

Fig. 2 LVDD and microalbuminuria

Table 4 Correlation between age, sex, BMI, duration of diabetes, HbA1c, UACR, and LVDD

	Age	Sex	BMI	Duration of diabetes	HbA1c	UACR
LVDD	0.1643493	− 0.1716	− 0.01916	0.0897713	0.058515	0.397783

BMI, body mass index; *UACR*, urine albumin-creatinine ratio; *LVDD*, left ventricular diastolic dysfunction

Correlation and regression analysis

The correlation testing of various parameters studied in this study revealed a moderate positive correlation of 0.397 between microalbuminuria and LVDD (Table 4 and Fig. 3). However, there was no strong correlation between the other parameters (Table 5).

Discussion

High prevalence of left ventricular diastolic dysfunction

After echocardiographic assessment of 90 diabetic patients, the prevalence of left ventricular diastolic dysfunction seen in this study was 59%. On further classification, 50% are grade 1 and 9% belong to grade 2. Grade 3 and grade 4 left ventricular diastolic dysfunction was not seen. This shows high prevalence of left ventricular diastolic dysfunction in our population as evidenced by this study and similar study by Jain et al. which showed prevalence of 63% (39% had grade 1, 14% had grade 2, and 10% had grade 3 diastolic dysfunction) [13]. A systemic review and meta-analysis by Bouthoorn et al. showed the pooled prevalence of LVDD in type 2 diabetics to be 48% and 35% in hospital and general population respectively [21].

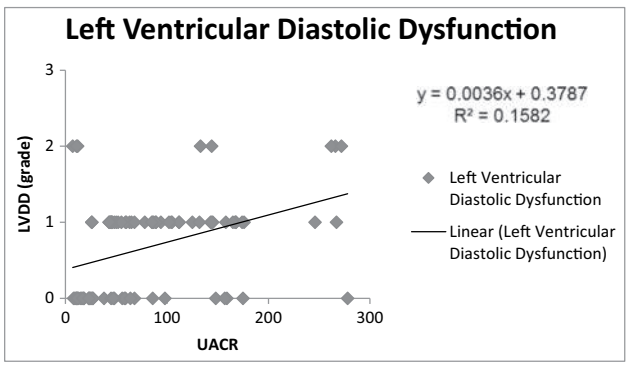

Fig. 3 Regression graph between UACR and grades of LVDD (UACR, urine albumin-creatinine ratio; LVDD, left ventricular diastolic dysfunction)

Age, sex, and obesity

In the subjects with left ventricular diastolic dysfunction, 26% were between 36 and 55 years, and 68% were between 56 and 75 years. This revealed that more number of patients were seen in diabetics with higher age group. However, whether this correlation between increasing age and diastolic dysfunction could be solely attributed to age factor or due to additive effect of increase in number of diabetic patients with advancing age cannot be differentiated by this study. Similarly in a study of 120 participants conducted by Sharavanan et al., diastolic dysfunction was found to be higher in the patients with more than 45 years of age compared to individuals in age group less than 45 years of age [14]. However, results of a study conducted by Shogade et al. in Nigerian population revealed that age remained independently associated with LVDD [22] and a similar study in Gujarat, India, by Mehta et al. showed that increase in age was significantly associated with LVDD [23].

A study in China on sex-specific associations of cardiac function by Wei et al. concluded that women had worse diastolic dysfunction and higher LV ejection function [24]. Similarly in India, Madhumathi et al. showed that prevalence of diastolic dysfunction was higher in diabetic females as compared to males [15]. Even our study showed slightly higher female preponderance in the subjects with left ventricular diastolic dysfunction (54%). However, no significant correlation between sex and left ventricular diastolic dysfunction was seen (p value 0.12).

Obesity is a major risk factor for cardiovascular diseases and is associated with abnormalities in cardiac function and is linked with heart failure. Jain et al. found that as BMI increases, LVDD increases [13]. In this study, out of 59 subjects with BMI $\geq$ 25 kg/m^2 (overweight and obese individuals), diastolic dysfunction was seen in 31 patients which showed no statistical significance between obesity and left ventricular diastolic dysfunction (p value 0.09).

Duration of diabetes and glycemic control

Diabetic complications show an increasing incidence as the duration of disease increase. Guria et al. concluded that every year of increase in the duration of diabetes increased the risk of diastolic dysfunction by 30% [16]. In subjects with left ventricular diastolic dysfunction, 81% had duration

Table 5 Regression analysis of urine albumin-creatinine ratio and left ventricular diastolic dysfunction

Regression statistics				
Multiple *R*	0.397783203			
R square	0.158231477			
Adjusted *R* square	0.148665926			
Standard error	0.584007748			
Observations	90			
	Coefficients	*Standard error*	*t Stat*	*p value*
Intercept (LVDD)	0.378659158	0.095908552	3.948127	0.000158
Urine albumin-creatinine ratio	0.003584644	0.000881363	4.067162	0.000103

LVDD, left ventricular diastolic dysfunction

of diabetes mellitus ≤ 15 years and 19% had duration > 15 years. As the duration of diabetes mellitus increases, other associated co-morbid conditions such as hypertension and ischemic heart disease become more prevalent, which were not included in our study, resulting in a lower number of patients with diabetes for more than 15 years. Correlation was thus not observed between left ventricular diastolic dysfunction and duration of diabetes mellitus.

HbA1c has been recommended as a standard of care for diagnosis and monitoring diabetes. It reflects the cumulative glycemic history of preceding 2 to 3 months and correlates well with the risk of long-term diabetes complications. Various studies conducted so far revealed a significant correlation between diastolic dysfunction and HbA1c which denotes that higher level of HbA1c has higher prevalence of left ventricular diastolic dysfunction [17, 18]. Poor glycemic control, i.e., HbA1c > 7.0, was seen in 40 patients out of 53 patients with left ventricular diastolic dysfunction in our study which showed that higher HbA1c levels show an increased prevalence of left ventricular diastolic dysfunction. However, this is not statistically significant, as opposed to a cohort study by Patro et al. that showed LV dysfunction has a strong correlation with poor glycemic control and duration of diabetes [28].

Microalbuminuria and left ventricular diastolic dysfunction

Microalbuminuria is an important independent risk factor for cardiovascular disease and renal impairment, which occurs due to endothelial dysfunction. Endothelial dysfunction has also been attributed as the pathogenesis of left ventricular diastolic dysfunction in diabetes patients. Our study showed a statistical significance between microalbuminuria and left ventricular diastolic dysfunction with p value < 0.001. This result was in accordance with a study by Liu et al. which concluded that albuminuria is independently associated with diastolic dysfunction in diabetes mellitus patients [19]. Similar results were also seen in various studies conducted across different countries, i.e., microalbuminuria is associated with increased likelihood of left ventricular diastolic dysfunction [25–27].

Conclusion

There is high prevalence of left ventricular diastolic dysfunction in normotensive, type 2 diabetes mellitus patients and significant correlation between microalbuminuria and left ventricular diastolic dysfunction, independent of other factors like age, sex, BMI, glycemic control, and duration of diabetes. Microalbuminuria can be used as a reliable screening test for left ventricular diastolic dysfunction. Early diagnosis by using non-invasive tools like echocardiography and microalbuminuria and initiation of appropriate treatment for diastolic dysfunction would lead to significant reduction in cardiovascular morbidity and mortality in patients with type 2 diabetes mellitus.

Study limitations

This study has few limitations. Type 1 diabetes mellitus patients were not included in this study. There was no control population to compare the results. Our study was performed in a tertiary care hospital in Western India. Given the ethnic group of the study population, the results cannot be generalized to other populations.

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Declarations

Ethical clearance This study was approved by the Institutional Ethics Committee of Bharati Vidyapeeth Medical College, Pune.

Conflict of Interest The authors declare no competing interests.

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ORIGINAL ARTICLE

The risk factors of early arterial stiffness in type 2 diabetes without diabetic macroangiopathy

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Abstract

Background Type 2 diabetes exposes the body to a state of high blood sugar for a long time and causes varying degrees of hardening of the arteries, making it more prone to cardiovascular emergencies.

Objective The aim of the study was to explore the risk factors of early arterial stiffness in patients with type 2 diabetes mellitus (T2DM).

Methods A retrospective study was conducted on 316 T2DM patients without macroangiopathy in The First Hospital of Qinhuangdao. Early arterial stiffness was evaluated by brachial-ankle pulse wave velocity (baPWV).

Results Ninety patients (28.5%) had baPWV≥1800cm/s. baPWV showed positive correlation with systolic blood pressure (r=0.456, p<0.001), diastolic blood pressure (r=0.133, p=0.018), urine albumin-creatinine ratio (UACR) (r=0.232, p<0.001), neutrophil lymphocyte ratio (NLR) (r=0.185, p=0.001), and visceral fat area (r=0.139, p=0.014). In multiple linear regression analysis, systolic blood pressure (β=6.240, p<0.001), UACR (β=3.805, p=0.019), NLR (β=43.722, p=0.013), and visceral fat area (β=0.778, p=0.030) were significant independent predictors for baPWV.

Conclusion The decline of arterial elasticity was common in T2DM patients without macroangiopathy. Elevated blood pressure, microangiopathy, chronic inflammation, and visceral fat accumulation were the risk factors of early arterial stiffness in patients with T2DM.

Keywords Type 2 diabetes · Early arterial stiffness · Risk factors

Introduction

In the last several decades, the prevalence of diabetes is increasing dramatically in China. Two national surveys showed that the prevalence of type 2 diabetes mellitus (T2DM) was around 10% [1, 2]. Recent research showed that the estimated prevalence of diabetes increased from 10.9% in 2013 to 12.4% in 2018 in China [3]. Diabetic macroangiopathy, such as cardiovascular disease and stroke, is a common comorbidity in T2DM. In meta-analysis, diabetic macroangiopathy affects approximately 32.2% of all patients with T2DM and is a major cause of mortality among patients with T2DM [4]. Diabetic macroangiopathy also has a substantial impact on the medical costs of T2DM [5]. Early-stage multifactorial intervention is important for reducing the risk of diabetic macroangiopathy in T2DM [6].

Brachial-ankle pulse wave velocity (baPWV) is a simple, noninvasive method with good validity and reproducibility for screening vascular damages [7]. Some studies have found that high baPWV was correlated with coronary artery stenosis and ischemic stroke in T2DM [8–11]. In cohort studies,

The present study showed that the decline of arterial elasticity was common in type 2 diabetes patients. Elevated blood pressure, microangiopathy, chronic inflammation, and visceral fat accumulation were the risk factors of early arterial stiffness in patients with type 2 diabetes.

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baPWV can predict of all-cause and expanded cardiovascular disease mortality in patients with T2DM [12–14]. BaPWV was independently associated with poorer short-term prognosis in T2DM patients with coronary artery disease [15]. In T2DM without macroangiopathy, baPWV can predict future cardiovascular events [16]. baPWV can be considered a surrogate marker of early arterial stiffness and is correlated with atherosclerotic cardiovascular disease in T2DM.

ba-PWV can predict the risk of new-onset T2DM [17]. Higher baPWV was also observed in new-onset diabetes [18]. These findings suggest that arterial stiffness already existed in new-onset diabetes. In this study, we evaluated the risk factors of early arterial stiffness in T2DM patients without diabetic macroangiopathy.

Methods

Study design

We performed a retrospective study. All subjects were adults with type 2 diabetes from the First Hospital of Qinhuangdao between June 2020 and June 2021. The inclusion criteria included the following: (1) all patients were type 2 diabetes and (2) subjects were men and women over 18 years of age. The definition of type 2 diabetes was according to the criteria of the Diabetes Society of Chinese Medical Association [19]. The exclusion criteria included the following: (1) subjects with type 1 diabetes, (2) subjects with other specific types diabetes, (3) pregnancy, (4) subjects with hematological diseases or infection, and (5) subjects with diabetic macroangiopathy, such as coronary atherosclerotic cardiopathy, stroke, or ankle brachial index$\leq$0.9. This study was approved by the ethics committee of the First Hospital of Qinhuangdao.

Data collection

All data were extracted from the Hospital Information System. Sociodemographic variables included age, sex, ethnicity, and smoking. Clinical data included the diagnosis and classification of diabetes and duration of diabetes. Physical examination included height, weight, and blood pressure. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Laboratory data included fasting plasma glucose (FPG), glycosylated hemoglobin A1C (HbA1c), blood lipid, complete blood count, and urine albumin-creatinine ratio (UACR). The neutrophil lymphocyte ratio (NLR) was calculated as the neutrophil count/the lymphocyte count.

Brachial-ankle pulse wave velocity (baPWV)

An arteriosclerosis tester (model: HBP-8000) produced by Omron Corporation (Japan) was used. The baPWV was measured by specially trained technicians. The room temperature of the examination room was maintained at 22–25°C. Before the measurement, the participants were told not to smoking and to remain in a resting state for >5 min. They were also told to wear thin clothes and stay quiet and keep their entire body relaxed at the beginning of the measurement. Subsequently, the participants were placed in a horizontal position without a pillow. The palms of both hands were upward and placed by the sides of the body. Blood pressure cuffs were bound to the upper arms and the ankles of the lower limbs. The cuff balloon mark on the upper arm was aligned with the brachial artery, and the lower edge of the cuff was 2–3 cm from the cross-striation of the cubital fossa. The cuff balloon mark on the lower limb was located on the medial lower limb, and the lower edge of the cuff was 1–2 cm from the medial malleolus. The measurements were taken twice by the device, and the second measurements were the final results. The average values of the baPWV on the left and right sides were calculated.

Visceral fat area (VFA)

VFA was measured by the InBody S10 (Biospace Co, Ltd, Seoul, Korea) as an indicator of visceral fat accumulation. The measurements were performed with the subjects in sitting position. Measurements were taken using the 4-electrode 8-point touch electrode method by wiping the areas where the 8 electrodes would be attached (one each on thumb and middle fingers on both hands and one each on both ankles) with electrolyte tissue and connecting the holder electrode.

The definition of arterial stiffness

Subjects were devided into three goups according to the levels of baPWV, normal arterial stiffness: baPWV <1400 cm/s; borderline arterial stiffness: 1400$\leq$baPWV <1800 cm/s; and elevated arterial stiffness: baPWV $\geq$1800 cm/s [20].

Statistical analyses

All analyses were performed using the SPSS 24.0 statistical software (SPSS 24.0 for Windows; SPSS, Inc., Chicago, IL). Numerical variables were reported as mean ± standard deviation. Comparisons were conducted between groups using the ANOVA. Categorical data were reported as abnormal subjects (%) and chi-square test was used. A pearson correlation coefficient was used to measure the strength of association between variables. Multiple linear regression analyses were performed to examine the relationships between baPWV and other variables. Multiple logistic regression analyses were performed to examine the

relationships between elevated arterial stiffness and other variables. $p<0.05$ was considered statistically significant.

Results

There were 482 patients who met the requirements fitted inclusion criteria within the period of the study. According to exclusion criteria, fifteen patients with type 1 diabetes were excluded, two patients with other specific types diabetes were excluded, no patients with in pregnancy were excluded, thirty-six patients with hematological diseases or infection were excluded, and one hundred and thirteen patients with diabetic macroangiopathy, such as coronary atherosclerotic cardiopathy, stroke, or ankle brachial index≤0.9, were excluded. Eventually, this study enrolled 316 patients with type 2 diabetes (185 males and 131 females), age 52.0±12.4 years. In these patients, 84 patients (26.6%) have baPWV<1400 cm/s and 90 patients (28.5%) had baPWV≥1800cm/s. There were no significant differences in gender and smoking between the three groups ($p>0.05$). The patients with borderline arterial stiffness had higher age, systolic blood pressure (SBP), diastolic blood pressure (DBP), and VFA than the patients with normal arterial stiffness ($p<0.05$). The patients with elevated arterial stiffness had higher DBP and VFA than the patients with normal arterial stiffness ($p<0.05$). The patients with elevated arterial stiffness had higher NLR than the patients with borderline arterial stiffness ($p<0.05$). The patients with elevated arterial stiffness had higher age, duration of diabetes, SBP, and UACR than the patients with normal arterial stiffness and borderline arterial stiffness ($p<0.05$). No significant differences were observed in any of the other variables between the three groups ($p>0.05$) (Table 1).

The correlation coefficients between baPWV and the other variables for all the subjects are shown in Table 2. The baPWV was positively correlated with age ($r=0.524$, $p<0.001$), duration of diabetes ($r=0.313$, $p<0.001$), SBP ($r=0.456$, $p<0.001$), DBP ($r=0.133$, $p=0.018$), UACR ($r=0.232$, $p<0.001$), NLR ($r=0.185$, $p=0.001$), and VFA ($r=0.139$, $p=0.014$) (Table 2). In multiple regression analysis, age ($\beta=10.933$, $p<0.001$), SBP ($\beta=6.240$, $p<0.001$), UACR ($\beta=3.805$, $p=0.019$), NLR ($\beta=43.722$, $p=0.013$), duration of diabetes ($\beta=7.064$, $p=0.011$), and VFA ($\beta=0.778$, $p=0.030$) maintained an independent association with baPWV (Table 3).

In multiple logistic regression analysis, age≥65 years (OR=15.356, $p<0.001$), duration of diabetes≥5years (OR=1.921, $p=0.036$), hypertension (OR=2.521, $p=0.004$), UACR≥3mg/mmol (OR=2.764, $p=0.002$), and VFA≥100cm^2 (OR=2.229, $p=0.022$) were independently associated with elevated arterial stiffness (Table 4). Patients with 3rd tertiles of NLR were more likely to have elevated arterial stiffness (OR=2.256, $p=0.009$) compared to patients with 1st tertiles of NLR in univariate logistic regression

Table 1 Clinical and laboratory characteristics of the type 2 diabetes patients with different levels of baPWV

Variables	baPWV			*p*
	<1400 cm/s (*n*=84)	1400–1799 cm/s (*n*=142)	≥1800 cm/s (*n*=90)	
Gender (male/female)	57/27	81/61	47/43	0.099
Age (y)	44.1±11.7	51.3±10.4a	60.6±10.5ab	<0.001
Duration of diabetes (y)	4.7±5.2	5.8±5.8	8.3±6.4ab	<0.001
Smoking [*n* (%)]	24 (28.6)	38 (26.8)	21 (23.3)	0.723
BMI (kg/m^2)	26.2±3.2	26.9±3.7	26.3±3.4	0.278
SBP (mmHg)	127.4±13.7	138.5±16.6a	148.9±19.4ab	<0.001
DBP (mmHg)	84.4±9.5	88.3±11.7a	88.6±12.4a	0.023
HbA1c (%)	8.7±2.2	8.5±2.0	8.5±1.8	0.710
FPG (mmol/L)	8.51±2.98	8.58±2.99	8.66±3.44	0.949
TG (mmol/L)	2.84±3.05	2.69±2.28	2.38±2.10	0.451
TC (mmol/L)	5.47±1.15	5.49±1.26	5.56±1.30	0.873
HDL-C (mmol/L)	1.11±0.23	1.10±0.22	1.12±0.22	0.750
LDL-C (mmol/L)	3.05±0.83	3.05±0.81	3.08±0.93	0.942
NLR	1.57±0.90	1.48±0.64	1.80±1.09b	0.022
UACR (mg/mmol)	2.69±6.30	2.65±4.93	9.42±14.84ab	<0.001
VFA (cm^2)	103.7±37.4	121.9±43.3a	123.8±44.5a	0.002

baPWV, brachial-ankle pulse wave velocity; *BMI*, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HbA1c*, glycosylated hemoglobin A1c; *FPG*, fasting plasma glucose; *TG*, triacylglycerol; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *NLR*, neutrophil lymphocyte ratio; *UACR*, urine albumin-creatinine ratio; *VFA*, visceral fat area. A compared with baPWV<1400 cm/s $p<0.05$, b compared with baPWV 1400-1799 cm/s $p<0.05$

Table 2 Simple correlations between the baPWV and other variables in type 2 diabetes

Variables	*r*	*p*
Age (y)	0.524	<0.001
Duration of diabetes (y)	0.313	<0.001
BMI (kg/m^2)	−0.029	0.602
SBP (mmHg)	0.456	<0.001
DBP (mmHg)	0.133	0.018
HbA1c (%)	−0.038	0.505
FPG (mmol/L)	0.068	0.230
TG (mmol/L)	−0.055	0.327
TC (mmol/L)	0.078	0.167
HDL-C (mmol/L)	0.053	0.349
LDL-C (mmol/L)	0.067	0.234
NLR	0.185	0.001
UACR (mg/mmol)	0.232	<0.001
VFA (cm^2)	0.139	0.014

baPWV, brachial-ankle pulse wave velocity; *BMI*, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HbA1c*, glycosylated hemoglobin A1c; *FPG*, fasting plasma glucose; *TG*, triacylglycerol; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *NLR*, neutrophil lymphocyte ratio; *UACR*, urine albumin-creatinine ratio; *VFA*, visceral fat area

analysis (Table 4). When BMI was further adjusted, the results were not changed (Table 4).

Discussion

Our study shows that arterial stiffness was common in T2DM without diabetic macroangiopathy. More than a quarter of T2DM without diabetic macroangiopathy have arterial stiffness. Age is the major influencing factor of baPWV and can explain 27.5% of the total variance of baPWV. The frequency of arterial stiffness reached nearly three quarters in ≥65 years group. Except for age, arterial stiffness also correlated with the duration of diabetes, elevated blood pressure, NLR, UACR, and VFA.

Arterial stiffness is highly correlated with blood pressure [21]. Hypertension is common comorbidity in T2DM patients [22]. Comorbid hypertension and diabetes further increase the risk of artery plaques [23]. Using multivariate regression model, we found that 11.8% of the total variance of baPWV was due to SBP. The frequency of arterial stiffness in T2DM patients with hypertension reaches up to 45.3%, and was significantly higher than T2DM patients without hypertension (15.6%).

T2DM may affect both macrovascular and microvascular at the same time. Previous studies demonstrated that UACR correlated with arterial stiffness as assessed by baPWV [24, 25]. Endothelial dysfunction plays a key role in the progression of arterial stiffness. UACR is an early marker of endothelial dysfunction [26]. Endothelial dysfunction may be the link between baPWV and UACR. T2DM worsens arterial stiffness through endothelial dysfunction [27]. In our study, more than half of the T2DM patients with UACR≥3mg/mmol have arterial stiffness. T2DM patients with UACR≥3mg/mmol have longer duration of diabetes. baPWV and UACR all correlated with duration of diabetes. To avoid the effect of duration of diabetes, we used the multiple logistic regression analysis. After adjusted for duration of diabetes, UACR was independently correlated with baPWV.

The relationship between obesity and baPWV was also evaluated in this study. As expected, baPWV closely correlated with VFA, and no relationships were observed between baPWV and BMI. The results were similar to those of previous studies in Asia [28, 29]. BMI is a marker of increases in overall adiposity. Compared with overall adiposity, body fat distribution is more important. Visceral fat is closely associated with an elevated outflow of free fatty acids [30]. Free fatty acids and hyperglycemia exert a synergistic adverse effect on the arterial stiffness [31].

Chronic inflammation plays a considerable role in the pathogenesis of arterial stiffness. NLR is a useful marker of chronic inflammation and a predictor for cardiovascular

Table 3 Multiple linear regression analyses for brachial-ankle pulse wave velocity in type 2 diabetes (stepwise method)

Model	Unstandardized coefficient B	Std. error	Standardized coefficient B	*t*	*p*	95%CI	R^2
(Constant)	−21.905	124.961		−0.175	0.861	−267.787 to 223.977	
Age (y)	10.933	1.316	0.385	8.309	<0.001	8.344–13.522	0.275
SBP (mmHg)	6.240	0.835	0.328	7.472	<0.001	4.597–7.883	0.393
UACR (mg/mmol)	3.805	1.617	0.104	2.354	0.019	0.624–6.985	0.413
NLR	43.722	17.581	0.107	2.487	0.013	9.129–78.315	0.428
Duration of diabetes (y)	7.064	2.751	0.120	2.568	0.011	1.650–12.478	0.438
VFA (cm^2)	0.778	0.358	0.095	2.174	0.030	0.074–1.481	0.446

Dependent variable: brachial-ankle pulse wave velocity. *SBP*, systolic blood pressure; *UACR*, urine albumin-creatinine ratio; *NLR*, neutrophil lymphocyte ratio; *VFA*, visceral fat area

Table 4 The relationship between elevated arterial stiffness and other variables in type 2 diabetes

Variables		Elevated arterial stiffness [*n* (%)]	Univariate logistic regression analysis		Multiple logistic regression analysis 1		Multiple logistic regression analysis 2	
			OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
Gender	Male (*n*=185)	47 (25.4)	1					
	Female (*n*=131)	43 (32.8)	1.435 (0.877–2.348)	0.151				
Age	<45 years (*n*=89)	10 (11.2)	1		1		1	
	45–64 years (*n*=172)	39 (22.7)	2.317 (1.096–4.896)	0.028	1.744 (0.776–3.919)	0.179	1.606 (0.705–3.661)	0.260
	≥65 years (*n*=55)	41 (74.5)	23.136 (9.454–56.616)	<0.001	15.356 (5.758–40.953)	<0.001	13.335 (4.874–36.482)	<0.001
Duration of diabetes	<5 years (*n*=171)	33 (19.3)	1		1		1	
	≥5 years (*n*=145)	57 (39.3)	2.709 (1.634–4.489)	<0.001	1.921 (1.044–3.536)	0.036	1.890 (1.025–3.486)	0.041
Smoking	No (*n*=233)	69 (29.6)	1					
	Yes (*n*=83)	21 (25.3)	0.805 (0.456–1.422)	0.455				
BMI	<24 kg/m^2 (*n*=68)	19 (27.9)	1					
	24–27.9kg/m^2 (*n*=162)	51 (31.5)	1.185 (0.634–2.214)	0.595				
	≥28kg/m^2 (*n*=86)	20 (23.3)	0.781 (0.377–1.619)	0.507				
Hypertension	No (*n*=179)	28 (15.6)	1		1		1	
	Yes (*n*=137)	62 (45.3)	4.458 (2.637–7.538)	<0.001	2.521 (1.352–4.702)	0.004	2.580 (1.377–4.833)	0.003
HbA1c	<7% (*n*=86)	20 (23.3)	1					
	≥7% (*n*=230)	70 (30.4)	1.444 (0.813–2.562)	0.210				
FPG	<7mmol/L (*n*=117)	32 (27.4)	1					
	≥7mmol/L (*n*=199)	58 (29.1)	1.093 (0.657–1.817)	0.733				
TG	<1.7mmol/L (*n*=133)	44 (33.1)	1					
	≥1.7mmol/L (*n*=183)	46 (25.1)	0.679 (0.415–1.111)	0.123				
TC	<4.5mmol/L (*n*=59)	15 (25.4)	1					
	≥4.5mmol/L (*n*=257)	75 (29.2)	1.209 (0.634–2.303)	0.564				
HDL-C	>1.0mmol/L for males or >1.3mmol/L for females (*n*=142)	40 (28.2)	1					
	≤1.0mmol/L for males or ≤1.3mmol/L for females (*n*=174)	50 (28.7)	1.028 (0.629–1.681)	0.912				
LDL-C	<2.6mmol/L (*n*=99)	24 (24.2)	1					
	≥2.6mmol/L (*n*=217)	66 (30.4)	1.366 (0.794–2.351)	0.260				
NLR	1st tertiles (*n*=106)	22 (20.8)	1					
	2nd tertiles (*n*=105)	29 (27.6)	1.457 (0.772–2.750)	0.245				
	3rd tertiles (*n*=105)	39 (37.1)	2.256 (1.221–4.169)	0.009				
UACR	<3mg/mmol (*n*=233)	46 (19.7)	1		1		1	
	≥3mg/mmol (*n*=83)	44 (53.0)	4.586 (2.677–7.858)	<0.001	2.764 (1.453–5.259)	0.002	2.823 (1.481–5.380)	0.002
VFA	<100cm^2 (*n*=119)	24 (20.2)	1		1		1	
	≥100cm^2 (*n*=197)	66 (33.5)	1.994 (1.166–3.411)	0.012	2.229 (1.125–4.415)	0.022	2.838 (1.267–6.358)	0.011

Elevated arterial stiffness was defined as baPWV ≥1800 cm/s. In multiple logistic regression analysis 2, BMI was further adjusted. *OR*, odds ratio; *CI*, confidence interval; *baPWV*, brachial-ankle pulse wave velocity; *BMI*, body mass index; *HbA1c*, glycosylated hemoglobin A1c; *FPG*, fasting plasma glucose; *TG*, triacylglycerol; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *NLR*, neutrophil lymphocyte ratio; *UACR*, urine albumin-creatinine ratio; *VFA*, visceral fat area

disease [32]. Many evidences suggested that higher NLR was independently associated with baPWV in patients with hypertension, acute coronary syndrome, diabetic retinopathy, and health examination population [33–36]. NLR is also an important determinant of baPWV in T2DM without diabetic macroangiopathy.

There are limitations to our study. FPG and HbA1c were not associated with baPWV in this study. The following are the possible causes. All subjects of this study were inpatients. HbA1c was not up to standard (HbA1c<7%) in more than 70% of them. This could lead to selective bias. Higher triglyceride-glucose index was not associated with increased

baPWV in another study with hospitalized T2DM patients [37]. Additionally, previous study reported that post-load plasma glucose level is closely associated with the change of arterial elasticity [38]. Glucose fluctuations evaluated by continuous glucose monitoring are significantly associated with baPWV in T2DM patients without history of apparent cardiovascular disease, but HbA1c was not [39]. Because we did not assess oral glucose tolerance test and continuous glucose monitoring, we cannot analyze the association between baPWV and glucose fluctuations in this study. In addition, the sample size was small in this study and the result should be confirmed by larger survey sample.

Conclusion

In summary, the decline of arterial elasticity was common in T2DM patients without macroangiopathy. Elevated blood pressure, microangiopathy, chronic inflammation, and visceral fat accumulation were the risk factors of early arterial stiffness in patients with T2DM.

Data Availability The data that support the findings of this study are available from the corresponding author, [Chun-Ming Ma], upon reasonable request.

Declarations

Consent for publication We declare that submitted manuscript does not contain previously published material, and are not under consideration for publication elsewhere. Each author has made an important scientific contribution to the study and is thoroughly familiar with the primary data. All authors listed have read the complete manuscript and have approved submission of the paper. The manuscript is truthful original work without fabrication, fraud, or plagiarism.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

Diabetic keto-acidosis in pancreatic diabetes – how is it different from DKA in type 1 or type 2 DM?

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Abstract

Objective There is scarcity of data on diabetic ketoacidosis (DKA) in patients with diabetes mellitus (DM) due to diseases of exocrine pancreas (T3DM).

Methods We did a retrospective record-review of patients admitted with DKA from 2017 to 2022. Clinical and biochemical characteristics were compared among DKA cases in type 1 DM (T1DM), type 2 DM (T2DM), and T3DM. Logistic regression was performed to find independent predictors of death and DKA complications.

Results Out of 107 cases, $n = 79$ were included (T1DM = 30, T2DM = 37, T3DM = 12). All cases of DKA in T3DM were of mild or moderate severity. The time to resolution of DKA was shortest for T3DM ($p = 0.04$). Death rates were similar in all groups but in T3DM, all deaths occurred after resolution of DKA. There was a precipitating factor for DKA in 11/12, 91.7% cases in T3DM group, most common being infections (58.3%) followed by glucocorticoid use (16.7%). T3DM cases had shorter duration of DM but higher CRP, procalcitonin, and qSOFA scores for infections precipitating DKA ($p_{all} < 0.03$). CRP was an independent predictor for DKA-related complications (OR: 1.01, 1.001–1.01, $p = 0.04$).

Conclusion This is the largest single-center cohort of T3DM with new-onset DKA, suggesting severe infections and glucocorticoid usage as major precipitating factors, while clinical course and outcomes were distinctly different from T2DM and T1DM patients with DKA.

Keywords Pancreatic DM · Diabetic ketoacidosis · DKA · T3cDM

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Introduction

Diabetic ketoacidosis (DKA) is one of the most frequent causes for mortality in children with type 1 diabetes mellitus (T1DM). However, DKA can occur commonly in adults as well with high rates of in-hospital mortality and complications. It was believed that even in adults, most cases of DKA occur in those with T1DM, with DKA being the presenting feature of adult T1DM in many cases [1]. But several recent studies on adult DKA found a high prevalence of type 2 diabetes mellitus (T2DM), specially with the predominance of sodium-glucose-linked-cotransporter 2-inhibitor (SGLT2i) use, and even gestational DM [2–4]. There has been a resurgence in the importance of DKA following the COVID-19 pandemic and the high prevalence of DKA in cases with DM and COVID-19 or even new onset DM following COVID-19 [5].

Though there have been several case reports of DKA in cases of diabetes of the exocrine pancreas,

systematic studies to characterize DKA in this type of DM are lacking. Cases of DM due to chronic pancreatitis with pancreatic parenchymal atrophy, specially fibrocalculous pancreatopathic DM (FCPD), are believed to be resistant to ketosis due to a number of reasons including less glucagon and lesser adipose tissue and carnitine deficiency in these individuals consequent to fat malabsorption [6, 7]. The prevalence of chronic pancreatitis is common in tropical countries like India, accounting for ~ 15–20% of cases with DM, and is commonly underdiagnosed or misdiagnosed [6, 8]. In a recent study from Taiwan, DM patients with chronic pancreatitis were found to have a higher incidence of hyperglycemic emergencies including DKA and hyperglycemic hyperosmolar state (HHS), and an overall lower survival [9]. In a recent study from Bangladesh, around 13.3% of patients with FCPD developed DKA [10].

We aimed to study the clinical and biochemical characteristics of DKA in patients with DM of the exocrine pancreas and compare these to DKA in adult patients with T1DM and T2DM.

Methods

Study design

We did a retrospective review of electronic medical records of all patients above 18 years of age presenting with diabetic ketoacidosis (DKA) at admission in a tertiary care hospital in West Bengal over a period of 5 years between 1st August 2017 and 31st July 2022. The objective of our study was to characterize clinical and biochemical features, precipitating factors, and outcomes in new onset DKA in patients with DM of the exocrine pancreas and compare them with those with T1DM or T2DM.

Clinical and biochemical parameters of the study subjects at admission were noted. Death or other complications during admission were recorded as per predefined criteria (see section on "Study definitions"). We excluded those with hyperglycemic hyperosmolar syndrome (HHS), DKA-HHS overlap, euglycemic ketosis, or ketoacidosis secondary to other factors like starvation or alcohol and pregnant women. The study design is outlined in Fig. 1. For those with recurrent episodes of DKA, the history and number of recurrences

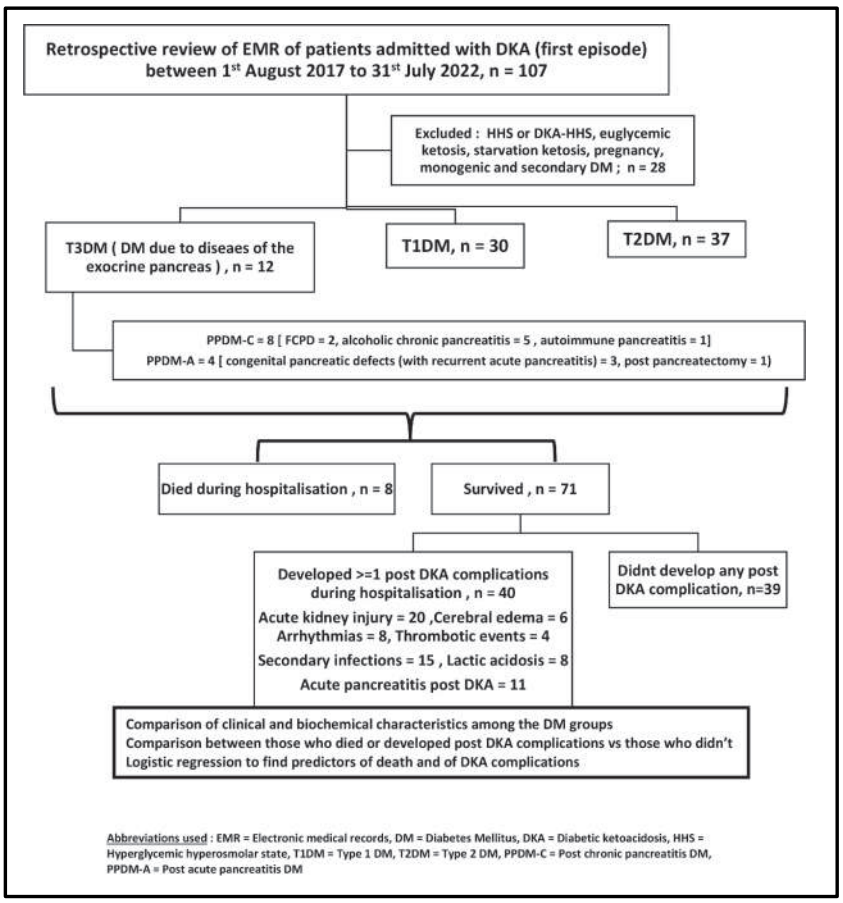

Fig. 1 Study design

were documented but clinical and biochemical parameters of the first episode of DKA alone were included for analysis.

Study definitions

The patients were divided into three groups — those with type 1 diabetes mellitus (T1DM), patients with type 2 diabetes mellitus (T2DM), and those with diabetes in the context of disease of the exocrine pancreas (T3DM). Diseases of exocrine pancreas (DEP) encompasses cases of post-pancreatitis diabetes mellitus (PPDM), pancreatic cancer-related diabetes (PCRD), and cystic fibrosis-related diabetes (CFRD) [11]. We did not encounter any case of PCRD or CFRD. PPDM included cases of post-acute pancreatitis DM and post-chronic pancreatitis DM — acronymized as PPDM-A and PPDM-C, respectively [11]. PPDM-C was diagnosed in the presence of imaging findings of intraductal and/or parenchymal pancreatic calcifications and/or strictures and dilatations in the main pancreatic duct on CT or USG or MRI abdomen. This was inclusive of tropical calcific pancreatitis (also known as fibrocalculous pancreatopathy), alcohol-induced chronic pancreatitis, autoimmune pancreatitis, and hereditary pancreatitis [12]. Those with DM following attack(s) of pancreatitis but without above morphological changes were considered as PPDM-A. The diagnosis of fibrocalculous pancreatic diabetes (FCPD), alcoholic CCP, and autoimmune pancreatitis was made using standard definitions for each of these [13–15]. Pancreatic exocrine insufficiency (PEI) was established by either a history of steatorrhea or a documented need for pancreatic enzyme supplements or documented low fecal elastase demonstrated any time since diagnosis of the disease. Fecal elastase, wherever available, was done by ELISA technique by Merck Millipore (Merck Ltd, Germany) on freshly passed stool sample and any value less than a cut-off of 200 mcg/g of stool was considered suggestive of PEI [16]. We excluded those with pre-existing DM admitted for their first episode of acute pancreatitis with DKA, those with monogenic forms of DM, and those with DM secondary to endocrinopathies or medications, gestational DM, or new onset DM (NODM) following COVID-19.

A diagnosis of diabetic ketoacidosis (DKA) was made when the blood glucose (BG) was greater than 250 mg/dl, arterial pH less than 7.3, serum bicarbonate less than 15 mEq/l, and ketonuria [17, 18]. Severity of DKA was defined according to pH and serum bicarbonate [18]. Classification of DM was made according to American Diabetes Association guidelines [19]. A history of established atherosclerotic cardiovascular disease (ASCVD) was determined from previous documented history of coronary artery disease or acute coronary syndrome, ischemic cerebrovascular accident, or peripheral arterial disease. Diabetic kidney disease (DKD) was diagnosed in presence of chronic kidney disease with albuminuria in absence of other non-diabetic causes for kidney disease. Neuropathy was defined in the presence of symptoms or abnormal monofilament tests or relevant electrodiagnostic tests. A diagnosis of diabetic retinopathy was made based on fundoscopy or fundus photograph findings by an ophthalmologist. Diabetes complication severity index (DCSI) was calculated based on the presence and severity of the complications [20]. Non-alcoholic fatty liver disease (NAFLD) was defined as presence of ultrasound imaging findings of hyperechogenic liver with/without raised liver enzymes.

In-hospital death was defined as death during the episode of hospitalization and includes death due to DKA (before resolution of DKA) and death after resolution of DKA. Any DKA complication refers to the presence of any of acute kidney injury (AKI), new onset arrhythmias, thrombotic events, cerebral edema, acute respiratory distress syndrome (ARDS), secondary infection, lactic acidosis, or acute pancreatitis occurring during the course of admission with DKA without any other underlying etiology to explain the particular complication. An illness that could potentially lead to DKA in a previously healthy patient was considered a precipitating factor for DKA. New onset acute pancreatitis arising due to DKA was defined as rise in amylase or lipase above three times the upper limit of normal (ULN) with corroboratory imaging evidence on ultrasound or CT imaging of the pancreas. banks [21]. Secondary infection was diagnosed when the total leukocyte count was higher than 25,000 with more than 10% band forms or persistent leukocytosis with fever [18]. Resolution of DKA was defined as blood pH $\geq$ 7.3 and serum bicarbonate $\geq$ 18 mEq/l with BG < 200 mg/dl [18]. Time to resolution was calculated from time of hospital admission for DKA to resolution of DKA. Total daily dose was calculated as the mean daily amount of insulin in units per kg of body weight required during the period from hospital admission until resolution of DKA. Values of the different parameters including complete blood count (CBC), urea, creatinine, liver function test parameters, electrolytes including sodium (Na), potassium (K), calcium (Ca), phosphate (P), magnesium (Mg) measured from venous blood, arterial blood pH, and bicarbonate refer to values measured immediately after admission. Anion gap was calculated as the difference between serum Na and the sum of chloride and bicarbonate values on automated arterial blood gas (ABG) analysis. An ABG pH below 7.3 was considered as acidosis, AG value above 10 mEq/L was considered as high AG, and urine ketone positivity greater than 3 + was considered as ketosis. qSOFA index was used as an estimate of the severity of sepsis in those where infection was identified as a precipitating factor for DKA.

Laboratory methods

Biochemical investigations done at admission included Na, K, Ca, P, Mg, renal and liver function tests, C reactive protein (CRP), interleukin-6 (IL-6), D-dimer, and procalcitonin. Complete blood count with peripheral blood smear for band forms and ABG were also done at diagnosis. Serum CRP was measured using particle-enhanced immunoturbidimetry by Integra 400 + analyzer (Roche Diagnostics, Rotkreuz, Switzerland, CV: 6.6%), serum IL-6 and procalcitonin by electrochemiluminescence assay using Cobas e411 analyzer (Roche Diagnostics, Rotkreuz, Switzerland, CV 10% and 7.6% for IL-6 and procalcitonin respectively), and D-dimer using quantitative ELISA by VIDAS® D-Dimer Exclusion™ (Biomerieux, India, CV: 8.2%). Capillary blood glucose measurements were done before and 2 h after every meal using Accu-Check Active glucometer and strips following standard measures and precautions. Semiquantitative estimation of urine ketones was done using KetoDiastix reagent strips by Bayer Diagnostics, India, and simultaneous urine sample analysis for ketone body estimation using Cobas—u411 analyzer. Capillary blood glucose measurements were done before and 2 h after every meal using Accu-Check Active glucometer and strips following standard measures and precautions. ABG analysis was done using Gem Premier 3500 from Instrumentation Laboratory. CBC was analyzed using Sysmex 6-part cell counter. HbA1c% was measured using HPLC via BIORAD D10 analyzer (BIO-RAD, India, CV: 2.8%) and expressed both as HbA1c% (NGSP) and in mmol/mol (IFCC). Blood glucose values were measured using Roche Integra 400 plus analyzer (Roche Diagnostics, Rotkreuz, Switzerland, CV 3.6%). Ethical clearance for the current project was obtained from the institutional ethical committee reference no HWH/IEC-BMHR/008/2022.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL). Quantitative parameters were expressed as mean ± SD for parametric and median (range) for non-parametric parameters and qualitative parameters as *n* (%).The three groups were compared using ANOVA analysis followed by Tukey's post hoc analysis with comparison of harmonic means to correct for unequal sample sizes in the three groups, and chi-square test, with Fisher's correction where appropriate, for categorical variables. A p value ≤ 0.05 was considered significant. Correlation coefficient is expressed using Spearman's rho for parameters with significant linear correlation. Binomial logistic regression was done to identify the independent predictors of death and complications of DKA.

Results

A total of 107 patients were admitted with DKA during the 5 years, of which $n = 79$ were included in the study. Of this, $n = 30$ (38%) had T1DM, $n = 37$ (46.8%) had T2DM, and $n = 12$ (15.2%) had T3DM. Out of the twelve cases in this group, $n = 8$ cases had PPDM-C and $n = 4$ had PPDM-A. Out of the eight cases with PPDM-C, fibrocalcific pancreatopathic DM [FCPD] was seen in $n = 2$, alcoholic chronic pancreatitis was seen in $n = 5$, and autoimmune pancreatitis was seen in one patient. Out of the four cases of PPDM-A, $n = 3$ had congenital structural anomalies of the parenchyma with history of recurrent acute pancreatitis (dorsal pancreatic agenesis = 2, pancreas divisum = 1) and one developed DM after distal pancreatectomy done for acute pancreatitis with pseudocyst formation. All cases of T3DM had clinical history and biochemical documentation of PEI at diagnosis and were on pancreatic enzyme replacements at inclusion in the study.

Overall, severe DKA occurred in $n = 19$ (24.1%), moderate DKA in $n = 40$ (50.6%), and mild DKA in $n = 20$ (25.3%) subjects. A precipitating factor for the episode of DKA was seen in $n = 59$ (74.7%) of the study population. Eight (10.1%) patients died during admission, of which three (3.7%) deaths occurred during the course of treatment of DKA while the remaining deaths occurred following resolution of DKA. Clinical and biochemical parameters of the study cohort are summarized in Table 1.

Comparison of DKA characteristics of T3DM with other DM groups

On comparative analysis, among the baseline characteristics, there were significant differences among the three groups with respect to age, BMI, and duration of DM. With regard to DKA-related parameters, there were differences in the severity of DKA, presence of a precipitating factor before DKA, the time to resolution of DKA, overall length of hospital stay, and incidence of secondary complications (Table 2).

On post hoc Tukey's comparison test between the three groups, those with T3DM had a significantly higher BMI (26.38 ± 1.66 kg/m^2) than those with T1DM (19.68 ± 1.57) and lower than T2DM (27.39 ± 2.45) ($p < 0.001$). The mean age of presentation for T3DM was similar to T1DM and significantly lower than for T2DM ($p < 0.001$). The duration of DM, in years before DKA, was shortest for T3DM (3.8 ± 2.73) followed by T1DM (8.2 ± 5.9) and T2DM (15.76 ± 5.57) ($p < 0.03$). There were no differences in the prevalence of pre-existing comorbidities and complications and mean DCSI scores among the three groups.

Table 1 Clinical and biochemical characteristics of the study cohort ($n = 79$)

Parameter	Values*
Age (years)	39.59 (9.38)
Gender	M: 42 (53.2%) F: 37 (46.8%)
BMI (kg/m^2)	24.31 (4.18)
Type of DM	T1DM = 30 (38%) T2DM = 37 (46.8%) T3DM = 12 (15.2%)
Hba1c% HbA1c in mmol/mol	11.02 (2.56) 97 (4)
Pre-existing comorbidities/complications	Established ASCVD: 10 (12.7%) Diabetic kidney disease: 10 (12.7%) Diabetic retinopathy: 9 (11.4%) Diabetic neuropathy: 13 (16.5%) Hypertension: 22 (27.8%) Dyslipidemia: 17 (21.5%) NAFLD: 11 (13.9%)
Median DCSI	1 [0–6]
Presence of precipitating factor	59 (74.7%) Infections: 32 Bacterial: 20 COVID: 9 PTB: 1 Fungal (mucormycosis): 1
Severity of DKA	Mild: 20 (25.3%) Moderate: 40 (50.6%) Severe: 19 (24.1%)
Death	Total: 8 (10.1%) Death prior to resolution of DKA: 3 (3.8%) Death after DKA resolution: 5 (6.3%)
Complications due to DKA	Acute kidney injury: 20 (2.3%) Cerebral edema: 6 (7.6%) New onset arrhythmias: 8 (10.1%) Thrombotic events: 5 (6.3%) Secondary infections: 15 (19%) Lactic acidosis: 8 (10.1%) Acute pancreatitis post-DKA: 11 (13.9%)
Need for ICU stay	22 (27.8%)
Need for ventilator	11 (13.9%)
Sodium abnormalities	Hyponatremia: 37 (46.8%) Hypernatremia: 14 (17.7%)
Potassium abnormalities	Hypokalemia: 25 (31.6%) Hyperkalemia: 14 (17.7%)
Other electrolyte abnormalities	Hypocalcemia: 42 (53.2%) Hypophosphatemia: 23 (29.1%) Hypomagnesemia: 22 (27.8%)
Median time to DKA resolution	4 [2–8]

*Qualitative parameters are expressed as *n* (%) and quantitative parameters as mean (SD) for normally distributed and median [range] for non-normally distributed variables. Abbreviations used: *DM*, diabetes mellitus; *BMI*, body mass index; *DKA*, diabetic ketoacidosis; *ASCVD*, atherosclerotic cardiovascular disease; *DCSI*, diabetes complication severity index

Severe DKA was not seen in the group with T3DM, while mean arterial pH at admission was higher and bicarbonate and anion gap were significantly lower in T3DM compared to T1DM and T2DM ($p_{all} < 0.004$). Those with T3DM had shorter time to resolution of DKA than T1DM and T2DM ($p_{all} < 0.001$). While mortality rates were similar among the three groups, the two deaths reported in the T3DM group occurred following resolution of DKA.

There was a strikingly high prevalence of the presence of an identifiable precipitating factor for DKA ($n = 11/12$,

Table 2 Comparison of clinical and biochemical characteristics at admission for DKA among the three groups

Parameter	T1DM ($n=30$)	T2DM ($n=37$)	T3DM ($n=12$)
Age (years)*	31.5 (8.53)	47.2 (2.84)[#]	36.42 (4.94)
Gender	M: 13, F: 17	M: 23, F: 14	M: 6, F: 6
BMI (kg/m^2)*	19.68 (1.57)[#]	27.39 (2.45)	26.38 (1.66)
Duration of DM*	8.2 (5.9)	15.76 (5.57)[#]	3.8 (2.73)
Hba1c%	10.82 (2.58)	11.23 (2.51)	10.88 (2.83)
Hba1c in mmol/mol	95 (5)	99 (4)	95 (7)
RBG at admission (mg/dl)	413.93 (94.69)	367.08 (76.12)	412.58 (78.13)
Pre-existing comorbidities/complications			
Established ASCVD	3 (10%)	6 (16.2%)	1 (8.3%)
Diabetic kidney disease	3 (10%)	7 (18.9%)	0
Diabetic retinopathy	3 (10%)	8 (21.6%)	1 (8.3%)
Diabetic neuropathy	3(10%)	5 (13.5%)	2 (16.7%)
Hypertension	6 (20%)	15 (40.5%)	1 (8.3%)
Dyslipidemia	6 (20%)	10 (27%)	1 (8.3%)
NAFLD	2 (6.7%)	7 (18.9%)	2 (16.7%)
Median DCSI [range]	1.13 [1.66]	1.97 [2.07]	0.92 [0.99]
Presence of precipitating factor	18 (60%)[#]	30 (81.1%)[#]	11 (91.3%)
Severity of DKA*			
Mild	6 (20%)[#]	5 (13.5%)[#]	9 (75%)
Moderate	16 (53.3%)	21 (56.6%)	3 (25%)
Severe	8 (26.7%)[#]	11 (29.7%)[#]	0
Death			
Total	3 (10%)	3 (8.1%)	2 (16.7%)
Deaths before resolution of DKA	2 (6.7%)	1 (2.7%)	0
Death after resolution of DKA	1 (3.3%)	2 (5.4%)	2 (16.7%)
Complications due to DKA	13 (43.3%)	18 (48.6%)	9 (75%)
Acute kidney injury	6 (20%)	10 (27%)	4 (33.3%)
Cerebral edema	3 (10%)	2 (5.4%)	1 (8.3%)
New onset arrhythmias	2 (6.7%)	4 (10.8%)	2 (16.7%)
Thrombotic events	2 (6.7%)	2 (5.4%)	1 (8.3%)
Secondary infections	6 (20%)	6 (16.2%)	3 (25%)
Lactic acidosis*	1 (3.3%)	3 (8.1%)	4 (33.3%)
Acute pancreatitis	6 (20%)	5 (13.5%)	0
Need for ICU stay	7 (23.3%)	10 (27%)	5 (41.7%)
Need for ventilator	2 (6.7%)	6 (16.2%)	3 (25%)
Recurrence	3	1	0
Sodium abnormalities			
Hyponatremia	14 (16.7%)	15 (40.5%)	8 (66.7%)
Hypernatremia	5 (16.7%)	8 (21.6%)	1 (8.3%)
Potassium abnormalities			
Hypokalemia	8 (26.7%)	11 (29.7%)	6 (50%)
Hyperkalemia	4 (13.3%)	9 (24.3%)	1 (8.3%)
Hypocalcemia	12 (40%)	19 (51.3%)	11 (91.6%)
Hypophosphatemia	6 (20%)	10 (27%)	7 (58.3%)
Hypomagnesemia	7 (23.3%)	10 (27%)	5 (41.7%)
ABG at admission			
pH*	7.12 (0.11)[#]	7.12 (0.1)[#]	7.23 (0.04)
Bicarbonate*	15.1 (3.84)[#]	13.51 (3.42)[#]	16.75 (1.22)
Anion gap*	16.3 (2.35)[#]	14.81 (1.81)	13.92 (1.83)
Length of stay*	5.57 (2.39)[#]	6.27 (2.97)[#]	10.83 (4.34)

Table 2 (continued)

Parameter	T1DM ($n=30$)	T2DM ($n=37$)	T3DM ($n=12$)
Total daily dose of insulin required for resolution of DKA*	0.97 (0.2)	1.44 (0.53)#	0.99 (0.26)
Time to resolution of DKA*	5.14 (1.35)#	4.61 (1.40)#	2.58 (0.79)
CRP*	44.47 (58.92)#	103.62 (93.79)#	243.6 (163.68)
Procalcitonin*	3.25 (8.09)#	6.73 (10.53)#	23.30 (19.56)
D-dimer	704.4 (1319.27)	895.01 (946.36)	1545.98 (1891.66)
25-hydroxy vitamin D*	25.14 (12.63)	22.24 (11.54)	15.26 (7.8)
qSOFA*	1.92 (0.64)#	2.15 (1.14)#	3.44 (0.53)
Total leukocyte count	12,754.47 (5200.82)#	11,916.03 (4464.16)#	18,663.33 (9722.32)
Platelet count	188.37 (45.84)	190.89 (55.36)	224.08 (105.9)
GGT*	88.76 (117.03)	109.68 (247.63)	278.64 (328.95)
Amylase	183.7 (384.04)	126.89 (264.3)	56.75 (66.67)
Lipase	391 (1137.59)	250.65 (813.48)	39.5 (20.87)

*Significant differences among the three groups on ANOVA, $p<0.05$. #Significant differences with respect to T3DM. *DKA*, diabetic ketoacidosis; *ASCVD*, atherosclerotic cardiovascular disease; *qSOFA*, quick sequential organ failure assessment; *BMI*, body mass index; *RBG*, random blood glucose at admission; *ABG*, arterial blood gas; *CRP*, C reactive protein; *DCSI*, diabetes complication severity index; *GGT*, gamma glutamyl transaminase

91.7%) in T3DM followed by T2DM ($n=31$, 83.8%) and lowest in T1DM ($n=18$, 60%) ($p=0.03$) (Fig. 2). The most common precipitating factors for DKA were infection ($n=32$, 40.51%), followed by insulin omission ($n=8$, 10.12%), trauma ($n=6$, 7.59%), vascular events ($n=5$, 6.33% (acute coronary syndrome = 2, cerebral hemorrhage = 1, portal vein thrombosis = 1, pulmonary embolism)), and glucocorticoid use for treatment of COVID-19-associated pneumonia ($n=5$, 6.33%). Those with T3DM had a significantly higher prevalence of infections ($n=7$) and glucocorticoid use ($n=2$) as a precipitating factor for DKA ($p=0.04$). Community-acquired bacterial pneumonia was the most common infection ($n=10$) followed by COVID-19 pneumonia ($n=9$), lower urinary tract bacterial infections ($n=7$), cellulitis with septicemia ($n=2$), necrotizing fasciitis ($n=1$), orbital mucormycosis ($n=1$), emphysematous pyelonephritis ($n=1$), and pulmonary tuberculosis ($n=1$). Insulin omission alone was identified as a precipitating factor in

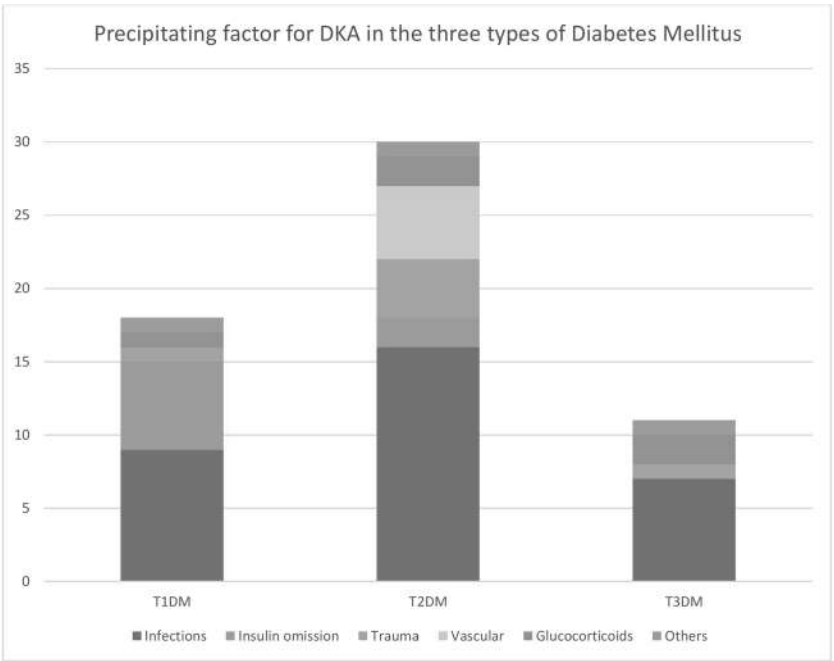

Fig. 2 Frequency of different precipitating factors for diabetic ketoacidosis (DKA) in the three groups of diabetes mellitus (DM)

six cases of T1DM and two cases of T2DM (out of $n=18$ cases of T2DM who were on insulin) but none in the patients with T3DM who were on insulin ($n=9$). The mean qSOFA score for cases with sepsis precipitating the DKA was higher in those with T3DM ($p=0.001$). All the cases of vascular events leading to precipitation of DKA occurred in T2DM.

Those with T3DM had higher incidence of lactic acidosis, hypocalcemia, and hypophosphatemia following DKA compared to T1DM and T2DM ($p_{all}<0.04$). Levels of 25OHD were also significantly lower in T3M compared to T1DM and T2DM. There were no differences in the rates of other complications among the three groups. Those with T3DM also had higher levels of the pro-inflammatory cytokines, CRP and procalcitonin, and higher total leukocyte count ($p_{all}<0.003$) (Table 2).

There were no significant differences in any parameter between cases of DKA in PPDM-C and PPDM-A.

Correlation analysis

We found a significant correlation of time to resolution of DKA with anion gap (Spearman's rho = 0.36, $p=0.001$), pH (Spearman's rho = −0.36, $p=0.01$), and bicarbonate (Spearman's rho = −0.34, $p=0.003$). There was a significant positive correlation of duration of hospital stay with procalcitonin and qSOFA scores (Spearman's rho = 0.25, $p=0.03$ and Spearman's rho = 0.39, $p=0.01$).

Comparison of between survivors and non-survivors following DKA

Compared to those who died during their hospital stay ($n=8$), those who survived ($n=71$) had significantly lower time to resolution of DKA (4.55 ± 1.58 vs 3.6 ± 0.55, $p<0.05$), while also exhibiting lower rates of DKA complications like AKI (18.3% vs 87.5%), cerebral edema (2.8% vs 50), new onset arrhythmias (1.4% vs 87.5%), thrombotic events (2.8% vs 25%), need for ICU stay (22.5% vs 75%), and hypophosphatemia (25.5% vs 62.5%). Those who died before the resolution of DKA ($n=3$) had higher DCSI scores (4 ± 2 vs 1.39 ± 1.77, $p=0.04$) AG at admission (18 ± 5.19 vs 15.13 ± 1.93, $p=0.03$) and noticeably lower procalcitonin (1.35 ± 0.2 vs 8.21 ± 13.45, $p=0.03$) levels.

Those who developed any DKA complications ($n=44$) had higher CRP (128.65 ± 140.5 vs 69.4 ± 63.94), total leucocyte count (16361.36 ± 63005.04 vs 9359.63 ± 2977.27), and qSOFA scores (2.48 ± 0.93 vs 0) than those who did not develop any DKA complication ($p_{all}<0.03$).

Results of logistic regression analysis

Upon multiple logistic regression using a model that included age, duration of DM, type of DM, precipitating factor, severity of DKA, and CRP, we could not find any independent predictors for in-hospital death, death due to DKA, or death after resolution of DKA.

For the occurrence of any DKA complication, CRP levels were found to be an independent predictor (OR: 1.01, 1.001–1.01, $p=0.04$). Type of DM was found to be an independent predictor for the occurrence of lactic acidosis (OR: 149.39, C.I 1.8–12,369.71, $p=0.03$).

Discussion

During the 5-year study period, we included seventy-nine cases of hospital admissions due to new-onset DKA among adults with T3DM, T2DM, and T1DM. In our study, majority (46.8%) of the patients had T2DM followed by T1DM (38%). Older studies estimated that newly diagnosed T1DM accounts for adult DKA in up to 30% of cases but recent estimates suggest this figure to be no larger than 3% [1–3]. Some studies suggest that T2DM could account for more than 50% cases of adult DKA [4].

We found twelve cases of DKA in patients with T3DM. Out of the twelve PPDM, nine had chronic pancreatitis (PPDM-C), and three had PPDM-A due to recurrent pancreatitis in patients with congenital structural anomalies of the pancreas. Most cases of DKA in diabetes of the exocrine pancreas have been reported in those with hypertriglyceridemia-induced pancreatitis and pancreatic adenocarcinoma [22–24]. However, our cohort is unique in that it contains predominantly subjects with chronic calcific pancreatitis including FCPD. The latter is a form of diabetes unique to the tropics and has traditionally been associated with resistance to ketosis/ketoacidosis due to a multitude of causes including decreased alpha cells and glucagon reserve, lack of subcutaneous fat leading to reduced non-esterified fatty acids (NEFA), carnitine deficiency, and partial preservation of beta cell functions [6, 25]. Owing to similar degrees of destruction of the pancreatic parenchyma in most other cases of chronic calcific pancreatitis and recurrent acute pancreatitis, they are by extension thought to have similarly reduced risks of developing DKA.

We found cases of T3DM with DKA had shorter duration since diagnosis of DM to DKA compared to other groups of DM. Majority of the cases of T3DM were PPDM-C and had imaging evidence of pancreatic atrophy. Most of the cases of PPDM had exocrine pancreatic insufficiency as evidenced by a history of steatorrhea, documented evidence of low fecal elastase, or need for pancreatic enzyme supplements. In PPDM, exocrine insufficiency precedes endocrine insufficiency [24]. In FCPD, there is progressive inflammation of the pancreatic islet cells followed by irreversible fibrosis. Among the endocrine cells, first the beta cells and the pancreatic

polypeptide (PP) cells are affected, followed by glucagon secreting alpha cells [26, 27]. One possibility could be that in PPDM, the risk for DKA is higher early in the disease course, before the alpha cells are destroyed and when there is still some residual insulin reserve. However, since we could not measure glucagon and C peptide levels for all, this postulation remains to be tested in future studies.

Eleven out of the twelve cases of T3DM (91.3%) had an identifiable precipitating factor prior to their DKA. This was strikingly higher compared to T1DM (60%) and T2DM cases (81.1%). Infections were the most common precipitating factor in all the groups but again were significantly more common in T3DM. Severity of sepsis (expressed as the qSOFA score) that precipitated DKA was higher in T3DM compared to the other two groups. The levels of pro-inflammatory cytokines — CRP and procalcitonin at admission — were also significantly higher in T3DM, possibly related to the greater severity of sepsis precipitating DKA in this group. The patients with T3DM in our cohort had body weight higher than those with T1DM, and DKA occurred relatively early in their disease course. It might be possible that only under conditions of significant stress like severe sepsis or any such precipitating factors with very high levels of pro-inflammatory cytokines, the residual insulin reserve was inadequate to counteract the increased adipose tissue lipolysis and release of glucagon from residual alpha cells. This culminated in ketosis/ketoacidosis in these patients with T3DM, who are otherwise less vulnerable to DKA. T3DM patients with DKA also had a significantly higher incidence of lactic acidosis following DKA resolution, which is likely due to higher severity of the infections, often bacterial, that lead to precipitation of DKA in these subjects.

Glucocorticoid use for treatment of COVID-19 led to precipitation of DKA in five of the seventy-nine cases of DKA, with significantly high prevalence in those with T3DM. In our previous study, we demonstrated a high prevalence of DKA following glucocorticoid use in COVID-19 patients [5]. Counter-regulatory hormones produced during stress like glucagon, cortisol, catecholamines, and growth hormone can upregulate ketone body production by stimulating lipolysis in adipocytes and providing free fatty acids to the liver [28, 29]. In T3DM, states with high counter-regulatory hormones like glucocorticoid use or thyrotoxicosis can lead to precipitation of DKA by stimulating lipolysis and glucagon release from remaining alpha cells in the pancreatic parenchyma. Since this study encompassed the COVID-19 pandemic period, COVID-19 or its treatment with glucocorticoids were identified as important etiologies for precipitating DKA in those with DM. COVID-19 infection has been shown to cause transient beta cell failure [30]. Our previous work has shown glucocorticoid use as one of the important factors associated with new-onset DKA in patients with DM following COVID-19 pneumonia, with levels of the pro-inflammatory cytokines like IL-6 being one of the significant predictors [31].

None of the cases of T3DM presented with severe DKA, and the pH, bicarbonate, and anion gap at admission for cases with T3DM and DKA were all lower than T1DM or T2DM. There was earlier resolution of DKA with lesser insulin dose requirement for T3DM. Though the death rates were similar among the three groups, none of the deaths in T3DM occurred prior to resolution of DKA. Those with T3DM had longer hospital stays despite earlier DKA resolution — possibly due to prolonged treatment required for associated complications like hypocalcemia, hypophosphatemia, and lactic acidosis.

We found a significant correlation of the duration of hospital stay with procalcitonin levels and qSOFA scores. Additionally, on multiple logistic regression analysis, we found the type of DM to be an independent predictor for lactic acidosis following the resolution of DKA. However, the rates of secondary infection developed during hospitalization for DKA were not significantly different among the three groups. It might be that the greater severity of infections, often bacterial, led to precipitation of DKA in these subjects. The prolonged hospital stay beyond the resolution of DKA was also mostly for the management of the underlying precipitating factor or for correction of post-DKA complications like lactic acidosis. The apparently increased severity of infections in T3DM compared to other types could be due to a selection bias, since we were only looking at cases of T3DM presenting with DKA and the more severe infections were most likely to precipitate DKA in this group. However, it is interesting to note that despite having more severe sepsis and higher pro-inflammatory cytokines, all the cases of DKA in T3DM were mild to moderate in severity, suggesting that the role of reduced glucagon and some residual insulin secretion function in these patients need to be further researched in future studies. Notably, there were no cases of acute pancreatitis and lower amylase, lipase levels in T3DM, likely due to very less residual pancreatic parenchyma in these patients.

Studies on DKA mortality across the world have reported a wide range from < 1% to more than 30%, depending on ethnicity and also time of the study [2, 5]. Our in-hospital mortality rate was 10%. An Indian study on 279 patients reported relatively high mortality rate of ~ 30% [33]. However, they included pediatric and elderly population with an age range between 13 and 80 years. The authors found gender, baseline APACHE II score, and serum phosphate levels to be important predictors of DKA-associated mortality. We could not find an independent predictor for death in our cohort, likely due to small numbers and a heterogenous population including different types of DM. We found CRP to be an independent predictor for DKA complication and the type of DM as an independent predictor for lactic acidosis.

To the best of our knowledge, this is the first and largest reported single-center cohort of patients with T3DM presenting with new-onset DKA, along with a comparative analysis with DKA in T1DM and T2DM to bring out their unique clinical and biochemical characteristics. While providing unique insights into the characteristics of DKA in this population, our study had the limitations of being a single-center study with relatively smaller sample size. Furthermore, serial measurements of insulin and glucagon secretory capacities and their correlations with severity of DKA would have enhanced our findings and are possible areas of future research in this unique population of T3DM/DEP.

Our study findings indicate that during a 5-year period, 15% (12 out of 79) cases of DKA were seen in T3DM patients. While traditionally considered at lower risk of DKA in comparison to T1DM and T2DM patients, T3DM patients can develop DKA early in the course of their disease, often precipitated by severe infections with high pro-inflammatory cytokines or usage of drugs like glucocorticoids for co-existing diseases. Though the severity of DKA is usually mild with earlier resolution than T1DM or T2DM, they can have higher rates of complications and prolonged hospital stay due to secondary complications like lactic acidosis or hypocalcemia.

Conclusion

Our findings from the largest single-centre reported cohort of pancreatic DM with new-onset DKA suggest that serious precipitating factors like severe infections, high doses of glucocorticoids can precipitate DKA in DM due to diseases of the exocrine pancreas. Though the DKA is mostly mild in such cases, but the hospital course and outcomes in these cases of DKA are distinct from T2DM and T1DM patients with DKA.

Author contribution Author 1 (SM) was involved in collection, analysis, and interpretation of data and formulating the initial draft. Author and corresponding author (RD) formulated the concept and design of this project and critically analyzed the data and edited the draft incorporating important intellectual content. Author 3 (ML) was involved in conduct of all relevant biochemical investigations in the laboratory and interpretation of data. Authors 4 (AKP) and 6 (AH) were actively involved in rigid implementation of study methodology and protocol in hospital wards and ICUs and raw data collection and data verification. Author 5 (MH) was involved in data collection and analysis. Author 8 (AG) was involved in data verification, supervision of the entire project, and revising the draft critically.

Data availability The raw data file and analyzed datasets can be available from the corresponding author upon reasonable request by email to riddhi_dg@rediffmail.com.

Declarations

Competing interests The authors declare no competing interests.

Ethical clearance For the current project was obtained from the institutional ethical committee reference no HWH/IEC-BMHR/008/2022.

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ORIGINAL ARTICLE

Achieving lipid goals in type 2 diabetic patients with dyslipidemia: barriers to treatment—the patient perspective

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Abstract

Objective Statin treatment compliance and achieving target levels differ between populations. This study aimed to determine the status of achieving the lipid targets and evaluate patients' compliance to statin treatment in type 2 diabetic patients with dyslipidemia.

Method This cross-sectional study included type 2 diabetes mellitus (T2DM) patients with dyslipidemia who applied for treatment at our polyclinics. Statin compliance was evaluated using the Modified Morisky Adherence Scale (MMAS-8) through a face-to-face interview. Cardiovascular (CV) risk was calculated according to the 2019 ESC (European Society of Cardiology) criteria by evaluating the patients' individual risk factors. LDL targets were determined according to risk categories with the same criteria.

Results A total of 504 patients (F/M: 274/230) were included. The serum LDL levels were 102.6 ± 39.2 mg/dL. Of the patients, 56.1% were under statin treatment. CV risk was very high in 73.6% of patients. LDL levels were significantly lower in users than in non-users (91.2 ± 26.0, 117.3 ± 38.4, $p < 0.0001$). The rate of reaching the LDL target was 14.8% in statin users. Treatment compliance was low for 40.6% of statin users. Discontinuation of statin treatment due to side effects was 15.7% ($n = 14$). $N = 49$ patients willingly discontinued statin treatment. They reported that 40.8% considered the treatment unnecessary.

Conclusion It was observed that 56.1% of type 2 diabetic patients were on statin therapy. A small percentage of them 14.8% ($n = 42$) reached the LDL target. Statin non-compliance and a lack of awareness of the statin treatment are the main reasons for high LDL levels in type 2 diabetic patients.

Keywords Diabetes mellitus · Hyperlipidemia · Medication adherence · Statin

Introduction

Hyperlipidemia is a major cardiovascular risk factor that is observed in 60–97% of type 2 diabetic patients [1, 2]. Primary and secondary CV prevention studies on statin therapy demonstrated significant benefits in cardiovascular protection in hyperlipidemic patients with T2DM [3]. As diabetes is accepted as equal to cardiovascular disease, guidelines recommend a lower LDL target for diabetic patients and moderate- and high-intensity statin therapy for patients with LDL levels above target. However, the rates of achieving LDL targets differ between populations. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) data, it is known that 70–80% of diabetic dyslipidemic patients cannot reach the recommended targets even in developed countries [4]. In the EUROASPIRE study, 33% of patients achieved their LDL target [5]. In the data of the Turkish arm of the CEPHEUS study, the rate of reaching the LDL target was found to be 35.1% [6]. Although there is strong evidence for statin treatment in the primary and secondary prevention of diabetes, there are concerns among patients and physicians about the initiation and continuation of statin therapy. Drug compliance is one of the major barriers to achieving LDL targets. Statin compliance studies show that more than 50% of

Highlights
- In diabetic patients, the LDL targets specified by the guidelines cannot be achieved.
- In diabetic patients, compliance with statin therapy is low.
- Diabetic patients consider statin therapy unnecessary.

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patients discontinued treatment in the first year, and statin adherence decreased to 25% [7–9]. The aim of this study was to determine the usage frequency of statin therapy, habits of use, and treatment compliance in patients with T2DM and to reveal the barriers to reaching target LDL levels.

Materials and methods

Study Design

The study was designed as a cross-sectional survey. Five hundred and four patients who applied to Pendik Training and Resarch Hospital of Marmara University School of Medicine for treatment at internal medicine, endocrinology, and cardiology outpatient clinics between November 2020 and August 2021 were included. Inclusion criteria were defined as the patient's diagnosis of T2DM for at least 1 year and diabetic dyslipidemia. Exclusion criteria were defined as the patient's diagnosis of malignancy, pregnancy, or being in the lactation period.

Demographic and clinical data collected through face-to-face interviews were with the patients and the patients' files. Medication history, the patient's statin use, dosage, duration, side effects, and smoking and alcohol habits were questioned. A questionnaire was completed for compliance with statin therapy. The duration of diabetes and hyperlipidemia, medications, and comorbid conditions were collected from patients' files. The total number of drugs used per day was recorded. Height was measured using a stadiometer to the nearest 0.5 cm. Bodyweight was measured using a digital electronic scale closest to 0.1 kg. BMI was calculated by dividing the weight in kilograms by the square of the height in meters.

Laboratory evaluation

Laboratory data from the last 3 months (fasting plasma glucose (FPG), HbA1c, total cholesterol (TC), high-density lipoprotein (HDL), LDL, and triglyceride (TG)) was recorded retrospectively from the patient's file.

Fasting blood glucose was studied with the Cobas 8000 Chemistry Analyzer Series (Roche Diagnostics, Switzerland) using an enzymatic method with hexokinase. HbA1c was studied with the premier Hb9210 (Trinity Biotech) by the boronate affinity chromatography method. The enzymatic colorimetric method studied TC, HDL, LDL, and TG with the Cobas 8000 Chemistry Analyzer Series (Roche Diagnostics, Switzerland).

CV risk calculation

Cardiovascular risk calculation was done according to the 2019 ESC "Diabetes, prediabetes, and cardiovascular diseases guideline" [10]. Each patient was divided into cardiovascular risk categories of moderate, high, and very high. Young patients with DM duration < 10 years with no risk factors were included in the moderate-risk group. Patients with DM duration ≥ 10 years without target organ damage plus any other additional risk factor were included in the high-risk group. Patients with DM and cardiovascular disease or other target organ damage, or three or more major risk factors were included in the very high-risk group. Using the same guideline, individual LDL targets (< 100 for the moderate-risk group, < 70 for the high-risk group, and < 55 for the very high-risk group) and whether the patient reached the LDL target were determined.

Evaluation of statin compliance

The 8-item Morisky Medication Adherence Scale (MMAS-8) questionnaire was used for determining statin compliance. It is a scale that evaluates drug use behaviors with eight questions based on the patient's self-report. This scale has been validated in Turkish studies [11–13]. The total score of the questionnaire was 8, with 1 point for each question; the high-compliance group had 8 points, the medium-compliance group had 6 to 8, and the low-compliance group had < 6 [14]. Patients were also asked whether they were aware they had hyperlipidemia and had been offered statin therapy before. Answers were recorded as yes or no.

Statistical methods

Continuous variables were summarized using descriptive statistics presented as the mean and standard deviation (SD). Categorical variables were summarized using counts and percentages. Categorical data was analyzed using the chi-square (χ^2) test. The independent *T* test and ANOVA test were used for the parametric variables. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the software Graphpad Instat.

Results

Clinical and demographic characteristics of type 2 diabetic patients are given in Table 1. A total of 504 patients, including 274 (54.3%) women and 230 (45.6%) men, were included in the study. The mean duration of diabetes mellitus (DM) among the patient was 12.3 ± 7.2 years, and 54.5% of them were on insulin therapy. The duration of DM was longer in female patients than in male patients ($p = 0.0144$). Macrovascular complications were more common in male patients than female patients. The mean BMI was 32.8 ± 6.9 kg/m^2 in the entire group, which was higher in female patients than in male patients ($p < 0.0001$). Fasting blood glucose was higher in male patients than in female patients. The mean LDL was

Table 1 Clinical and demographic characteristics of type 2 diabetes mellitus patients

Parameters	Total T2DM patients ($n=504$)	Female T2DM patients ($n=274$, 54.3%)	Male T2DM patients ($n=230$, 45.6%)	p value
Age (years)	58.2 ± 9.2	58.1 ± 9.2	58.2 ± 9.2	0.995
DM duration (years)	12.3 ± 7.2	13 ± 7.4	11.5 ± 6.9	0.0144
Insulin use, *n* (%)	275 (54.5)	152 (55.4)	123 (53.4)	0.7200
Macrovascular complications				
CAD, *n* (%)	127 (25.1)	48 (17.5)	79 (34.3)	< 0.0001
CKD, *n* (%)	54 (10.7)	23 (8.3)	31 (13.4)	0.0904
BMI (kg/m^2)	32.8 ± 6.9	34.5 ± 7.1	30.8 ± 6.2	< 0.0001
FPG (mg/dL)	154.7 ± 67.7	150.6 ± 68.4	159.5 ± 66.7	0.0336
HbA1c (%)	7.9 ± 2.0	7.7 ± 1.9	8.0 ± 2.1	0.0750
TC (mg/dL)	183.5 ± 51.0	192.0 ± 52.9	173.4 ± 46.7	< 0.0001
HDL (mg/dL)	44.6 ± 11.7	47.5 ± 12.6	41.2 ± 9.4	< 0.0001
LDL (mg/dL)	102.6 ± 39.2	107.9 ± 42.6	96.5 ± 33.8	0.0060
TG (mg/dL)	190.0 ± 178.2	194.0 ± 175.0	183.6 ± 182.3	0.1805

Continuous data were presented as mean and standard deviation (SD), and categorical data were presented as numbers (%). Differences between female and male individuals were analyzed using the independent *T* test and chi-square test for categorical data

T2DM type 2 diabetes mellitus, *DM* diabetes mellitus, *CAD* coronary artery disease, *CKD* chronic kidney disease, *BMI* body mass index, *FBG* fasting plasma glucose, *TC* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride

102.6 ± 39.2 mg/dL in the entire group; it was higher in the female patients than in the male patients ($p=0.006$).

Of the patients, 56.1% were using statins. A comparison of demographic and clinical data between statin users and non-users is shown in Table 2. Statin users were older ($p=0.0004$). DM duration was longer ($p=0.0014$), and CAD frequency was higher ($p<0.0001$) in statin users than non-users. The mean LDL was lower in the statin users than in the non-users ($p<0.0001$). Of the non-users, 32.1% ($n=71$) had a high CV risk, and 66% ($n=146$) had a very high CV risk. The rates of achieving LDL targets were 14.8% in statin users, 4% in non-users, and significantly higher in statin users ($p<0.0001$).

Clinical data according to CV risk categories is shown in Table 3. The mean LDL levels were similar in the moderate-, high-, and very high-risk groups. Patients categorized in the very high-risk group were older ($p<0.0001$) and had a longer duration of diabetes ($p<0.0001$) compared to patients in the high-risk and moderate-risk groups. The statin use rate was higher (60.6%) in the very high-risk group than in the high- and moderate-risk groups. Of the patients, 16.1% were receiving high-intensity statin treatment. The number of daily drugs they used was higher in the very high-risk group than in the other groups.

Table 4 shows the statin treatment duration, intensity, and compliance results. The patients' mean duration of statin use ($n=283$) was 6.9 ± 6.2 years, and 75.9% of them were on moderate-intensity statin treatments. Although 86.2% of the patients stated that they used statin therapy regularly, only 17.6% showed high compliance, according to the MMAS-8 questionnaire.

Statin users were evaluated according to statin compliance (Table 5). The high-compliance group has lower LDL ($p=0.0216$), lower HbA1c ($p=0.001$), lower BMI ($p=0.0039$), and the highest age ($p=0.0005$) compared to other groups.

A group of 221 patients (43.8%) were not using statin treatment. When questioned, 18% ($n=91$) of the patients did not know that they had hyperlipidemia, and 22.4% ($n=113$) of them self-reported that they had not been offered a statin treatment before. The remainder of patients, 17.6% ($n=89$) used statins before but quit treatment for different reasons. Figure 1 shows the reasons for discontinuing treatment in the group who quit statin therapy. 43.8% of patients stopped the statin treatment following doctors' orders, 55% stopped under their own free will, and 0.1% stopped for unknown reasons. The two main reasons for dropout from statin treatment due to patients' willingness were that they decided treatment was unnecessary (40.8%) or they were using multiple drugs (36.7%). Proven side effects were found in 17.9% of the patients, while 14.2% of patients attributed muscle, gastrointestinal, and headache side effects to statin treatment as a reason to stop the treatment.

Discussion

Patients with T2DM are often accompanied by a lipid abnormality, even if they have good glycemic control. In the coexistence of both clinical conditions, CV risk is thought to be higher and is associated with increased morbidity and

Table 2 Comparison of patients using and not using statins

Parameters	Statin users ($n=283$, 56.1%)	Statin non-users ($n=221$, 43.8%)	p value
Age (years)	59.5 ± 8.5	56.4 ± 9.8	0.0004
Sex			
Female	155 (54.7)	119 (53.8)	0.9072
Male	128 (45.2)	102 (46.1)	
BMI (kg/m^2)	33.1 ± 7.2	32.5 ± 6.5	0.4655
DM duration (years)	13.3 ± 7.3	11.2 ± 6.8	0.0014
Macrovascular complications			
CAD, *n* (%)	101 (35.6)	26 (11.7)	< 0.0001
CKD, *n* (%)	28 (9.8)	26 (11.7)	0.5622
Number of drugs (*n*)	8.0 ± 3.2	5.2 ± 2.7	< 0.0001
Blood parameters			
FPG (mg/dL)	152.2 ± 62.5	157.9 ± 73.9	0.8376
HbA1c (%)	7.8 ± 1.8	8.0 ± 2.2	0.6180
TC (mg/dL)	169.4 ± 44.3	201.5 ± 53.4	< 0.0001
HDL (mg/dL)	43.5 ± 10.6	46.1 ± 12.8	0.0496
LDL (mg/dL)	91.2 ± 26.0	117.3 ± 38.4	< 0.0001
TG (mg/dL)	183.9 ± 148.1	197.9 ± 210.6	0.8775
CV risk category			
Moderate risk	4 (1.4)	4 (1.8)	0.0029
High risk	54 (19)	71 (32.1)	
Very high risk	225 (79.5)	146 (66)	
LDL target reaching status			
On-target	42 (14.8)	9 (4)	< 0.0001
Off-target	240 (84.8)	210 (95)	

Continuous data were presented as mean and standard deviation (SD), and categorical data were presented as numbers (%). Differences between female and male individuals were analyzed using the independent *T* test and chi-square test for categorical data

BMI body mass index, *DM* diabetes mellitus, *CAD* coronary artery disease, *CKD* chronic kidney disease, *FBG* fasting plasma glucose, *TC* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *CV* cardiovascular

mortality, so it is very important to determine treatment targets well. Statins are the most important antihyperlipidemic agents with proven safety and efficacy in the prevention of atherosclerotic cardiovascular disease. Despite this, studies conducted with T2DM showed that 44–67% of patients could not reach the recommended treatment goals [15, 16]. According to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) data, it is known that 70–80% of diabetic dyslipidemic individuals cannot reach the recommended targets, even in developed countries [4]. In the EUROASPIRE study conducted in Europe, it was reported that 33% of the patients had the desired target LDL level [17]. According to the data from the Turkish arm of the CEPHEUS study investigating the target LDL level in patients using statins, the rate of reaching the LDL target was 35.1% [18]. In our study, the rate of reaching the LDL target value in statin users was 14.8%, which was lower than the literature data. Of the $n=504$ patients who applied to our hospitals' outpatient clinics with the diagnosis of T2DM and dyslipidemia, 56.1% were under statin treatment, 26.1% had never used statins before, and 17.6% had discontinued statin therapy. Only 17.6% ($n=50$) of patients using statins had high compliance. The plasma LDL value of 84.8% ($n=240$) of the patients using statins was higher than the target LDL value determined for that individual; in other words, patients were undertreated. The results obtained from our study show that the rate of compliance with statin therapy in type 2 diabetic patients and reaching lipid targets is low. Our study's lower target realization rates may be because the target values determined in the current guidelines are lower than the previous ones.

Patients' knowledge and drug compliance are important in initiating and continuing statin therapy. It is known that there are problems with compliance with statin treatment in Turkey and the rest of the world. Insufficient knowledge of the patients' diseases and their treatments, the development of side effects or the fear of their development, the fact that the patients use multiple drugs due to their comorbidities, public perception, and prescription/report problems can cause treatment non-compliance. As a result of MMAS-8 performed to evaluate compliance with statin therapy, 40.6% of our patients showed low compliance, 41.6% moderate compliance, and 17.6% high compliance. Considering the mean age, it was seen that the patients in the high-compliance group were older than the patients in the moderate- and low-compliance groups. At the same time, the metabolic data of the patients in this group is better due to the lower body mass index and mean TC, LDL, and HbA1c.

In the EPHESUS study, which is a multicenter study conducted in Turkey, it was observed that 52.6% of the patients did not know that they had hyperlipidemia [19]. In our study, 18% ($n=91$) of the patients did not know that they had hyperlipidemia. This low rate may be due to fact that the hospital where the study was conducted was a third-level center and the patients were under follow-up.

In a study by Ergin et al., in a single center, 45.3% of patients who were started on statin treatment stated that they used it irregularly [20]. In our study, 13.7% of patients using statins stated that they used statin therapy irregularly. The majority (38.4%) of the group who used the treatment irregularly said they took it when they thought of it. It was determined that they received the treatment at lower rates: once every 2 days, after heavy meals, and twice a week. Although our irregular use rate seems low, 38.1% of the patients who stated that they use it regularly show low compliance, 42.6% moderate compliance, and 19.2% high compliance.

Physicians' hesitations in the application of statin treatment seem to be an obstacle to the initiation of treatment. Their doctors did not recommend statin treatment to 82.5%

Table 3 Clinical and demographic parameters according to cardiovascular risk in type 2 diabetes mellitus patients

Parameters	Moderate risk ($n=8$, 0.01%)	High risk ($n=125$, 24.8%)	Very high risk ($n=371$, 73.6%)	p value
Age (years)	44.6 ± 4.2	55.3 ± 9.6	59.4 ± 8.7	< 0.0001
Sex				
Female	2 (25)	61 (48.8)	211 (56.8)	0.0713
Male	6 (75)	64 (51.2)	160 (43.1)	
BMI (kg/m^2)	25.8 ± 3	30.3 ± 6.5*	33.8 ± 6.8**	< 0.0001
DM duration (years)	5.0 ± 1.6	10.6 ± 6.0	13.1 ± 7.4	< 0.0001
Insulin use	2 (25)	55 (44)	218 (58.7)	0.0039
Statin use				
User	4 (50)	54 (43.2)	225 (60.6)	
Never use	4 (50)	55 (44)	74 (19.9)	< 0.0001
Quit	0 (0)	16 (12.8)	72 (19.4)	
Statin intensity				
Low	0 (0)	0 (0)	4 (10.7)	
Moderate	4 (50)	52 (41.6)	161 (43.3)	0.0028
High	0 (0)	2 (1.6)	60 (16.1)	
Total number of drugs (n)	3.1 ± 2.1	4.9 ± 2.4^	7.5 ± 3.2^^	< 0.0001
LDL (mg/dL)	90.8 ± 15.4	99.7 ± 34.3	103.8 ± 41	0.7440
LDL target reaching status				
On-target	5 (62.5)	23 (18.4)	23 (6.1)	< 0.0001
Off-target	2 (25)	102 (81.6)	346 (93.2)	

Continuous data were presented as mean and standard deviation (SD), and categorical data were presented as numbers (%). Comparisons between the two groups were analyzed using the ANOVA test, and comparisons between the three groups were analyzed using the independent T test and chi-square test for categorical data

BMI body mass index, *DM* diabetes mellitus, *LDL* low-density lipoprotein

*$p > 0.05$ compared to the moderate-risk group

**$p < 0.001$ compared to the moderate- and high-risk groups

^$p > 0.05$ compared to the moderate-risk group

^^$p < 0.001$ compared to the moderate- and high-risk groups

Table 4 Statin treatment compliance of type 2 diabetes mellitus patients using statin therapy

Parameters	Statin users Total ($n=283$)	Statin users Female ($n=155$, 54.7%)	Statin users Male ($n=128$, 45.2%)	p value*
Duration of statin use (years)	6.9 ± 6.2	7.5 ± 6.8	6.1 ± 5.2	0.3668
Statin intensity				
Low	4 (1.4)	2 (1.2)	2 (1.5)	0.9413
Moderate	215 (75.9)	118 (76.1)	99 (77.3)	
High	64 (22.6)	35 (22.5)	27 (21)	
Statin use				
Regular	244 (86.2)	133 (85.8)	111 (86.7)	0.8639
Irregular	39 (13.7)	22 (14.1)	17 (13.2)	
Statin compliance according to MMAS-8 (n)				
Low	115 (40.6)	72 (46.4)	43 (33.5)	0.0766
Moderate	118 (41.6)	60 (38.7)	58 (45.3)	
High	50 (17.6)	23 (14.8)	27 (21)	

Continuous data were presented as mean and standard deviation (SD), and categorical data were presented as numbers (%). Differences between female and male individuals were analyzed using the independent T test and chi-square test for categorical data

MMAS-8 Modified Morisky Adherence Scale-8

Table 5 Comparison of patients using statins according to their statin treatment compliance

Parameters	Low compliance ($n=115$, 0.05%)	Moderate compliance ($n=118$, 41.6%)	High compliance ($n=50$, 17.6%)	p value
Age (years)	57.2 ± 8.5^{a}	60.4 ± 7.7^{b}	62.9 ± 9	0.0005
Sex				
Female	72 (62.6)	60 (50.8)	23 (46)	0.0766
Male	43 (37.3)	58 (49.1)	27 (54)	
BMI (kg/m^2)	34.5 ± 7.5^{d}	32.6 ± 6.9^{e}	31.1 ± 6.8	0.0039
DM duration (years)	13.9 ± 9.3	12.5 ± 7	13.7 ± 8.2	0.3046
Laboratory data				
HbA1c (%)	8.3 ± 2^{m}	7.5 ± 1.7^{n}	7.3 ± 1.4	0.001
LDL (mg/dL)	98.5 ± 38.8	86 ± 30.2	86.7 ± 39.5	0.0216
Total number of drugs (n)	8.2 ± 3	8 ± 3.3	7.3 ± 3.1	0.2456
Insulin use	78 (67.8)	71 (60.1)	24 (48)	0.0538
CV risk category				
Moderate	2 (1.7)	2 (1.6)	0 (0)	0.8608
High	24 (20.8)	21 (17.7)	9 (18)	
Very high	89 (75.4)	95 (80.5)	41 (82)	

Continuous data were presented as mean and standard deviation (SD), and categorical data were presented as numbers (%). Comparisons between the two groups were analyzed using the ANOVA test, and comparisons between the three groups were analyzed using the independent T test and chi-square test for categorical data

BMI body mass index, *DM* diabetes mellitus, *LDL* low-density lipoprotein, *TG* triglyceride, *CV* cardiovascular

[a] $p < 0.05$ compared to the medium-compliance group, $p < 0.01$ compared to the high-compliance group

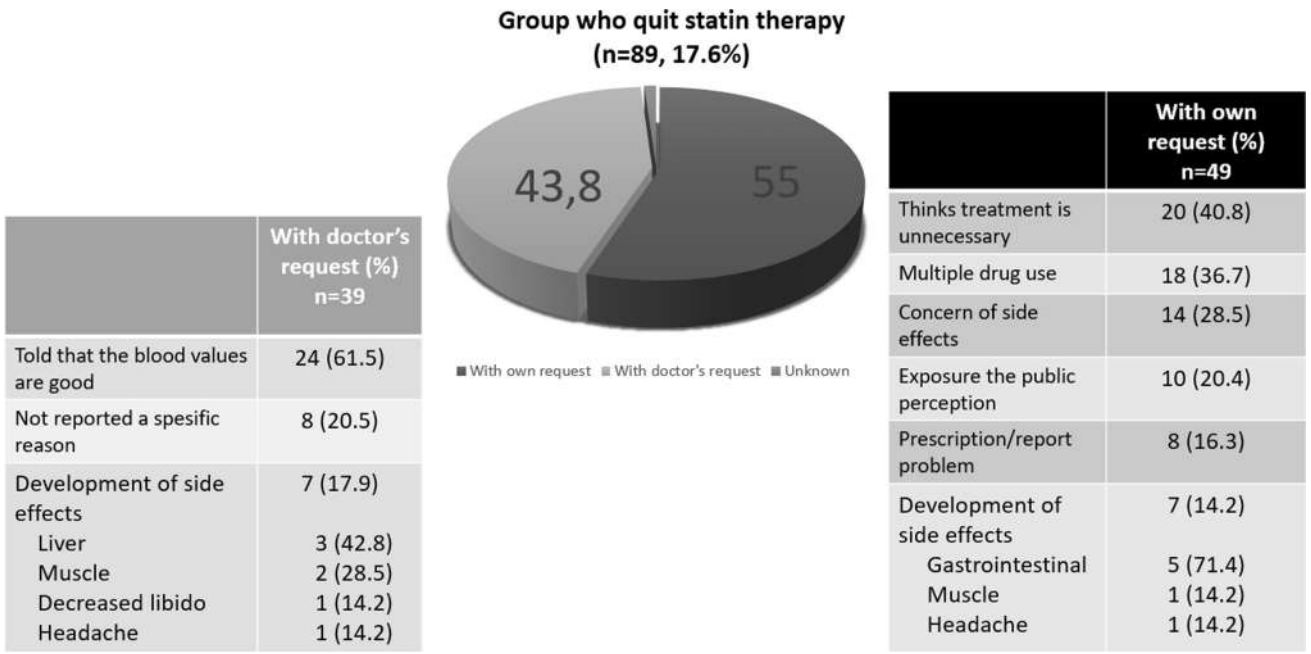
[b] $p > 0.05$ compared to the high-compliance group

[d] $p > 0.05$ compared to the medium-compliance group, $p < 0.01$ compared to the increased compliance group

[e] $p > 0.05$ compared to the increased compliance group

[m] $p < 0.01$ compared to the moderate- and high-compliance groups

[n] $p > 0.05$ compared to the high-compliance group



	With doctor's request (%) n=39
Told that the blood values are good	24 (61.5)
Not reported a spesific reason	8 (20.5)
Development of side effects	7 (17.9)
Liver	3 (42.8)
Muscle	2 (28.5)
Decreased libido	1 (14.2)
Headache	1 (14.2)

	With own request (%) n=49
Thinks treatment is unnecessary	20 (40.8)
Multiple drug use	18 (36.7)
Concern of side effects	14 (28.5)
Exposure the public perception	10 (20.4)
Prescription/report problem	8 (16.3)
Development of side effects	7 (14.2)
Gastrointestinal	5 (71.4)
Muscle	1 (14.2)
Headache	1 (14.2)

Fig. 1 Reasons for discontinuation of statin therapy

of our patient group who had never received statin treatment before. It is noteworthy that the rate of those in the group who were not recommended statin treatment was higher than the rate of those who refused. It is known that most patients in Turkey can stop their treatment without their doctors' knowledge.

In a study conducted by Yiğiner et al. on statin use, the rate of discontinuation of treatment without the knowledge of their doctor was 56.2%, and in a study by Tokgözoğlu et al., it was 73.7% [21, 22]. Patients discontinuing statin therapy without their doctor's knowledge may result from inadequate visits.

Although the frequency of statin-related side effects appears to be low in randomized controlled studies, real-life data show that the frequency of side effects can reach 30% [23]. In the study of Tokgözoğlu et al., the reason for the discontinuation of statin treatment was primarily liver-related side effects, and the rate of discontinuation of treatment was 13.9% because they considering the treatment unnecessary [22]. In a study by Kocas et al., it was observed that statin use decreased with the treatment of statin topics in social media [24]. In our study, side effects were observed in 14.2% of those who voluntarily discontinued the treatment; 57.1% of these patients discontinued treatment due to gastrointestinal system side effects. 40.8% of the patients who quit voluntarily quit the treatment because they thought it was unnecessary (22.4% of the total group who started the treatment and stopped it because they thought it was unnecessary). 36.7% of patients discontinued treatment due to using too many drugs; 28.5% of patients due to fear of developing side effects; 20.4% of patients due to adverse effects from social media; and 16.3% of patients due to report-prescription problems.

In 61.5% of the group who discontinued statin treatment at the request of their doctor, the patients were told that their blood values were good as the reason for discontinuation of statin treatment. This rate shows that the recommendation that statin therapy should be a lifelong treatment has not been adopted. The fact that 20.5% of patients' doctors did not know why they discontinued statin treatment reveals that patients should be included in the treatment process. It was determined that 17.9% of the patients had their treatment discontinued by their doctors due to the development of side effects. Elevated liver enzymes were the cause of 42.8% of the group whose treatment was discontinued due to side effects. The rate of patients whose treatment cannot be continued due to side effects is lower than the rate of patients who do not know the need for treatment and are worried about possible side effects. This is evidence of poor communication with patients.

Lipid-lowering treatments for primary prevention of diabetes patients were given to 61% of patients in the USA and 24% of patients in Germany; for secondary protection, it has been shown that these rates increase to 80% in the USA and 46% in Germany [25]. The rate of patients who received statin therapy among our patients with diabetes and CAD was 79.5%, while the rate of patients without CAD receiving statin therapy was 48.2%. This shows that we use statin therapy more in our diabetes patients with CAD.

In a study by Gant et al., it was observed that the target LDL level was reached in approximately 75% of patients with type 2 diabetes with high cardiovascular risk, and the use of statins was higher in the target group [26]. In a study by Özkan et al., 84.9% of patients with diabetes were started on statins, but only 8.7% of the patients received effective statin treatment, while 24.3% received an acceptable level of statin treatment [27]. Although 73.6% of our patients were in the study's very high-risk group, the rate of patients receiving high-intensity statin therapy was only 22.6%. Although our rate of using high-intensity statin therapy is higher than similar studies, the fact that this rate should be even higher shows that we are insufficient in the titration of therapy. This may be due to physicians' hesitations about LDL targets.

Our study had some limitations. It was carried out at a time when the COVID-19 pandemic was effective worldwide. It is thought that the negative effects of the pandemic, such as inaccessibility to health services, an increase in sedentary life, and a deterioration in drug use due to a tendency to depression, may affect the results.

Conclusion

It seems that the rates of compliance with statin therapy and reaching our treatment goals in our patients are very low. Our patients often consider statin therapy unnecessary. The patient-physician relationship is very important in statin treatment management, as in all treatments. It is necessary to explain the scientific data about statins to the patients and to eliminate their concerns. Although the guidelines explain what the target LDL levels should be in diabetic patients, the different risk classifications and targets in different country and group guidelines may cause confusion. Therefore, a global consensus is needed.

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Data Availability Data sharing not applicable.

Declarations

Ethics approval and consent to participate The clinical study was approved by the Ethics Committee of Marmara University Faculty of Medicine (Approval Date: 12.06.2020, Approval Number: 09.2020.626). The research protocol was implemented according to the principles expressed in the World Medical Association Declaration

of Helsinki and under the International Ethical Guidelines for Biomedical Research Involving Subjects (GIOMS, Geneva, 1993). Informed consent was obtained from all subjects.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Triglyceride variability affects diabetic kidney disease in middle-aged and elderly people with type 2 diabetes mellitus in the Guangxi Zhuang population

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Abstract

Objective Dyslipidemia has been implicated in the development and progression of renal disease. To our knowledge, no reports have demonstrated an association between blood lipid level variability and diabetic kidney disease (DKD) in China. Our objective is to investigate the influence of variability in triglyceride levels on DKD incidence in a middle-aged to elderly Chinese Zhuang population with type 2 diabetes mellitus.

Methods In all, 276 participants with type 2 diabetes mellitus aged ≥ 45 years were followed up for 2 ~ 5 years and the results were analyzed. Variability in their triglycerides was evaluated using standard deviation, coefficient of variation, and variability independent of the mean, and the mean was calculated, and the outcome was DKD. We applied a Cox proportional hazard model to determine the relationship between variability TG levels and DKD.

Results During the mean 3-year follow-up, 74 participants developed DKD. In a multivariable cox regression model, triglyceride variability was a significant risk factor for DKD. The hazard ratios (HRs) (95% confidence intervals [CI]) for each increase in SD, CV, and VIM of triglycerides by 1 SD were 1.257 (1.038–1.522), 1.525 (1.059–2.195), and 1.007 (1.004–1.011), respectively. Compared to the lowest quartiles of SD of triglycerides, the HRs (95%CI) were 1.858 (1.359–2.542), 1.881 (1.354–2.612), and 1.858 (1.343–2.570) in Q2, Q3, and Q4. Consistency was seen when CV and VIM were used for calculating variability.

Conclusion High TG variability in middle-aged and elderly Chinese Zhuang patients with type 2 diabetes mellitus was associated with a significantly increased risk of developing DKD.

Keywords Triglyceride variability · Diabetic kidney disease · Type 2 diabetes · Zhuang population

Introduction

Diabetic kidney disease (DKD) is a severe irreversible complication of diabetes mellitus and is defined as a persistent rise in albuminuria excretion, a reduction in the estimated glomerular filtration rate (eGFR), or both [1, 2]. Data show that approximately 20–40% of diabetic patients have DKD in a Chinese population [3–5]. In China, DKD has become a major cause of ESRD in middle-aged and older age-groups [6]. Unfortunately, the awareness rate of DKD in our country is less than 20%, and the treatment rate is less than 50% [7]. In addition to low awareness of the disease, poor patient compliance and treatment inertia are the main reasons for inadequate DKD control [2, 8]. Despite improvements in diabetes risk factor control and management, only a small proportion of patients with type 2 diabetes achieve target levels of glucose, blood pressure, and lipids, especially if they are also affected by CKD [9–11]. Hypertriglyceridemia (HTG) is a common type of dyslipidemia, and the prevalence of HTG is particularly high in the Chinese population. Insulin resistance

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(IR) refers to the reduced ability of insulin target tissues to respond to physiological levels of insulin due to various reasons, and the body compensates by secreting too much insulin to maintain blood glucose stability [12]. Studies have shown that hypertriglyceridemia can cause insulin resistance and at the same time, insulin resistance, as a key causative factor in many metabolic diseases, can exacerbate hypertriglyceridemia [13]. Patients with type 2 diabetes mellitus (T2DM) frequently have concurrent lipid metabolism disorders, and abnormalities in lipid metabolism can be involved in kidney disease development [14–16]. In the World Health Organization's Multi-Country Follow-Up Study of Diabetic Vascular Disease, plasma triglycerides were found to be a significant predictor of renal failure in patients with type 2 diabetes [17]. Recent evidence has suggested that lipid variability can be used to predict renal insufficiency. Specifically, a Korean study involving 8 493 277 individuals reported an association between high total cholesterol (TC) variability and ESRD [18]. In the same year, a multicenter cohort study in Italy reported that low-density lipoprotein-C (LDL-C) and triglyceride (TG) variabilities led to reduced eGFRs in individuals with type 2 diabetes, and interestingly, lipid variability may have a greater impact on low-risk populations, such as young subjects without metabolic disease [19]. A recent Japanese study suggested that the standard deviation (SD), adjusted SD, and maximum minus minimum deviation of postprandial triglyceride (PTG) may increase the likelihood of patients with type 2 diabetes having reduced eGFR or microalbuminuria [20]. It is therefore unclear, based on the above evidence, what effect lipid variability has on renal insufficiency, which may be due to the differences in the populations, metabolic characteristics and ethnicities, and potential confounding factors in the studies. Moreover, few studies have focused on Chinese diabetic patients, and it is unclear whether previous findings can be translated to the high risk of DKD in middle-aged or elderly people from China with type 2 diabetes. Hence, we need additional consistent data that support the effect of lipid variability on DKD to draw more concrete conclusions. China is composed of 56 ethnic groups, with the Han nationality accounting for the main part and the remaining 55 ethnic minorities accounting for 9% of the national population. The ethnic minorities show distinct genetic backgrounds, socioeconomic status, disease burdens, languages, eating habits, and living environments [21, 22]. However, research into the risk factors of noncommunicable diseases in China often integrates ethnic minorities into a single ethnic category, ignoring ethnic differences, and the survey population is relatively limited. Therefore, we used a longitudinal cohort study to explore the link between TG variability and DKD development in middle-aged and elderly T2DM patients in a Chinese Zhuang population.

Material and methods

Study design

This was a single-center observational cohort study on middle-aged and elderly (age ≥ 45 years old) Chinese Zhuang individuals with T2DM. The participants were recruited from among ambulatory and hospitalized patients presenting at the Second Affiliated Hospital of Guilin Medical University in Guangxi Zhuang Minority Autonomous Region, China. We first confirmed that they had Guangxi household registration, that their nationality was shown as Zhuang on their ID cards, and they had lived there for more than five years. Patients with type 2 diabetes were identified, followed by baseline data collection, from January to December 2014. The follow-up for each patient ranged from 2 to 5 years (median 3.0 years). In 2014, there were 485 diabetic patients. Participants that had other conditions (congestive heart failure, infections of the urinary system, and primary kidney disease) or other forms of diabetes, complied with less than 2-year of follow-up, whose records were lacking blood lipid data, and eGFR of < 60 mL/min/1.73 m^2 were excluded. Data on 276 diabetic patients in the final cohort were used in data analysis. The research flow chart was shown in Fig. 1.

Measurement of anthropometric, clinical, and biochemical parameters

At each check-up, the participants were questioned regarding their medical history, focusing on metabolic diseases, such as hypertension, diabetes, and hyperlipidemia. We took readings of each person's height (cm) and weight (kg), with 0.1 cm or 0.1 kg accuracy, when they were barefoot and in light clothing. To calculate BMI, we divided the patient's weight (kg) by their squared height (m). Using an automatic electronic device (Model HEM-7117, OMRON Company, Dalian, China), three blood pressure readings were taken within 1 min after the individual had rested for 5 min, and the average of three measurements was calculated. Participants were categorized as having hypertension when their systolic BP (SBP) was ≥ 140 mmHg or diastolic BP (DBP) was ≥ 90 mmHg or they used antihypertensive agents. Fasting venous blood specimens were collected between 07:30 and 08:30 on the day of the test. Smoking, drinking, the consumption of high-sugar and high-fat food, and strenuous activities were prohibited during the blood sampling period. After overnight fasting for at least eight hours, blood was collected in the morning. Concentrations of plasma fasting

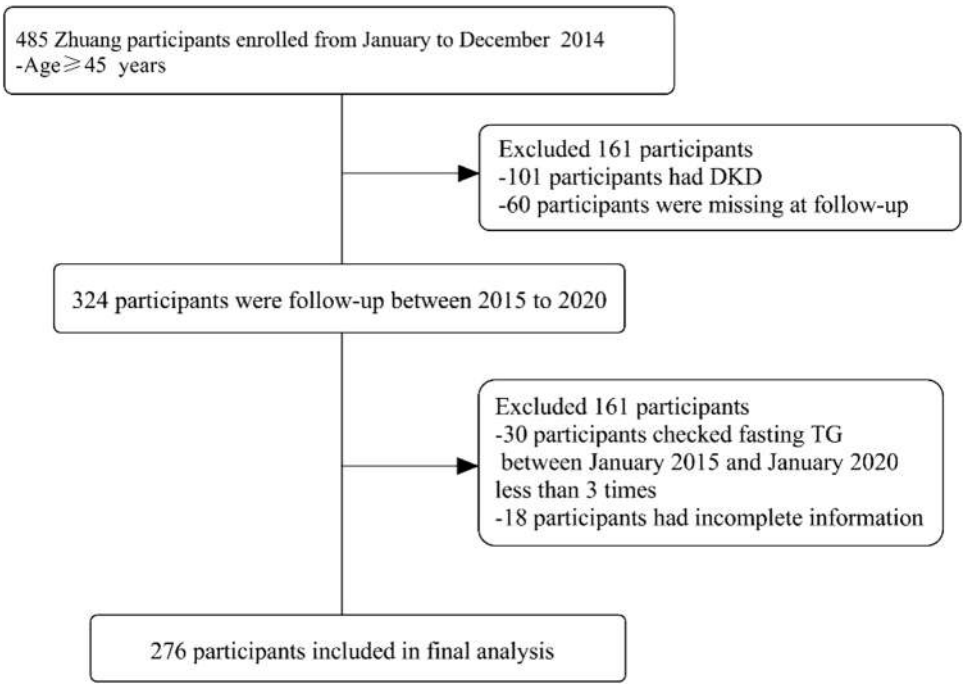

Fig. 1 Flowchart of included/excluded type 2 diabetic patients of Zhuang ethnicity. Abbreviations: DKD, diabetic kidney disease; TG, triglyceride

blood glucose (FBG), the two-hours postprandial blood glucose (2hPBG), TC, TG, LDL-C, and high-density lipoprotein-C (HDL-C) were measured with commercial colorimetric kits on an Architect c8000 analyzer (Abbott, IL, USA) using the manufacturer's protocol. HbA1c was determined by high-pressure liquid chromatography.

Outcomes and follow-up

DKD was the outcome of the present study, and diagnoses were formed using National Kidney Foundation Kidney Disease Outcomes Quality Initiative clinical practice guidelines. Patients were considered to have DKD when three standard criteria were applied to them: (1) a diagnosis of type 2 diabetes; (2) two consecutive urine tests within 6 months with urine albumin-to-creatinine ratio (UACR) $\geq$ 30 mg/g and/or eGFR $\leq$ 60 ml·min^{-1}·(1.73 m^2).$^{-1}$; (3) there was no clinical or laboratory evidence for other kidney diseases. The eGFR was evaluated based on revised Japanese Society of Nephrology Chronic Kidney Disease Initiative equations:$eGFR\ (ml/min/1.73m2) = 194 \times serum\ creatinine - 1.094 \times age - 0.287$ [if female, $\times$ 0.739] [23]

DKD status was reassessed annually during follow-up.

Parameters of lipid variability

We drew blood for fasting plasma lipids measurements the morning after the individual had fasted overnight ($\geq$ 8 h) during the annual physical examination. For each patient, TG parameter variability was evaluated using three indices: SD, coefficient of variation (CV), and variability independent of the mean (VIM). The CV was obtained as the SD-to-mean ratio. $VIM = 100 \times SD/mean\beta$, in which β represents the regression coefficient based on the natural log of SD divided by the natural log of the mean. Therefore, we included only those individuals with at least four lipid variable readings in the final analyses. The variabilities of the TG parameters were described in quartiles.

Statistical analyses

Data were analyzed with SPSS 20 and other statistics formulae and tables. We represented continuous variables as means ± SD or median (interquartile range) and categorical variables as numbers (percentages). We used Student's t-test to compare normally distributed data between groups, the Wilcoxon test for non-normally distributed data, and the chi-square for categorical data. A Cox proportional hazards model was used to explore the relationships between different TG variabilities and the development of DKD; covariates included age, sex, disease duration, BMI, FBG, 2hPBG, HbA1c, TC, LDL-C,

and HDL-C, amongst others. The variabilities of the TG parameters were allocated to quartiles. Standardized Kaplan–Meier curves were employed for survival analysis, and log-rank tests were run to compare cumulative event rates differences according to TG variability quartiles. All tests were two-sided. p-values < 0.05 were deemed statistically significant.

Results

Baseline characteristics of participants

Table 1 shows an outline of the clinical characteristics of the study population categorized by DKD status. Patients were assigned to two groups according to their DKD status.

Table 1 Baseline and follow-up characteristics of the study participants

Characteristic	Overall ($n=276$)	Without DKD ($n=202$)	With DKD ($n=74$)	p-value
Baseline				
Age (y)	60.49 ± 11.05	61.63 ± 11.05	59.92 ± 11.03	0.740
Diabetes duration (y)	7.00 (2.00–12.00)	5.00 (1.00–9.00)	7.00 (2.50–14.00)	< 0.001
Female (*n*, %)	110 (39.86%)	77 (38.11%)	33 (44.59%)	0.330
Smoking (*n*, %)	97 (35.14%)	78 (38.61%)	19 (25.67%)	0.046
Alcohol-drinking (*n*, %)	79 (28.62%)	60 (29.70%)	19 (25.67%)	0.512
Education levels (*n*, %)				< 0.001
Primary school	51 (18.48%)	35 (17.32%)	16 (7.92%)	
High school	152 (55.07%)	100 (49.50%)	52 (70.27%)	
College or above	73 (26.45%)	67 (33.17%)	6 (8.10%)	
Rural residence (*n*,%)	86 (31.16%)	60 (29.70%)	26 (35.10%)	0.388
Diabetic retinopathy (*n*,%)	50 (18.12%)	31 (15.35%)	19 (25.67%)	0.048
Use of lipid-lowering agents (*n*,%)	84 (30.43%)	60 (29.70%)	24 (32.43%)	0.662
Insulin	148 (53.62%)	110 (54.46%)	38 (51.35%)	0.647
Antihypertensive drugs	118 (42.75%)	93 (46.03%)	25 (33.78%)	0.038
Hypoglycemic agents	196 (66.67%)	154 (77.72%)	48 (64.86%)	0.059
Family history of diabetes (*n*,%)	47 (17.03%)	35 (17.32%)	12 (16.21%)	0.828
BMI (kg/cm^2)	24.44 (22.19–26.75)	23.63 (21.91–26.35)	24.84 (22.50–26.74)	0.386
TC (mmol/L)	4.83 (4.07–5.65)	4.91 (4.12–5.65)	4.80 (4.01–5.67)	0.284
TG (mmol/L)	1.50 (1.00–2.36)	1.40 (1.00–2.31)	1.59 (1.00–2.35)	0.905
LDL cholesterol (mmol/L)	3.04 (2.40–3.56)	3.50 (3.06–4.04)	3.59 (3.09–4.04)	0.126
HDL cholesterol (mmol/L)	1.27 (1.08–1.43)	1.26 (1.03–1.45)	1.29 (1.11–1.42)	0.015
SBP(mmHg)	135.07 ± 21.75	137.75 ± 24.44	133.72 ± 20.21	0.017
DBP (mmHg)	78.83 ± 11.40	78.48 ± 12.68	79.00 ± 10.74	0.143
FBG (mmol/L)	7. 80 (6.29–9.87)	7.55 (6.40–9.60)	8.00 (6.31–9.90)	0.882
2hPBG(mmol/L)	12.50 (9.35–15.43)	13.62 (11.20–18.40)	12.00 (8.90–14.70)	0.001
HbA1c (%)	7.60 (6.50–9.20)	7.45 (6.50–8.80)	7.80 (6.60–9.40)	0.095
eGFR (mL/min/1.73m^2)	80.09 ± 8.19	86.40 ± 17.75	76.94 ± 16.51	< 0.001
UACR(mg/g)	3.92 (11.85–18.44)	7.29 (2.35–13.03)	14.59 (6.63–19.5)	< 0.001
Follow-up Variability				
Triglyceride_Mean	1.60 (1.14–2.40)	2.34 (1.42–3.17)	2.39 (1.70–3.84)	0.439
Triglyceride_SD	1.14 (0.74–1.48)	1.31 (0.76–1.79)	1.55 (1.26–1.96)	< 0.001
Triglyceride_CV	0.62 (0.22–0.84)	0.22 (0.14–0.66)	0.73 (0.56–0.89)	< 0.001
Triglyceride_VIM	100.93 (70.14–113.03)	79.20 (33.98–104.75)	107.21 (90.27–114.23)	< 0.001

Data represent percentages, means ± SD, or medians (25th–75th percentile), as appropriate. *DKD* diabetic kidney disease, *SD* standard deviation, *CV* coefficient of variation, *VIM* variability independent of the mean, *BMI* body mass index, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting plasma glucose, *2hPBG* two-hours postprandial blood glucose, *HbA1c* hemoglobin A1c, *eGFR* estimated glomerular filtration rate, *UACR* urine Albumin-to-Creatinine Ratio

In comparison with those without DKD, patients with DKD had a greater likelihood of being female and having diabetes for longer, a lower literacy level, higher HDL-C, lower 2hPBG levels, and were more likely to have diabetic retinopathy (all $p < 0.05$). Fewer patients with DKD were on insulin or antihypertensive drugs; although there were lower proportions of lipid-lowering drugs in the DKD group, these differences were not significant. In addition, we found that DKD patients had higher TG variability compared to those without DKD ($p < 0.05$), but the mean TG values did not differ significantly between the two groups ($p > 0.05$).

Risk factors for DKD in follow-up

To investigate how different TG variability influenced the emergence of DKD, Cox regression was used to conduct a multivariate factor analysis among related factors. After further adjustments for confounders, the results (Table 2) were relatively consistent. Overall, TG variability was a DKD risk factor of significance. After further adjustments for age, sex, diabetes duration, BMI, SBP, DBP, FBG, HbA1c, TC, HDL, and LDL, the hazard ratios (HRs) and 95% confidence intervals (CIs) of an increase by 1 SD of SD, CV, and VIM of TG were 1.235 (1.017–1.499), 1.481 (1.028–2.133), and 1.008 (1.004–1.011), respectively ($p < 0.05$).

TG variability and diabetic kidney disease

When the lowest quartile of TG variability was included as a reference (Table 3) across the models, we found a significant rise in the risk of DKD in the upper quartiles (Q2-Q4) compared with the lowest (Q1). In the unadjusted Model 1, the HRs and 95%CIs for the highest quartiles of SD, CV, and VIM of TG variability were 1.826 (95%CI: 1.429, 2.333), 1.485 (95%CI: 1.174, 1.879), and 1.572 (95%CI: 1.317, 1.875). In Model 3 (after adjusting for age, sex, BMI, SBP, DBP, FBG, 2hPBG, HbA1c, TC, LDL-C, and HDL-C levels, etc.), the HRs and 95%CIs for the highest quartiles of SD,

Table 2 Multivariate Cox proportional hazards regression model to study the relationship between TG variability and DKD

Variables	Model 1		Model 2		Model 3	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	*p*-value
TG-SD	1.106 (1.005–1.217)	0.039	1.204 (1.026–1.412)	0.023	1.235 (1.017–1.499)	0.033
TG-CV	1.365 (1.056–1.763)	0.017	1.417 (1.055–1.903)	0.021	1.481 (1.028–2.133)	0.035
TG-VIM	1.007 (1.004–1.010)	0.000	1.007 (1.004–1.010)	0.000	1.008 (1.004–1.011)	0.000

Data presented as HR and 95%CI. *HR* hazard ratio, *CI* confidence interval, *DKD* diabetic kidney disease, *SD* standard deviation, *CV* coefficient of variation, *CV* coefficient of variation, *VIM* variability independent of the mean

Table 3 Relationships between TG variability and diabetic kidney disease

Variable			Model 1		Model 2		Model 3	
			HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
TG-SD	Q1	ref						
	Q2		1.601 (1.251–2.047)	< 0.001	1.484 (1.156–1.906)	0.002	1.668 (1.226–2.269)	< 0.001
	Q3		1.800 (1.412–2.295)	< 0.001	1.649 (1.279–2.125)	< 0.001	1.750 (1.282–2.389)	< 0.001
	Q4		1.826 (1.429–2.333)	< 0.001	1.785 (1.313–2.428)	< 0.001	2.051 (1.428–2.945)	< 0.001
TG-CV	Q1	ref						
	Q2		1.729 (1.367–2.188)	< 0.001	1.592 (1.251–2.027)	< 0.001	1.651 (1.251–2.180)	< 0.001
	Q3		1.684 (1.322–2.128)	< 0.001	1.592 (1.245–2.035)	< 0.001	1.655 (1.225–2.237)	0.001
	Q4		1.485 (1.174–1.879)	0.001	1.491 (1.155–1.925)	0.002	1.574 (1.153–2.150)	0.004
TG-VIM	Q1	ref						
	Q2		1.233 (0.944–1.612)	0.124	1.303 (0.990–1.715)	0.059	1.612 (1.168–2.224)	0.004
	Q3		1.256 (1.007–1.566)	0.043	1.32 (1.060–1.661)	0.013	1.456 (1.116–1.901)	0.006
	Q4		1.572 (1.317–1.875)	< 0.001	1.563 (1.300–1.879)	< 0.001	1.733 (1.389–2.164)	< 0.001

Data presented as HR and 95%CI. Model 1: non-adjusted. Model 2:adjusted for adjusted for age, sex, BMI, diabetes duration, mean TG. Model 3: adjusted for age, sex, BMI, diabetes duration, mean TG, smoking, drinking, family history of diabetes, SBP, DBP, FBG, 2hPBG, HbA1c, TC, HDL, and LDL. **Abbreviations:** *HR* hazard ratio, *CI* confidence interval, *DKD* diabetic kidney disease, *SD* standard deviation, *CV* coefficient of variation, *VIM* variability independent of the mean, *BMI* body mass index, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *eGFR* estimated glomerular filtration rate

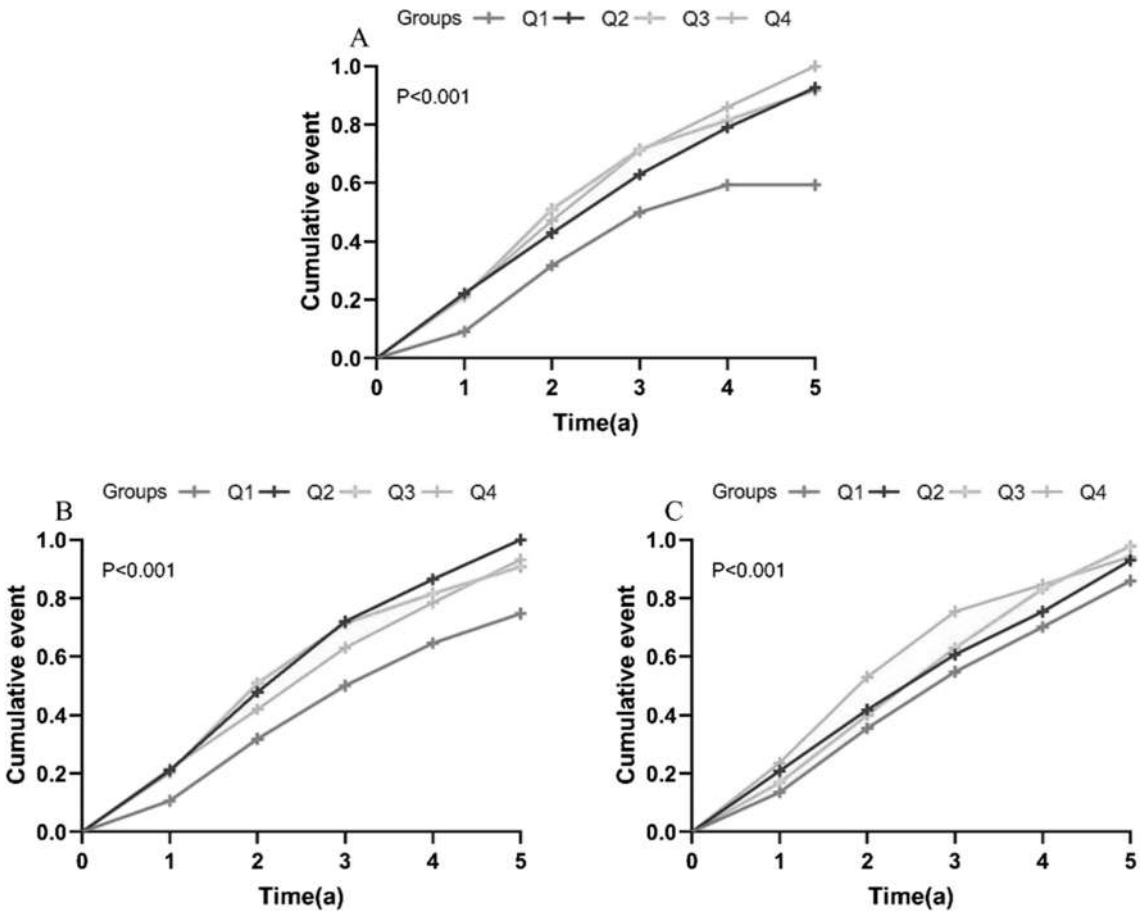

Fig. 2 Kaplan–Meier curves of cumulative events of diabetic kidney disease by quartile variability of lipid parameters (A: TG variability by SD; B: TG variability by CV; C: TG variability by CV)

CV, and VIM of TG variability were 2.051 (95%CI: 1.428, 2.945), 1.574 (95%CI: 1.153, 2.150), and 1.733 (95%CI: 1.389, 2.164). In addition, when the variability of TG was calculated using CV, the risk of DKD occurrence was still increased in the Q4 group, but it was lower than those in the Q2 and Q3 groups. Figure 2 shows the survival curves of cumulative incidence of DKD grouped by quartiles of TG variability, and higher TG variability (Q2-Q4) had an increased probability of association with an increased cumulative incidence of DKD.

Discussion

In our investigation, we found a link between higher TG variability and an increased prevalence of DKD, with the retention of significance after adjustment for demographic and clinical features. This concurs with the outcomes of a previous Italian study, although we used a different index for calculating variability[24]. However, there are known biological differences in lipid responses [25, 26]. Thus, it is important to explore the specific characteristics of particular populations, and in our study, we focused on middle-aged and elderly residents of Guangxi Zhuang, China, for whom both dietary habits and customs differ substantially from those of the Han Chinese. A Japanese report [20] claimed that PTG variability was a new risk factor for decreased eGFR and microalbuminuria incidence in type 2 diabetic individuals, but the study did not perform simultaneous blood collection and lacked a standardized diet for quality control, which may have directly affected the lipid variability measurements. TG concentrations are susceptible to dietary patterns, for example, short-term very-low-calorie factors can induce myocardial triglyceride accumulation. Lai et al. used the Environment-Wide Association Study (EWAS) approach to characterize dietary factors associated with triglycerides and found that alcohol consumption, cigarette smoking, and carbohydrate intake were the main influences on triglycerides [27], and that changes in TG concentrations were also affected by mealtimes, exercise, heredity, and free fatty acids (FFA) in the body [28]. It has been reported that inter-day variations in TG concentrations are closely

related to diet composition and time of measurement, that similar food compositions can lead to different results in PTG responses [29, 30], and that peak TG often occurs at different time points [31, 32]. Currently, the oral fat tolerance testing (OFTT) protocol is most commonly utilized for the assessment of PTG and recommends the use of a dietary meal standard containing 75 g of fat to assess PTG [33–35]. According to the Greek consensus, a PTG level of > 220 mg/dL after the consumption of high-fat food is considered high [36]. Recently, researchers in China showed this threshold to be effective for assessing dyslipidemia in those with a fasting triglyceride (FTG) level of 1.0–1.7 mmol/L [37]. In contrast, a Mexican study showed that a cut-off value of 280 mg/dL PTG at any timepoint after a meal following the OFTT test was effective for differentiating patients with fasting hypertriglyceridemia (> 150 mg/dL) [38]; however, their calculated PTG threshold is inconsistent with the consensus and, despite the inclusion of only 50 g of fat in the meal, a similar FTG response was seen in the Chinese study following the consumption of75 g of high-fat food, suggesting the existence of population variability in the definition of PTG thresholds. Furthermore, this recently discovered threshold cannot be directly correlated with cardiovascular or mortality events. Consistent with previous studies, this study found that TG variability in women was more strongly associated with the risk of diabetic nephropathy, which may be related to both estrogen and gene expression [39]. However, in previous studies, some important sex differences were observed in the management of risk factors. Compared with men with T2 DM, women with T2 DM had worse cardiovascular risk factor management, especially when they had cardiovascular disease [39]. These results suggest that stabilization of lipid levels is important in diabetic patients, especially women. Currently, patients' fasting lipids are still normative measurements in China [40]. Although some recent guidelines and consensus statements suggest that PTG is more reliable than FTG for predicting cardiovascular disease risk [35, 41, 42], the PTG does not have wide clinical application and is disadvantaged by an absence of standard clinical guidelines for the detection of postprandial lipid levels and population-based normal reference values. There have been many studies demonstrating the correlation between TG and DKD [43, 44], but no causal factors linking TG and DKD have been demonstrated. It has recently been reported that altered TG only, without other concomitant metabolic abnormalities, does not seem to have any causal association with kidney disease development [45, 46]. However, a Mendelian randomization (MR) analysis from China showed that higher TG levels were linked to a higher likelihood of developing chronic kidney disease [47]; it is possible that this lack of consensus may be the result of discrepancies in sample sizes, ethnicities, and outcome classification of the studies. Diabetic dyslipidemia and different stages of nephropathy differ in their outcomes, indicating that the complexity of the pathogenesis and the likelihood of several biologically plausible mechanisms to explain the development of DKD. When adipose tissue becomes insulin resistant, it releases large amounts of free fatty acids (FFA), and increased FFA levels cause the liver to synthesize very low-density lipoproteins (VLDL) and increase the relative amount of low-density lipoproteins (LDL); in addition, the clearance of total triglycerides (TG) by lipoprotein lipase (LPL) is prolonged, resulting in the main manifestations of lipid metabolism disorders in DKD patients being hypertriglyceridemia and steatohepatitis [48]. TG levels exceeding the upper limit of its storage in adipose tissue aggravate lipid deposition in the glomerulus, and ectopic deposition of lipids leads to endothelial cell injury, which, in turn, penetrates into adjacent endothelial tissues, such as mesangial cells, pedunculated cells, and renal tubular epithelial cells (RTECs), further exacerbating glomerular injury and sclerosis [49]. In addition, the accumulation of lipid can further aggravate glomerular damage and sclerosis. Lipid accumulation can also lead to podocyte damage [50], increased extracellular matrix deposition [51], and macrophage infiltration [52], which is associated with pathways involving inflammatory responses [53], oxidative stress [54], endoplasmic reticulum stress [55], and mitochondrial damage [56].

Therefore, whether higher TG variability is a pathogenic factor of or a compensatory response to DKD, our current understanding of this regulatory network is incomplete and further studies are still needed to elucidate its features.

Study limitations

This was a study to assess the relationship between TG variability and DKD among Chinese minorities at follow-up, but a number of limitations warrant consideration. First, our sample size was relatively small, some of the data were lost during follow-up, and the cohort study was based in a tertiary care hospital, thus lacking broad representation and having the potential of a certain degree of sampling bias. Second, although patient medication information was collected at baseline, we did not monitor patient glycemic variability, elevated blood uric acid, inflammation, effects of nephrotoxic drugs and dietary habits during follow-up, which are known to lead to faster progression of renal damage. Moreover, because diet has strong regional and ethnic variations, this may have affected our actual results. Third, we conducted a short-term follow-up period of a maximum of only 5 years, and it is necessary to continue to conduct cohort studies with larger samples for longer periods of time to more clearly define the link between TG variability and DKD in the short and late stages. Despite these limitations, our study also has some advantages in that less attention was paid to ethnic minorities at home

and abroad. Furthermore, it was based on a natural cohort of ethnic minorities in Guangxi, where the Zhuang population lives in clusters, is less mobile, and the areas of residence are relatively concentrated, and thus have a better representation. Our study has practical reference value for the development of prevention and control strategies and measures for diabetic nephropathy in the Zhuang population.

Conclusion

In conclusion, after adjusting for confounders, three different high TG variants were risk-associated with DKD development in middle-aged and elderly type 2 diabetic patients in Guangxi Zhuang. This suggests that achieving lipid stabilization, especially TG, may help to reduce the propensity for renal dysfunction. Future studies should focus on whether interventional tools have beneficial effects on TG variability.

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Declarations

Ethical clearance The study was given approval by the Ethics Committee of Second Affiliated Hospital of Guilin Medical University and First Affiliated Hospital of Guangxi Medical University and followed the Declaration of Helsinki. All participants submitted their written informed consent.

Conflict of interest The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Unpacking the burden of hypertension and diabetes in Karnataka: implications for policy and practice based on NFHS-5 findings

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Abstract

Objective To investigate the prevalence, risk factors, and healthcare-seeking patterns of hypertension and diabetes in Karnataka, India, and to offer knowledge that might guide public health initiatives intended to lessen the burden of these illnesses.

Methods In order to examine the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka, India, a cross-sectional study is carried out using the information gathered from 26,574 households on 30,455 women and 4516 men (who were in their reproductive period) from the National Family Health Survey (2019–20). The information was summarised using descriptive statistics, which included frequencies and percentages. The association between different risk variables and the likelihood of getting diabetes and hypertension was examined using the chi-squared test and a logistic regression model. Data were analysed using STATA software version 16.

Results The study found that age, gender, education level, religion, and BMI are all significantly associated with hypertension and diabetes ($p < 0.001$). Tobacco use and alcohol consumption were not significantly associated with hypertension, but tobacco use was significantly associated with diabetes ($p < 0.001$). However, alcohol consumption was not found to be significantly associated with diabetes whereas the older age groups, males, underweight, overweight and obese, and tobacco use were all associated with increased odds of diabetes. On the other hand, females, secondary education or higher, and alcohol consumption were associated with decreased odds of diabetes.

Conclusion In conclusion, the study found a high prevalence of hypertension and diabetes in Karnataka, India, and identified several risk factors associated with these diseases. The study also highlighted the need for improved healthcare-seeking behaviour among people with hypertension and diabetes. The findings can inform public health interventions aimed at reducing the burden of these diseases in Karnataka and similar settings.

Keywords Hypertension · Diabetes · Prevalence · Risk factors · Healthcare-seeking behaviour

Introduction

Non-communicable diseases (NCDs), including hypertension and diabetes, have become a significant public health issue worldwide. These illnesses contribute significantly to the burden of disease globally and have negative effects on the quality of life of those who are afflicted. Hypertension and diabetes are two of the most common NCDs, and their prevalence has been rapidly increasing in recent years. According to the World Health Organization (WHO) [1], hypertension affects over one billion people worldwide, and diabetes affects over 420 million people. Additionally, these disorders are significant contributors to cardiovascular disease, the world's leading cause of mortality.

India is one of the countries with the highest burden of NCDs, and hypertension and diabetes are major public health challenges in the country. The prevalence of hypertension in India has increased from 25% in 1990 to 29% in 2019, and the prevalence of diabetes has increased from 2.2% in 1990 to 8.8% in 2019 [2]. The burden of these conditions is particularly high in the southern states of India, including Karnataka, which has a population of over 60 million people.

Karnataka is an important state in southern India and has a diverse population with a range of cultural and socioeconomic backgrounds. The state has experienced rapid economic growth in recent years, which has led to changes in

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lifestyle and dietary habits. The prevalence of NCDs, such as diabetes and hypertension, has increased as a result of these changes. In India, non-communicable diseases account for over 63% of all fatalities, with cardiovascular disease accounting for 27% of these deaths and affecting 45% of adults between the ages of 40 and 69.

The Government of India has launched the Indian Hypertension Control Initiative (IHCI) to fast-track access to treatment services for over 220 million people in India who have hypertension, and India has set a target of 25% relative reduction in the prevalence of hypertension (raised blood pressure) by 2025. According to the National Family Health Survey (NFHS-4), Karnataka had a prevalence of hypertension that was higher than the country's average of 29.8% at 34.6%. Similarly, the state had a diabetes prevalence of 8.4%, which was higher than the 7.3% norm for the country. The NFHS fifth cycle, which was conducted in 2019–2020 [3], offers the chance to look at the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka. The NFHS-5 is a large-scale survey that collects data on various health indicators, including NCDs, and provides a representative sample of the population of Karnataka. This study seeks to investigate the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka using data from the NFHS-5.

The study also explores the association between age, gender, education level, religion, BMI, tobacco use, and alcohol consumption with hypertension and diabetes. These variables are important determinants of NCDs and can help identify population subgroups at higher risk of these conditions. Understanding the relationship between these variables and hypertension and diabetes can inform the development of targeted public health interventions to prevent and manage these conditions.

The results of this study have significant ramifications for Karnataka politicians and healthcare professionals. Targeted public health interventions are required to prevent and manage NCDs in the state due to the high incidence of hypertension and diabetes in the region and the unfavourable healthcare-seeking behaviour for these disorders. The study can also inform the development of evidence-based strategies to improve healthcare-seeking behaviour for hypertension and diabetes in the study population.

Overall, this study provides important insights into the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka. The study can contribute to the development of evidence-based public health policies and programs to address the high burden of NCDs in the state.

Literature review

Hypertension and diabetes are major non-communicable diseases with significant public health implications. The literature on the prevalence, risk factors, and healthcare-seeking behaviour of these conditions in India and Karnataka is extensive. This section provides a review of recent studies that have investigated these issues.

According to Midha et al. [4], both urban and rural residents of a north Indian district have high prevalence of hypertension. Age and gender were risk factors for hypertension in both urban and rural populations. The risk of hypertension was also significantly influenced by diabetes, higher BMI values, a decline in physical activity, and a rise in waist circumference, necessitating primary healthcare-level intervention. Ranasinghe et al. [5] examined the prevalence and trends of the diabetes epidemic in urban and rural India; the prevalence of diabetes grew in both areas from 2.4% and 3.3% in 1972 to 15.0% and 19.0%, respectively. The prevalence of diabetes and pre-diabetes is rather high, according to a pooled analysis of estimates, with the urban–rural divide becoming more narrow. For this reason, they recommended developing urgent primary and secondary prevention initiatives to reduce further growth in areas with high frequency. In India's adult rural population, there is regional variation in the prevalence of overweight/obesity, hypertension, and diabetes as well as their correlates, according to Meshram et al. [6]. In contrast to diabetes, which was more prevalent in the Southern and Western regions, overweight/obesity and hypertension were more common in the Southern region. Age, male sex, being overweight or obese, and abdominal obesity were all significant risk factors for hypertension and diabetes. Geldsetzer et al. [7] examined the sociodemographic features of each individual as well as state-level variations in the prevalence of diabetes and hypertension in India. The study found that, despite the fact that household wealth and urban location were positively associated with both conditions, the prevalence of diabetes and hypertension among adults over 40 in the quintile with the lowest household wealth in rural areas was still high. Anchala [8] conducted a region-specific (urban and rural parts of north, east, west, and south India) systematic review and meta-analysis of the prevalence, awareness, and control of hypertension among Indian patients, which has never been done before, and came to the conclusion that about 33% of urban and 25% of rural Indians are hypertensive. In total, 42% of Indians living in cities and 25% of rural Indians are aware of their hypertension status. In India, just 38% of urban residents and 25% of rural residents receive

treatment for hypertension. One-fifth of hypertensive urban Indians and one-tenth of those living in rural areas get their blood pressure under control. Kurjogi et al. [9] assessed the prevalence of hypertension and related risk factors, and their findings showed that hypertension was more common among people who consumed alcohol and who chewed or smoked tobacco. Sedentary lifestyles were also found to be a significant risk factor for hypertension. Raja et al. [10] examined the prevalence of hypertension in individuals in rural areas and evaluated the risk factors that go along with it. They found that the whole population had a 26.2% prevalence of hypertension whereas males were at higher risk than females. They also found that the age, body mass index, diet, and family history of hypertension all significantly (p 0.05) correlated with hypertension. According to a study by Gupta et al. [11] on the prevalence, awareness, treatment, and control of diabetes and hypertension among elderly people in a rural area of Haryana, the high prevalence, low awareness, and low proportion of people with controlled disease populations highlight the significance of bolstering primary care and raising awareness about diabetes and hypertension among elderly people in rural areas. In a high-altitude area in rural Uttarakhand, India, senior residents were examined for the prevalence of hypertension and diabetes as well as related risk factors by Kapil et al. [12], who came to the conclusion that these conditions were present in 54.5% and 14.6% of the population, respectively. Ageing, having a high level of education, and having a body mass index (BMI) of less than 25 kg/m^2 for HTN were revealed to be important risk factors, as were having a higher level of education and a BMI of less than 25 kg/m^2 for DM.

Nair et al. [13] carried out a cross-sectional study in Kerala to ascertain the prevalence of diabetes and hypertension among inhabitants of urban slums. The study indicated poor healthcare-seeking behaviour as well as a high prevalence of both illnesses. The study emphasises the requirement for focused interventions for populations that are underserved. A systematic evaluation of studies on the prevalence of hypertension in India was carried out by Patel et al. [14]. The review discovered considerable regional variations and a high prevalence of hypertension in both urban and rural areas. The analysis highlights the demand for focused public health initiatives. A study on the prevalence of diabetes in Tamil Nadu, a state that borders Karnataka, was undertaken by Anjana et al. [15]. According to the report, diabetes is highly prevalent and is poorly understood and managed. The study emphasises the demand for improved diabetes control initiatives. In a few urban slums in Bangalore, Ramani et al. [3] looked at the incidence of hypertension and diabetic morbidity among adults, as well as the factors that determine risk factors and control options. They discovered that 13.8% of people had diabetes, 21.5% of people had hypertension, and 30.4% had both conditions concurrently. In total, 5.1% of the study participants used tobacco (in any form), whereas 32.4% were overweight, and 20.0% were obese. The study population makes up 18.96% of the source, and a lack of programmatic awareness was the main cause of the low utilisation.

Overall, the literature review highlights the high burden of hypertension and diabetes in India and its states. The studies reviewed also emphasize the need for targeted interventions to improve healthcare-seeking behaviour, awareness, and control of these conditions. The present study aims to contribute to this body of literature by examining the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka.

Methods

This study used data from the National Family Health Survey (NFHS-5) conducted in 2019–2020 in Karnataka, India. The NFHS-5 is a large-scale survey that collects information on various health indicators, including hypertension and diabetes, among a representative sample of households in each state. The NFHS-5 used a multi-stage stratified sampling design. In the first stage, villages in rural areas and census enumeration blocks in urban areas were chosen as primary sampling units (PSUs). Using a systematic sample technique, families were chosen within each PSU in the second step. From each family, eligible candidates were chosen for the third round.

The study population consists of 30,455 women and 4516 men who were in their reproductive period and had complete information on hypertension and diabetes status, demographic characteristics, and lifestyle factors residing in Karnataka. The prevalence of hypertension and diabetes was estimated by calculating the proportion of individuals who reported having been diagnosed with these conditions by a healthcare provider.

The data analysis is done using STATA version 16.0 (StataCorp, College Station, TX, USA). The chi-squared test is used to compare the distribution of categorical factors (age, gender, education level, religion, BMI, cigarette and alcohol usage) between people with and without diabetes/hypertension. In order to examine the relationship between numerous risk variables and the likelihood of having either condition (diabetes/hypertension) and to determine the major predictors of diabetes/hypertension, the logistic regression model is used, and to quantify the relationship between each risk factor (age, gender, education level, religion, BMI, tobacco use, and alcohol consumption) and the likelihood of having diabetes/hypertension, the odds ratios (ORs) and 95% confidence intervals (CIs) were also determined.

The logistic model showing the relationship between the probability p of having diabetes/hypertension and the several predictors (continuous/categorical) can be represented by the logit function as:

$$\begin{aligned}\text{logit}(p) &= \log\left(\frac{p}{1-p}\right)\\ &= \beta_0 + \beta_1 \text{age}_2 + \beta_2 \text{age}_3 + \beta_3 \text{age}_4 + \beta_4 \text{female} + \beta_5 \text{primary education}\\ &+ \beta_6 \text{secondary and above education} + \beta_7 \text{Muslim}\\ &+ \beta_8 \text{Christian} + \beta_9 \text{other religion} + \beta_{10} \text{Underweight}\\ &+ \beta_{11} \text{Overweight} + \beta_{12} \text{Obase}\\ &+ \beta_{13} \text{Tobacco use} + \beta_{14} \text{Alcohol use}\end{aligned}$$

where, p is the probability of having diabetes/hypertension, β_0 is the intercept term.

$\beta_1, \beta_2,$ and β_3 are the coefficient associated with age groups 30–39, 40–49, and 50–59 respectively, and age group 18–29 is the reference category.

β_4 is the coefficient associated with female gender, where female is coded as 1, and male is coded as 0 as male is taken as reference category.

$\beta_5$and β_6 are the coefficients associated with education levels primary and secondary education and above education respectively which take value as 1, and no education is taken as reference category takes value 0.

$\beta_7, \beta_8,$ and β_9 are the coefficients associated with Muslim, Christian, and other religions respectively and are coded as 1, whereas Hindu is considered as reference category coded as 0.

$\beta_{10}, \beta_{11},$ and β_{12} are the coefficients associated with BMI categories underweight, overweight, and obese respectively which are coded as 1 and 0 for reference category which is normal BMI.

β_{13} is the coefficient associated with tobacco use, where 'yes' is coded as 1 and 'no' is coded as 0 (no tobacco use as reference category).

β_{14} is the coefficient associated with alcohol use, where 'yes' is coded as 1 and 'no' is coded as 0 (no alcohol use as reference category).

This model allows us to estimate the effect of each categorical predictor on the probability of having diabetes/hypertension, while adjusting for the effects of other predictors in the model.

Results and discussion

Table 1 presents the findings of the chi-squared test for the connection of hypertension and diabetes with secondary variables and to ascertain whether there is a statistically significant association between the two primary variables (hypertension and diabetes) and the secondary variables. The chi-squared statistic, degrees of freedom (df), and p-value for each secondary variable for hypertension and diabetes individually are displayed in the Table 1. A smaller p-value (less than threshold value 0.05 or 0.01) indicates more evidence against the null hypothesis i.e. there is no link between the variables, whereas the p-value greater than the threshold value indicates the evidences against alternative hypothesis i.e. there is association between the variables. The results of the chi-squared test show that age, gender, education level, religion, and BMI are all significantly associated with hypertension and diabetes ($p < 0.001$). Specifically, older age groups were more likely to have hypertension and diabetes. Females had a higher prevalence of diabetes as compared to males. Higher education level and non-Hindu religions were associated with a lower prevalence of hypertension and diabetes. Individuals with higher BMI were found to be more likely to have hypertension and diabetes.

Tobacco use and alcohol consumption were not significantly associated with hypertension, but tobacco use was significantly associated with diabetes ($p < 0.001$). However, alcohol consumption was not found to be significantly associated with diabetes.

Table 1 The chi-squared test statistics showing the prevalence of hypertension and diabetes across several categories of risk factors

Secondary variables	Hypertension-chi-squared statistic (df, p-value)	Diabetes-chi-squared statistic (df, p-value)
Age	2332.51 (6, $p < 0.001$)	2830.52 (6, $p < 0.001$)
Gender	10.86 (1, $p = 0.001$)	155.89 (1, $p < 0.001$)
Education level	121.48 (3, $p < 0.001$)	238.31 (3, $p < 0.001$)
Religion	305.72 (4, $p < 0.001$)	140.69 (4, $p < 0.001$)
BMI	1462.99 (6, $p < 0.001$)	1715.09 (6, $p < 0.001$)
Tobacco use	0.25 (1, $p = 0.617$)	16.28 (1, $p < 0.001$)
Alcohol consumption	1.15 (1, $p = 0.283$)	1.14 (1, $p = 0.286$)

DF degrees of freedom

Table 2 Logistic regression showing the impact of risk factors on hypertension in Karnataka

Variable	Odds ratio (OR)	95% Confidence interval (CI)	*p*-value
Age			
18–29*			
30–39	1.87	(1.48, 2.36)	0.000 < 0.001
40–49	3.01	(2.38, 3.79)	0.000 < 0.001
50–59	4.62	(3.67, 5.82)	0.000 < 0.001
Gender			
Male*			
Female	1.22	(1.06, 1.40)	0.006 < 0.05
Education attainment			
No education*			
Primary	0.86	(0.69, 1.06)	0.152
Secondary and above	0.67	(0.54, 0.83)	0.000 < 0.001
Religion			
Hindu*			
Muslim	0.88	(0.72, 1.08)	0.209
Christian	0.73	(0.52, 1.04)	0.085
Others	0.66	(0.44, 0.98)	0.042 < 0.05
BMI			
Normal*			
Underweight	1.37	(0.98, 1.91)	0.068
Overweight	2.36	(2.00, 2.79)	0.000 < 0.001
Obese	3.66	(3.08, 4.36)	0.000 < 0.001
Tobacco use			
No*			
Yes	1.20	(1.04, 1.38)	0.014 < 0.05
Alcohol consumption			
No*			
Yes	1.05	(0.91, 1.21)	0.455

*the reference category

Table 3 Logistic regression showing the impact of risk factors on diabetes in Karnataka

Variable	Odds ratio (OR)	95% Confidence interval (CI)	*p*-value
Age			
18–29*			
30–39	2.46	(1.90, 3.18)	0.000 < 0.001
40–49	3.88	(2.98, 5.05)	0.000 < 0.001
50–59	5.79	(4.44, 7.56)	0.000 < 0.001
Gender			
Male*			
Female	0.80	(0.70, 0.91)	0.000 < 0.001
Education attainment			
No education*			
Primary	0.92	(0.78, 1.09)	0.345
Secondary and above	0.65	(0.55, 0.77)	0.000 < 0.001
Religion			
Hindu*			
Muslim	1.03	(0.86, 1.22)	0.771
Christian	0.81	(0.61, 1.07)	0.137
Others	1.04	(0.78, 1.38)	0.788
BMI			
Normal*			
Underweight	1.23	(0.89, 1.71)	0.207
Overweight	1.74	(1.50, 2.02)	0.000 < 0.001
Obese	2.61	(2.23, 3.06)	0.000 < 0.001
Tobacco use			
No*			
Yes	1.28	(1.12, 1.47)	0.000 < 0.001
Alcohol consumption			
No*			
Yes	0.93	(0.80, 1.09)	0.361

*indicates the reference category

Overall, the results suggest that age, gender, education level, religion, BMI, and tobacco use are important factors associated with hypertension and diabetes in Karnataka. These findings can have implications for healthcare providers and policymakers to develop targeted interventions to reduce the burden of these chronic diseases in the population. Further after knowing the association between the variables, the logistic regression is used to identify the impact of the risk factors on hypertension and diabetes.

Tables 2 and 3 show the logistic regression findings for both hypertension and diabetes based on the other risk factors. The analysis includes the odds ratios of having diabetes/hypertension for each category of the variables relative to its respective reference category and their 95% confidence intervals. The *p*-value indicates the statistical significance of the odds ratio if it is less than 0.05. The odds ratio represents the change in the odds of the having (hypertension or diabetes) associated with a one-unit increase in the variable. The odds ratio values greater than 1 indicate increased odds, whereas the odds ratio values less than 1 indicate decreased odds. The confidence interval gives a range of values within which the true odds ratio falls with 95% confidence level.

The findings from Table 2 infer that all age groups, females, secondary or above education level, other religions, overweight and obese, and tobacco consumption are significantly associated with the probability of having hypertension in Karnataka as their respective *p*-values are less than 0.05. We can also see that age groups (30–39, 40–49, 50–59), females, overweight and obese BMI, and tobacco use were all associated with increased odds of hypertension relative to its reference category, whereas the risk of having hypertension in secondary and above education and other

religion is lower than their respective reference category. In contrast, primary education, Muslim and Christian religion, underweight BMI, and alcohol consumption did not show significant associations with hypertension. Also, as the age increases, the probability of having hypertension increases (as the odds of developing the hypertension is increasing) relative to its reference category. From Table 3, we can see that all the age groups (30–39, 40–49, and 50–59) are significantly associated with diabetes and as the age increases the risk of having diabetes also increases. Males are more prone to risk of developing diabetes than females, and people who have attained secondary or higher level of education have less chances of having diabetes than those who have attained primary and no education. BMI also plays a significant role in developing the risk of diabetes; here, we can see that as BMI increases the chances of having diabetes also increases. Also those who consume tobacco have more changes of having diabetes relative to no tobacco use. Table 3 also indicates that there is no significant impact of religion and alcohol consumption on having diabetes.

These findings suggest that efforts to prevent and manage hypertension and diabetes in Karnataka should focus on modifiable risk factors such as tobacco use, alcohol consumption, and BMI. Additionally, interventions targeted towards specific demographic groups, such as older individuals and males for diabetes, and females for hypertension, could help improve the management of these conditions in the state.

Conclusion

In conclusion, this study aimed to investigate the prevalence, risk factors, and healthcare-seeking behaviour of hypertension and diabetes in Karnataka, India. The study used data from the latest National Family Health Survey (NFHS-5) and analysed the data using chi-squared test and logistic regression analysis.

The study found that the prevalence of hypertension and diabetes in Karnataka was high, with significant regional and socioeconomic disparities. The risk factors associated with hypertension and diabetes included age, gender, education level, religion, BMI category, and tobacco use and alcohol consumption. The study also found that healthcare-seeking behaviour was low among those with hypertension and diabetes, with a large proportion of individuals not receiving treatment or not being aware of their condition. The study also reveals that age, gender, education level, religion, BMI category, and tobacco use and alcohol consumption were significant predictors of hypertension and diabetes in Karnataka.

The findings highlight the need for targeted public health interventions to address the modifiable risk factors associated with hypertension and diabetes and to improve healthcare-seeking behaviour among individuals with these conditions in Karnataka state of India. This study can be used to inform and guide the development of public health policies and programs targeted at the prevention and management of hypertension and diabetes especially in Karnataka state. Policymakers can use this information to allocate resources and prioritize interventions in areas and communities with the highest burden of these conditions. The study underscores the low healthcare-seeking behaviour among individuals with hypertension and diabetes in Karnataka, indicating a need for interventions aimed at improving access to healthcare services and raising awareness of these conditions. Policymakers can use this information to develop and implement targeted awareness campaigns and health education programs to improve healthcare-seeking behaviour and increase treatment uptake.

Overall, the findings of this study provide a valuable resource for policymakers in the health sector to design effective policies and programs to reduce the burden of hypertension and diabetes in Karnataka and ultimately improve the health outcomes of the population. The findings of the study are based on the NFHS-5 data, and even though the NFHS-5 survey employs a multi-stage stratified sampling design to improve representativeness, it is possible that certain population groups may be underrepresented or omitted. This can limit the generalizability of the findings to the entire population of Karnataka, India. The study focuses on the population of Karnataka, India. Therefore, the findings may not be directly applicable to other regions or countries with different sociodemographic characteristics, healthcare systems, or lifestyle factors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01241-0.

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Data Availability Data is publically available on DHS website.

Declarations

Conflict of Interest I declare no conflicts of interest related to this research.

Ethical Clearance Statement Data Source: The data utilized in this study is publicly accessible from the District Level Household and Family Survey, India website.

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ORIGINAL ARTICLE

Nutrition Health interventions and Quality of life following Mini Gastric Bypass surgery- a randomized control trial

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Abstract

Background Laparoscopic mini gastric bypass (LMGB) bariatric surgery, characterized by a single ante colic gastro-jejunostomy (GJ) anastomosis procedure, requires specialized post-surgery care.

Objective The aim of the study is to evaluate the impact of personalized counseling using nutrition health education (NHE) material, developed based on the Bariatric Analysis and Reporting Outcome System (BAROS), as compared to standard hospital care, on post-operative patient's quality of life (QoL).

Methodology A prospective randomized control trial was conducted, wherein 120 patients registered for the LMGB surgery were enrolled and followed up over 3 months after surgery. Based on alternate allocation randomization, odd number patients were allocated to the experimental group (Group E $n = 60$) receiving the personalized NHE material, and even number patients were allocated to the control group (Group C $n = 60$) receiving the standard hospital care. QoL and health outcomes data were analyzed pre- and post-surgery using SPSS-23 software.

Results Post-operative findings indicated better weight loss and improved quality of life scores in group E patients. The mean %excess weight loss at 3 months post-surgery of group E patients was 18% more compared to group C patients. Notably, the BAROS scores for group E patients were within the good category (4.80 ± 1.63) versus group C patients' scores being in the fair category (3.00 ± 1.64), representing a statistically significant difference ($p < 0.001$).

Conclusion Based on the type of bariatric surgery (be it restrictive, mal-absorptive, or combined procedure), a focused bariatric surgery-specific nutrition and education leads to better weight loss, resolution of comorbidity, improved quality of life, prevention of weight regains, and minimal post-surgical complications as compared to general counseling.

Keywords Laparoscopic mini gastric bypass · Bariatric surgery · Nutrition health education · Bariatric nutrition · Quality of life · BAROS

Introduction

Overweight and obesity, characterized by abnormal or excessive fat accumulation resulting from a positive energy balance, is emerging as a colossal health epidemic worldwide. As per the World Health Organization (WHO) in 2016, non-communicable diseases (NCDs) accounted for approximately 63% of deaths, with obesity being a significant contributor. Furthermore, obesity has an adverse impact on the quality of life, indicating psychological dysfunction and poor social interaction [1]. In response to the escalating burden of NCDs, The National Program for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS) (Ministry of Health & Family Welfare, Government of India) was launched by the government of India.

Pertaining to the strategies to fight overweight and obesity, people try conventional weight-loss therapies and fail to achieve the desired results. Recognizing this, the National Institute of Health has identified bariatric surgery as a last resort for effective long-term treatment of severe obesity [2]. Clinically induced long-term weight loss and reduction in obesity-related comorbidities are the primary targeted outcomes of a "Bariatric Surgery" [3].

Robert Rutledge introduced the mini gastric bypass surgery and the laparoscopic mini gastric bypass (LMGB) surgery with latero-lateral anastomosis. This technique being

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straightforward and more refined by having one anastomosis, it offers multiple benefits like easy revision and reversal if needed, along with considerable weight loss and remission of comorbidities [4]. Weight loss in LMGB surgery is achieved by both restriction and malabsorption of food due to the reduced stomach capacity along with reduced calorie absorption in the intestines.

Few studies have shown the pivotal relationship between weight loss surgery and the improvement in patient's quality of life (QoL). A study by Rutledge et al. (2005) and Carbajo et al. (2005) noted enhancements in QoL post-surgery, but comprehensive assessments were lacking [5, 6]. A long-term study by Bruzzi et al. (2015) further affirmed the QoL improvements, illustrating elevated scores in social, psychological, and physical domains [7]. Notably, QoL improvements were also observed in patients with improved obstructive sleep apnea syndrome (OSAS) after the LMGB surgery [8]. Several studies have reported a positive outcome of LMGB surgery [6, 9, 10]. However, the data on the quality of life by the Bariatric Analysis and Reporting Outcome System (BAROS) post-LMGB surgery in India is limited. The technical success of bariatric surgery has overshadowed the subsequent impact on patient's QoL. In order to address the gaps, the present study reviews the pivotal role of QoL in the context of LMGB surgery. To further delve deeper into the relationship between post-surgery interventions and its implications on patients' overall well-being, the current study aimed to evaluate weight loss and QoL of individuals who underwent LMGB surgery. The assessment was conducted in the context of receiving personalized nutrition health education (NHE) material in contrast to standard hospital care.

Methodology

Study design

A prospective randomized controlled trial was conducted to assess the impact of personalized NHE material on QoL and weight loss in patients who underwent LMGB surgery.

Location

The study was conducted at the Asian Bariatrics Hospital located in Ahmedabad, Gujarat, India.

Sample size and sampling method

In total, 120 patients who were admitted for LMGB surgery were enrolled in the study. For sample selection, an alternate allocation approach of randomization was adopted. Based on the registration, patients were assigned unique identification numbers. Odd number of patients were assigned to the experimental group (group E), and an even number of patients were assigned to the control group (group C). Each group contained 60 patients. This method was chosen to distribute patients equally in each group.

Data collection and data analysis

Baseline data, including demographic information, body composition (Inbody 370 Body Composition Analyzer), medical history, and preoperative QoL score using the Bariatric Analysis and Reporting Outcome System (BAROS), was collected before surgery. Post LMGB surgery, similar data was collected and reported comorbidities and minor complications, if any. Weight loss, %excess weight loss, medical condition, and QoL data were tracked using the BAROS tool. Follow-up was taken after 3 months of the surgery. Data was analyzed using SPSS-23 software.

Intervention

Group E patients received personalized NHE material, and group C patients received general counseling as per standard hospital care practice. The NHE material, prepared using the American Society for Metabolic and Bariatric Surgery (ASMBS) 2016 guidelines, covered information about the after-LMGB surgery care in terms of weight loss/maintenance, nutrition therapy, potential complications post-surgery and their appropriate mitigation strategies through appropriate diet, customization of meal plans with food substitution lists, explanation of issues like weight loss reaching its plateau, post-LMGB surgery supplement use, and a regular follow-up regime. The NHE material was delivered to group E patients using a variety of channels encompassing e-mail and WhatsApp, along with traditional offline platforms, including in-person counseling and PowerPoint presentations. The counseling was delivered 24×7 through WhatsApp if needed by group E patients for 3 months.

Results

Pre-operative characteristics

A total of 120 patients were enrolled in the study, comprising 50 males and 70 females. The age range of the cohort ranged from 19 to 77 years, representing a diverse age group. Notably, 76% of the patients were from Gujarat, with an additional 9.2% from Maharashtra.

According to the World Health Organization's 2016 classifications, 33.3% of group E patients and 36.7% of group C patients suffered from Grade III obesity. The body fat content was 3-fold higher, and the body fat percentage was 30 times higher in both groups compared to the normal range (Inbody370 Body Composition Analyzer). The pre-operative health statistics also reported significant comorbidities in both groups: 73% in group E patients and 75% in group C patients with conditions such as diabetes mellitus, hypertension, dyslipidemia, thyroid, and cardiac issues. Anthropometrically, the group E patients had a mean body weight of 113.38 ± 21.92 kg, in contrast to group C patients' mean weight of 125.8 ± 27.20 kg. The baseline data revealed significant differences between both groups. The mean BMI was elevated in both groups, with group E patients at 42.85 ± 6.15 kg/m^2 and group C patients at 46.98 ± 10.18 kg/m^2 (Table 1).

Weight loss post surgery

Body weight reduction

A very high significant ($p < 0.001$) impact on the body weight loss was observed in group E patients as compared to group C patients (24 kg vs. 17 kg, respectively) 3 months post LMGB surgery. Weight loss in group E patients exceeded group C patients by approximately 7 kg, highlighting the positive impact of personalized NHE material. Consequently, the reduction in excess body weight (26.26 ± 14.74 kg) and body mass index (33.58 ± 5.66 kg/m^2) of group E patients was reduced significantly ($p < 0.001$ for each). Remarkably, group E patients exhibited less difference between ideal body weight and their weight at 3 months post-surgery than group C patients (26.24 kg vs. 44.7 kg) (Table 2).

Table 1 Demographic details of patients who underwent laparoscopic mini gastric bypass surgery

	Group E (mean ± SD)	Group C (mean ± SD)
Age (yrs)	44.9 ± 11.7 yrs	48.4 ± 12.7 yrs
Sex ratio (M/F)	21/39	29/31
Weight (kg)	113.38 ± 21.92 kg	125.8 ± 27.20 kg
Height (cm)	162.52 ± 10.06 cm	163.60 ± 11.86 cm
Body mass index (kg/m^2)	42.85 ± 6.15 kg/m^2	46.98 ± 10.18 kg/m^2
Body fat mass (kg)	56.74 ± 12.42	60.97 ± 18.47 (36–113)
% body fat	50.65 ± 5.80	50.32 ± 8.14
Waist/hip	0.97 ± 0.16	0.96 ± 0.18

Table 2 Post-operative anthropometric data comparison between group E patients and group C patients

Parameter	Group E Mean ± SD	Group C Mean ± SD	*T* value
Weight (kg)	88.74 ± 17.34	108.38 ± 27.32	4.61***
Excess body weight (kg)	26.26 ± 14.74	44.52 ± 25.24	4.75***
Body mass index (kg/m^2)	33.58 ± 5.66	40.42 ± 9.71	4.62***

$p < 0.05$*; $p < 0.01$**; $p < 0.001$***

Reduction in obesity grades and body mass index

In terms of the obesity grades, a significant difference ($p < 0.004$) was noted in group E patients as compared to group C patients. Over 3 months post-surgery, the reduction in Grade III obesity was notably higher in group E patients than group C patients, about 25.4% and 15.5%, respectively. A noteworthy outcome emerged from the reduction of BMI, where 1.8% of group E patients attained normal BMI values. Additionally, the gender-based analysis revealed that grade III obesity was notably elevated in group C females than the group E females (13.1% vs. 5.2%) (Table 3).

Percentage of Excess Weight Loss (%EWL)

The excess weight loss percentage in group E patients was significantly higher (50.82 ± 19.09) than in group C patients (33.23 ± 20.09) (Table 4). An 18% higher %EWL was observed in group E patients, satisfying the enhanced effectiveness of the personalized NHE material. Notably, in group E patients, a significant portion of patients achieved intermediate %EWL; 44.1% and 37.3% of patients achieved 25–49% and 50–74% of %EWL, respectively. Conversely, higher portions of patients, approximately 80% of patients from group C, achieved a lower %EWL ranging from 0 to 49%. These distribution patterns suggest more promising %EWL outcomes amongst group E patients compared to group C patients. A higher %EWL between 75 and 100% was observed in 13.6% and 5.5% of patients in groups E and C, respectively.

Table 3 Post-operative obesity grade distribution comparison between group E patients and group C patients

Obesity grades	Group E (%)	Group C (%)	Pearson chi-square value
Normal	1.8	0	15.1**
Overweight	14	5.3	
Grade I	15.8	11.4	
Grade II	12.3	9.6	
Grade III	7.9	21.9	

$p < 0.05$*; $p < 0.01$**; $p < 0.001$***

Table 4 Post-operative % excess weight loss comparison between group E patients and group C patients

Parameter	Group E Mean ± SD	Group C Mean ± SD	*T* value
%EWL	50.82 ± 19.09	33.23 ± 20.09	4.79***

$p < 0.05$*; $p < 0.01$**; $p < 0.001$***

Comorbidity and minor complications resolution

Following a 3-month interval post-LMGB surgery, both patient groups exhibited notable reductions in comorbidities. Group E patients demonstrated a 66% decline compared to group C patient's 62% reduction. This trend was evident across several comorbidities, including diabetes mellitus, hypertension, dyslipidemia, thyroid, cardiac, and pulmonary issues. Considering the outcomes, group E saw 71% of improvement in patients' comorbid conditions, with an additional 22% experiencing resolution in their comorbid condition. In contrast, group C patients marked a 67% in improvement rate and 20% in resolution rate of the comorbid conditions (Fig. 1). The results indicate the efficacy of the LMGB surgery, particularly when personalized post-operative care is complemented. Enhancement in health conditions was measured based on the improvement in the mean values of all biochemical parameters like cholesterol, triglycerides, low-density lipoprotein (LDL), HbA1c, serum glutamic pyruvic transaminase (SGPT), creatinine, and total proteins. However, there was no change in the hemoglobin and RBC count post-surgery. Interestingly, both the group patients saw an improvement in their gastrointestinal conditions like acidity, gas, diarrhea, constipation, and vomiting.

Quality of life (QoL) as per the Bariatric Analysis and Reporting Outcome System (BAROS)

The Bariatric Analysis and Reporting Outcome System's (BAROS) mean score was 4.8 ± 1.63 (good) for group E patients and 3.0 ± 1.64 (fair) for group C patients. The overall BAROS score outcome was very statistically significant ($p < 0.001$) in group E patients compared to group C patients.

In evaluating QoL, parameters encompassing daily activities, self-esteem, physical activity, social engagement, work activity, and sexual activity are considered. Post LMGB surgery, along with the receiver of the NHE material, the efficiency of doing daily activities notably improved by 22.8% in group E patients versus 10.5% improvement was seen in group C patients after receival of standard hospital care. Similar trends were seen in enhancement of social activities (27.2% for group E versus 10.5% for group C) and in eagerness to perform any physical activity (23.7% for group E versus 14.9% for group C) (Table 5). Furthermore, a statistically significant upliftment in the self-esteem metric was recorded in group E patients, with nearly a third of patients (29.8%) expressing high satisfaction post-LMGB surgery.

Post-operative dietary practices

Group C patient's standard care: On the second postoperative day of surgery, group C patients received a clear liquid diet, and continued for 3 days if devoid of any complications. Subsequently, patients transitioned to a full liquid diet for an additional 3 days, followed by a pureed diet for the next 5 days. A gradual shift to a soft diet was planned for the next 15 days. At the time of discharge, patients were handed a booklet explaining all the dietary phases and a prescription for essential multivitamins and protein supplementation to support their recovery.

Group E patient's personalized NHE material and dietary advice: Similar to group C, patients in group E also adhered to a structured dietary routine received from the hospital. Beyond this, they also received a personalized NHE material kit specific to LMGB surgery. This education material encompassed diverse topics to enhance post-operative care and well-being. It covered details about LMGB surgery, medical nutrition therapy guidelines, post-LMGB surgery possible complications, and their resolution using adequate diet and nutrients. It also included topics on dietary diversity based on traditional meal choices, food exchange lists, and early and late gastrointestinal complications arising

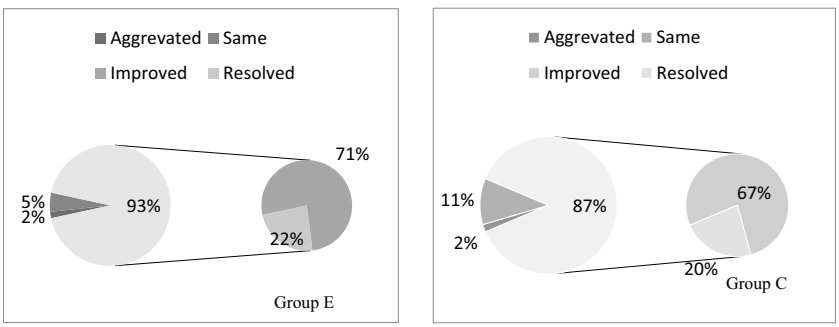

Fig. 1 Post-operative comorbidity resolution in group E patients and group C patients

Table 5 The Bariatric and Reporting Outcome System (BAROS) outcome post-surgery

BAROS outcome categories	Group E (%)	Group C (%)	Pearson chi-square value
Failure	0.9	4.4	23.8**
Fair	10.5	26.3	
Good	23.7	13.2	
Very good	14.9	2.6	
Excellent	1.8	1.8	

The outcome categories are measured based on the following scores: Failure 1 point or less, fair >1 to 3 points, good >3 to 5 points, very good >5 to 7 points, and excellent >7 to 9 points

$p < 0.001$***

post-LMGB surgery. Furthermore, information on weight loss plateau and the use of supplements was also addressed in the education material. Notably, patients in group E benefited from the continuous support through 24 × 7 WhatsApp-based counseling, if needed.

Regarding dietary diversity, greater quantities of legumes (25.3% vs. 8.7%), dairy products (10.7% vs. 9.7%), and vegetables (9.7% vs. 3.9%) were consumed by patients in group E compared to those in group C.

Discussion

The present study explored the multifaceted impact of personalized NHE material on the QoL of individuals undergoing LMGB surgery. Our findings underscore a substantial connection between post-operative weight loss and improved QoL, aligning with a growing body of research that recognizes the profound influence of LMGB surgery on patients' overall well-being. Guided by the Bariatric Analysis and Reporting Outcome System (BAROS), the present study evaluated the outcomes encompassing %excess weight loss, comorbidity/medical condition resolution, and QoL post-surgery along with recording the changes in the dietary diversity based on the personalized NHE material. The following discussion section delves into the comprehensive assessment of postoperative outcomes and addresses the complexities and challenges introduced by post-surgical factors.

To date, only one randomized control trial is available between Roux-en-Y Gastric Bypass (RYGB) and Mini Gastric Bypass (MGB), revealing MGB's favorable performance [11]. Following surgery, the outcome assessment should not only be based on weight loss but should include parameters like improvement in medical conditions and change in the patient's QoL [12]. Additionally, bariatric nutrition is also one of the evolving domains to manage the nutrition requirements of post-surgery patients [13].

The study observed a significant pre-operative mean weight of 113.38 ± 21.92 kg and mean BMI of 42.85 ±6.15 kg/m^2 in group E patients, showcasing a noteworthy mean %EWL of 51% post-3 months of surgery compared to 33% in group C. Regarding weight post-surgery, group C's mean weight was 19% higher than group E with remarkably significant values. These findings were along the lines of past studies. For instance, a study reported 30 kg weight loss in 3 months, resulting in mean 75% EWL after 1 year [5]. Significantly, current findings align with literature illustrating 75% of excess weight loss after 1 year [14]. Additionally, the first study from India on mini gastric bypass surgery reported 63% excess weight loss at 1 year of LMGB surgery, which was higher than the %EWL after sleeve gastrectomy surgery [15]. In a series of 1163 mini gastric bypass surgery patients, results reported 72.9% EWL at the end of 5 years of follow-up [11].

Notably, the current study's NHE intervention exhibited favorable outcomes enhancing % excessive weight loss, resolution of comorbidities, reduction of minor comorbidities, and QoL in group E patients. Though after surgery weight loss tends to happen to a certain extent, it is a challenging job for a nutritionist to deliver proper knowledge regarding dietary practices and a regular follow-up regime to maintain the excess weight loss and avoid weight regain. Chances of weight regain are high if patients do not follow the regime properly.

Furthermore, pre-LMGB surgery, comorbidity prevalence was reported to be more than 70% in both the groups. Following the surgical procedures, rates of resolution and improvement were higher in group E patients (93% to 22%) compared to group C patients (87% to 20%). Our outcomes align with the results of Wang et al. (2005), Rutledge et al. (2005), and Noun et al. (2012), further supporting the positive impact of LMGB on comorbidities [9, 10, 13]. Kular et al. (2014) reported 92% remission rates of diabetes in LMGB patients, which was highly significant ($p < 0.05$) compared to laparoscopic sleeve gastrectomy (LSG) patients [16]. In particular, group E patients experienced improvements in the biochemical results, particularly in cholesterol, LDL, SGPT, and HbA1c levels. This trend resonates with Milone et al.'s (2015) findings of improved cholesterol and triglyceride levels in mini gastric bypass surgery patients as compared to LSG patients [17]. Additionally, it is crucial to note that concerns related to bile reflux are common post-LMGB surgery but cannot be solely attributed to anatomical alterations. Factors such as smoking, use of NSAIDs, late night eating, consumption of fried foods, and alcohol intake can be one of many reasons for bile reflux complications post-surgery. This observation is in accordance with the insights of Rutledge, Kular et al. (2016), highlighting the multifaceted nature of post-surgical complications related to bile reflux [18]. Consequently, LMGB is claimed to be the

preferred surgical option as its results have proven less incidence of complications, which is acceptable after comparing it with other surgeries for morbid obesity [19].

Also, it is important to note that the length of the bypassed limb is a variable factor in this procedure, and which significantly influences factors like weight loss, comorbidity resolution, and nutrition absorption. In the present study, 150–200 cm of the limb was bypassed, which was also similar to the study by Piazza et al. (2011). Surgeons generally use a fixed limb length of 200 cm [20].

Moreover, patients' QoL is impacted during the management of obesity. In the current study, enhancements in QoL were observed in group E patients in comparison to group C patients, particularly concerning daily activities, social engagement, physical activity, and self-esteem status following a 3-month period post-LMGB surgery. The BAROS scale was used to analyze the QOL of the patient's post 3 months of surgery. Within the experimental group, noteworthy proportions of patients (23.7% and 14.9%) achieved good and very good BAROS scores for LMGB outcomes, respectively, in contrast to the control group, where such scores were less prevalent. The overall outcome of BAROS will tend to be in good categories if the dietitian is well-trained to carry out proper follow-up sessions. The rarity of studies regarding QOL based on BAROS in the current study underscores the novelty of our Indian investigation in this context.

It is to be noted that the findings of the present study revealed significant differences in QoL outcomes between group E patients and group C patients. It is important to consider the role of personalized NHE material education, which was exclusively given to group E patients. The continuous 24 × 7 counseling offered through WhatsApp exhibited considerable improvements in QoL. This contrasted with group C patients, who only received standard hospital care without benefiting the constant counseling. Offering personalized counseling on demand, 24 × 7 played a pivotal role in addressing concerns, clarifying doubts, and providing support.

Additionally, research based on dietary aspects following LMGB surgery has not yet been reported. Information regarding 4 phases of diet post-surgery is available in which ideally, the first phase starts with a clear liquid diet (1–2 days) followed by a full liquid diet (10–14 days), pureed diet (10–14 days), and soft diet (> 14 days) and then continued with regular diet [2]. The current study's personalized NHE material focused specifically on protein and multivitamin supplementation, given the decreased dietary intake, and compromised nutrient absorption due to bypassed intestines. Nutrition knowledge reinforcement after surgery is very important to avoid any nutritional complications. A knowledgeable bariatric nutritionist can help in resolving any post-surgical complications related to health and nutrition. Though vitamin B12 deficiency is common after bariatric surgery, our patients reported an acceptable range which was due to good compliance with supplements. The mean iron values were less than the pre values, yet in the normal range. Iron deficiency anemia was the common nutrition deficiency seen in 5% of patients after mini gastric bypass surgery after 1 year of LMGB surgery [13].

LMGB surgery is believed to be more malabsorptive than RYGB surgery, reporting malnutrition in 31/2410 patients of one study and 4/1000 patients of another study [9, 13, 21]. The general guidelines which recommend starting multivitamin chewable tablets after the 3rd day of surgery, followed by protein supplements from the 7th day of the surgery, were followed by the patients of the present study. Bariatric surgery requires super specialty care. In the same way, super special nutritionists/dietitians are required. Best counseling methods help in treating and caring for the patient post-surgery. It is a tool, not a cure, hence patient participation to resolve the morbid condition and their continuous follow-up and interaction with the dietitian is necessary. Therefore, the current study's NHE material focused on motivational counseling (psychological and social). This approach presented notable efficacy in attaining a significant %EWL and positive overall well-being in group E patients. The counseling also encouraged and motivated patients to follow a post-surgery nutrition regime along with taking long-term follow-ups. Thus, it is important that obesity management guidelines [22, 23] are drafted keeping in mind all surgical procedures of bariatric surgery for providing a better quality of life to the patients.

Limitations

One potential limitation of the current study is the relatively short follow-up period of only 3 months post-surgery. While this timeframe may be sufficient to evaluate the short-term outcomes of the intervention, a longer-term follow-up would be needed to assess the sustainability of the weight loss and other outcomes.

Another potential limitation is the lack of blinding of the study participants and the researchers to the intervention, which could introduce bias into the results. Additionally, the study only included patients from a single center, which may limit the generalizability of the findings to other settings. In terms of the results, the study suggests that personalized nutrition health education focused on LMGB leads to better weight loss, comorbidity resolution, and quality of life scores as compared to general counseling.

Conclusion

In conclusion, the present study reports the effectiveness of personalized nutrition health education (NHE) intervention post laparoscopic mini gastric bypass (LMGB) surgery. The findings highlighted the potential benefits of NHE in facilitating weight loss, improving QoL, and resolving comorbidities among patients. The tailored approach utilized in NHE not only addressed dietary aspects but also encompassed psychological and social aspects, contributing to more favorable outcomes. The comparison between group E patients and group C patients revealed better results in terms of %EWL, comorbidity resolution, and QoL for group E patients, justifying the pivotal role of personalized NHE material in managing obesity. Remarkably, both groups suffered from low rates of minor complications post-LMGB surgery. The nutritional deficiencies were also low pertaining to a regular adherence to the prescribed dietary regime and supplementations, as needed.

Thousands of LMGB procedures have been performed, but one with a nutrition interventional plan in Indian settings is hardly available. Specific counselling and patient education programs should be recommended after the bariatric surgeries to bring about long-lasting positive outcomes. Considering our research as a pilot study, further studies can be carried out.

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Declarations

Ethical clearance The study commenced following clearance from the department research committee and institutional ethical clearance from the academic institute (The Department of Foods and Nutrition) and place of the study (Asian Bariatrics, Ahmedabad), with written consent obtained from all patients.

Conflict of interest The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Usefulness of the Hypoglycemia awareness questionnaire in characterizing the Hypoglycemia events, proactive behaviors, and healthcare services used in patients with type 2 diabetes treated with insulin

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Abstract

Objective Hypoglycemia is a serious complication of type 2 diabetes (DM2), but its severity and incidence and the healthcare services used are usually underreported in clinical practice. We aimed to describe the severity and frequency of hypoglycemia episodes, the proactive behaviors adopted, and healthcare services used by administering the Hypoglycemia Awareness Questionnaire (HypoA-Q).

Methods In this cross-sectional, multicenter, validation study, demographic data and clinical variables were retrieved from electronic records, and the HypoA-Q questionnaire was filled out. Questions 1 and 2 assessed the frequency of hypoglycemia events and the healthcare services used. Questions 4c, 4d, 9, 16b, and 16c evaluated the proactive behaviors of the patient and/or caregiver during the occurrence of hypoglycemic events.

Results In total, 502 DM2 patients treated with insulin and with a history of hypoglycemia were included in this study [54.6% women, median age: 65 years (interquartile range: 58–73); 55.4% receiving the basal-bolus regimen]. 281 (56%) patients reported at least one hypoglycemic event in the last year; 25% reported at least one severe hypoglycemia event; and 8.7% required hospitalization. Only 52% of patients monitored their capillary blood glucose levels to assess for hypoglycemia.

Conclusion The use of HypoA-Q questionnaire in usual clinical practice could facilitate the characterization of hypoglycemia events and the identification of at-risk populations by healthcare professionals and payers.

Keywords Hypoglycemia · Type 2 diabetes mellitus · Hypo A-Q · Insulin

Introduction

Severe hypoglycemia is a serious insulin therapy complication associated with adverse cardiovascular outcomes and increased healthcare costs [1, 2]. Additionally, the quality of life decreases with increase in the severity of hypoglycemic events [3, 4]. Approximately 25% of patients with type 2 diabetes (DM2) treated with insulin for more than 5 years experienced severe hypoglycemia, which is comparable to the incidence of severe hypoglycemia among adults with type 1 diabetes (DM1) diagnosed within 5 years [5].

Hypoglycemia unawareness is associated with a 6–9-fold increase in the risk of severe hypoglycemia [6]. The proportion of individuals with recurrent severe hypoglycemia, defined as occurrence of more than one hypoglycemic event, has increased to 6.2% in those with sufficient knowledge and

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nearly 35% in those with hypoglycemia unawareness [7]. Inadvertent hypoglycemia affects 20%–25% of patients with DM1 [6, 8], but its prevalence in patients with DM2 ranges from 10% [9] to 50% [10] due to the healthcare personnel's lack of knowledge and the absence of validated instruments for its diagnosis in this population. Hypoglycemia unawareness is diagnosed clinically and requires the use of validated scales such as Gold Score [11], Clarke Hypoglycemia Index [12], Pedersen-Bjergaard Questionnaire [9], and Hypoglycemia Awareness Questionnaire (HypoA-Q) [13]. The HypoA-Q evaluates three subscales that reflect altered consciousness and the level and frequency of symptoms; unlike the other instruments, it also provides information on the severity and frequency of nocturnal episodes of hypoglycemia, the proactive behaviors adopted, and healthcare services used for its treatment [14].

In clinical practice, hypoglycemic events are not usually the recognized and reported, limiting the implementation of measures by the individual and the healthcare professionals and payer to reduce the frequency of new events [5]. Colombia is one of the countries in Latin America with the highest prevalence of DM2 [15]. According to the 2019 data, 50% of patients with DM2 treated with insulin have reported at least one hypoglycemic event in the last 6 months [10]. However, information about the severity and frequency of hypoglycemia events, the behaviors that favor the reduction of severe hypoglycemia adopted by the patient and/or caregiver, and the healthcare services used for its treatment are limited.

This study aimed to describe the severity and frequency of hypoglycemic episodes, the proactive behaviors adopted and the health services used by patients with DM2 treated with insulin and history of hypoglycemia evaluated through the HypoA-Q questionnaire.

Methods

A cross-sectional, multicenter, validation study was conducted using the HypoA-Q questionnaire in patients with DM2 under follow-up in three hospitals (Hospital de San José, Hospital Universitario San Ignacio, and Hospital Universitario Clínica San Rafael) and in primary care centre (Cafam IPS) in the city of Bogotá, Colombia, from March 2018 to March 2022. Patients diagnosed with DM2, aged >18 years, treated with basal insulin or basal bolus regimens following the usual clinical practice, and with a history of hypoglycemia were included in this study. Patients diagnosed or suspected with DM1 or with gestational, monogenic, or drug-induced diabetes, endocrinopathy, or diseases of the exocrine pancreas were excluded. Patients who were pregnant or with a history of active neoplasms were also excluded. This study was considered risk free, and all participants signed an informed consent form. The data were collected in each center by healthcare professionals who were duly trained to gather this information and were combined in a single database for the analysis. The study was approved by the Ethics Committee of each participating center.

All participants were followed up by a comprehensive diabetes management group and underwent nutritional assessment, education by a diabetes nurse educator, and quarterly follow-up by a first-level doctor or specialist. The demographic data and clinical and laboratory variables (hemoglobin A1c measured by high-performance liquid chromatography, urine albumin-creatinine ratio, and creatinine level) were retrieved from the electronic records systematically compiled in standardized formats. The participants filled out the HypoA-Q questionnaire, which was used to evaluate several aspects; the scores for each subscale were obtained instead of providing a single composite score, thus enabling users to choose the aspects or groups of aspects relevant to their objectives. The questionnaire also provides an algorithm to score the subscales. Items 1 and 2 were analyzed to assess the frequency of hypoglycemia events and healthcare services used in the last 6 months, respectively. Items 4c, 4d, 9, 16b, and 16c were scored on a Likert-type scale with 5 or 6 response options. Questions 4c and 4d evaluated the frequency of interventions for hypoglycemic events performed by the patient and the caregiver during the day, such as monitoring and correction of oral glucose levels and use of glucagon, whereas items 16b and 16c evaluated these behaviors during the night. Item 9 assessed the capillary blood glucose readings obtained during hypoglycemic events. The Colombian Spanish version of the questionnaire was used in this study [13].

The severity of hypoglycemic events was classified according to the International Hypoglycaemia Study Group criteria adopted by the American Diabetes Association (ADA) 2022 [16, 17]. Severe hypoglycemia was defined as any hypoglycemic event with loss of consciousness or requiring third-party assistance [16]. Level 1 hypoglycemia is any event with a capillary blood glucose reading lower than 70 mg/dL, whereas level 2 event or clinically significant hypoglycemia is defined as a capillary blood glucose reading of less than 54 mg/dL [17]. With regard to the healthcare services utilized, emergency care was defined as a care delivered in less than 24 hours, while hospitalization was defined as the admission of patients to the hospital for more than 24 hours. Inadvertent hypoglycemia was assessed using a 5-item subscale, with scores ranging from 0 to 20. A higher score was associated with the changes in the awareness of hypoglycemia symptoms [13]. The use of more than 5 medications per day was defined as polypharmacy.

Quantitative variables were analyzed using the measures of central tendency and dispersion (means and standard deviations or medians and interquartile ranges (IQR), depending on the data distribution); meanwhile, qualitative

Table 1 Characteristics of the study population according history of hypoglycemia

Characteristics of the study population	History of Hypoglycemia event in the last year				Total (n = 502)	
	Yes (n = 281, 56%)		No (n = 221, 44%)			
Women, n (%)	151	(53.7)	123	(55.6)	274	(54.6)
Age in years, median (IQR)	66	(59–74)	64	(56–71)	65	(58–73)
Diabetes duration in years, median (IQR)	16	(10–24)	15	(8–21)	15	(9–22)
Charlson Comorbidity Index, medicine (IQR)	4	(2-6)	3	(2-5)	4	(2-6)
Polypharmacy	197	(55.2)	160	(44.8)	357	(71.1)
Body mass index (kg/m^2), median (IQR) (n = 490) *	26.6	(23.2–30.4)	26.9	(24.2–30.4)	26.8	(23.8–30.4)
Normal weight (BMI ≤24.9 kg/m^2) (%)	99	(35.7)	60	(28.2)	159	(32.4)
Overweight (BMI ≥25–29.9 kg/m^2) (%)	103	(37.2)	95	(44.6)	198	(40.4)
Obesity Grade I (BMI ≥30–34.9 kg/m^2) (%)	54	(19.5)	47	(22.1)	101	(20.6)
Obesity Grade II (BMI ≥35–39.9 kg/m^2) (%)	14	(5.1)	9	(4.2)	23	(4.7)
Obesity Grade III (BMI ≥40 kg/m^2) (%)	7	(2.5)	2	(0.9)	9	(1.8)
Microvascular complications						
Nephropathy	129	(64.5)	71	(35.5)	200	(39.4)
Retinopathy	117	(62.9)	69	(37.1)	186	(37)
Neuropathy	67	(60.9)	43	(39.1)	110	(21.9)
Diabetic foot	44	(63.8)	25	(36.2)	69	(13.8)
Macrovascular complications						
Peripheral arterial disease	34	(38.6)	128	(30.9)	162	(32.3)
Coronary heart disease	25	(28.4)	107	(25.8)	132	(26.3)
Heart failure	6	(6.8)	24	(5.8)	30	(6)
Cerebrovascular disease	4	(4.5)	26	(6.3)	30	(6)
HbA1c %, median (IQR) (n = 357) *	8	(7-10)	8	(7-9)	8	(7-9)
Glomerular filtration rate (ml/min/1.73 m^2) (n = 376) *						
Stage 1 (GFR >90)	57	(44.5)	71	(55.5)	128	(25.5)
Stage 2 (GFR 60–90)	66	(59.5)	45	(40.5)	111	(22.1)
Stage 3a (GFR 60–45)	29	(60.4)	19	(39.6)	48	(9.6)
Stage 3b (GFR 45–30)	20	(52.6)	18	(47.4)	38	(7.6)
Stage 4 (GFR 30–15)	25	(80.6)	6	(19.4)	31	(6.2)
Stage 5 (GFR <15)	14	(70)	6	(30)	20	(3.9)
Insulin regimen						
Basal-Bolus	178	(64)	100	(36)	278	(55.4)
Basal-plus	27	(48.2)	29	(51.8)	56	(11.2)
Basal	76	(45.2)	92	(54.8)	168	(33.4)
Basal insulin (n = 476) *						
Neutral protamine Hagedorn (NPH) insulin	2	(66.7)	1	(33.3)	3	(0.6)
Insulin glargine	178	(55.3)	144	(44.7)	322	(64.1)
Insulin detemir	14	(58.3)	10	(41.7)	24	(4.8)
Insulin degludec	70	(55.1)	57	(44.9)	127	(25.3)
Basal insulin dose (n = 465) *						
<0.5 units/Kg/day	206	(56)	162	(44)	368	(80.3)
≥0.5 units/Kg/day	52	(53.6)	45	(46.4)	97	(19.7)
Hypoglycemic agent						
Metformin	107	(48.4)	114	(51.6)	221	(44)
Dipeptidyl peptidase-4 inhibitor	51	(48.6)	54	(51.4)	105	(20.9)
Sodium-glucose cotransporters inhibitors	55	(45.8)	65	(54.2)	120	(23.9)
Glucagon-like peptide-1 receptor agonist	56	(54.4)	47	(45.6)	103	(20.5)
Number of blood glucose readings per day (n = 461) *						
1 blood glucose reading per day, n (%)	48	(52.8)	43	(47.2)	91	(18.1)

Table 1 (continued)

Characteristics of the study population	History of Hypoglycemia event in the last year				Total (n = 502)	
	Yes (n = 281, 56%)		No (n = 221, 44%)			
2 blood glucose readings per day, n (%)	69	(51.5)	65	(48.5)	134	(26.7)
3 blood glucose readings per day, n (%)	68	(51.9)	63	(48.1)	131	(26.1)
≥ 4 blood glucose readings per day	82	(78.1)	23	(21.9)	105	(20.9)
Use of real-time blood glucose monitoring	27	(71)	11	(29)	38	(7.6)

*Analyzes of available data are reported

IQR interquartile range, *BMI* body mass index, *GFR* glomerular filtration rate

variables were expressed as absolute and relative frequencies. The tests were performed using the statistical package STATA 16 licensed to the Foundation University of Health Sciences.

Results

A total of 502 participants were included in this study. Table 1 outlines the general characteristics of the population, of which 54.6% were women, with a median age of 65 years (interquartile range [IQR]: 58–73). The mean age at diabetes diagnosis was 47.9 ± 13.3 years, and the median time since diagnosis was 15 years (IQR: 9–22). As for treatment, 55.4% patients were treated with a basal-bolus regimen. In addition to insulin, the most used hypoglycemic agent was metformin (in 44% of patients). Among the microvascular and macrovascular complications, the most frequent was nephropathy (39.4%), followed by retinopathy (37%), peripheral arterial disease (32.3%), coronary heart disease (26.3%), neuropathy (21.9%), diabetic foot (13.8%), and cerebrovascular disease (6%). 56% of the patients had a history of hypoglycemic events in the last year. In this group, polypharmacy, microvascular complications, GFR <30 ml/min/1.73 m2 and the use of the Basal-Bolus insulin regimen were more frequent (Table 1).

In the previous year, the median number of hypoglycemic episodes was 1 (IQR: 0–3). Table 2 outlines the number and frequency of global hypoglycemia events; approximately 41% of the patients presented at least one hypoglycemic event a week prior to their study inclusion, while 23% reported at least one severe hypoglycemia event. As regards the hypoglycemic events reported when awake, 25.8% of the patients reported at least one severe hypoglycemic event in the last 6 months, whereas 34.4% of the patients presented at least one hypoglycemic event when asleep, of whom 36.1% had symptomatic events and 12.2% had severe episodes. In addition, 3.2% of the patients presented complications related to a nocturnal hypoglycemia event, including falls and seizures.

Among the patients who presented with hypoglycemia during the day, 15.5% reported the use of oral glucose load at least once a month, while 3.5% used glucagon to treat hypoglycemia. When the frequency of nocturnal events were evaluated, 12.2% of the patients reported using an oral glucose load during a hypoglycemic event, while 1.6% reported using glucagon to manage a nocturnal hypoglycemic event. Furthermore, 37 patients performed real-time continuous glucose monitoring, of whom 8 reported alerts during the night. The mean capillary blood glucose readings were 3.3 readings per day (IQR: 0–8); thirteen patients did not perform self-monitoring of capillary blood glucose levels, while 52% of the patients performed capillary blood glucose readings when experiencing symptoms of hypoglycemia. The median score of the subscale for inadvertent hypoglycemia was 6 points (IQR: 3–10).

When the healthcare services utilized were evaluated, 1.2% of the patients required help from paramedics, 6.6% visited the emergency room, and 8.7% were hospitalized due to severe hypoglycemia. The analysis of events presented when awake showed that 2.68% and 5.7% of the patients presented at least one hypoglycemic event requiring emergency treatment or hospitalization, respectively.

Discussion

Hypoglycemia is the primary barrier to achieving glycemic control goals in patients with DM2 treated with insulin and impairs their quality of life, thus increasing healthcare costs. Additionally, severe hypoglycemia increases the cardiovascular and all-cause mortality rates [1, 5]. The global prevalence of nocturnal hypoglycemia reached 73%, as reported in observational studies [18]. Despite the numerous potential sources of information on the frequency and severity of global and nocturnal hypoglycemic episodes, the primary source of information is the individual. Unfortunately, people with diabetes do not report episodes of hypoglycemia to their healthcare professionals [5].

More than 70% of the patients with DM2 who filled out the HypoA-Q questionnaire presented a hypoglycemic event in the last 6 months, of whom 25% were classified as having severe episodes. In addition, a third of the events occurred at night. Notwithstanding their access to self-monitoring

Table 2 Description of the events reported and use of healthcare services (questions 3, 4, 15, and 16) and incidence of inadvertent hypoglycemia during sleep [17, 18]

Number of events when awake, *n* (%) *	Once or twice		Three to four times		One to four times a month		Once a week		More than once a week	
Hypoglycemia in the past 6 months (q3)	184	(38.6)	93	(19.5)	51	(10.7)	24	(5.0)	30	(6.3)
Had symptoms and able to treat self (q4a)	156	(32.7)	63	(13.2)	36	(7.5)	22	(4.6)	27	(5.6)
Had symptoms and unable to treat self (q4b)	81	(16.9)	24	(5.0)	13	(2.7)	4	(0.8)	2	(0.4)
Needed someone else to provide sugar (q4c)	74	(15.5)	20	(4.2)	16	(3.3)	4	(0.8)	3	(0.6)
Needed someone else to administer the glucagon injection (q4d)	14	(2.9)	1	(0.2)	2	(0.4)	0		0	
Number of events when asleep, *n* (%) *	Less than once a month		Once to twice a month		Once a week		Twice a week		Most days	
Hypoglycemia in the past 6 months (q15)	87	(18.2)	46	(9.6)	13	(2.7)	15	(3.1)	4	(0.8)
Unable to treat self when woke (q16a)	31	(6.5)	16	(3.4)	1	(0.2)	5	(1)	3	(0.6)
Someone else provided sugar by mouth (q16b)	31	(6.5)	18	(3.7)	3	(0.6)	2	(0.4)	5	(1)
Someone else administered the glucagon injection (q16c)	6	(1.2)	2	(0.4)	0		0		0	
Major problem (q16d)	10	(2.2)	2	(0.4)	0		1	(0.2)	2	(0.4)
Did not waken and only later realized hypoglycemia had occurred (q16e)	29	(6.1)	10	(2.1)	5	(1)	6	(1.2)	2	(0.4)

*Data analyzed from 477 patients. q: Question

capillary blood glucose devices, only half of the patients performed blood glucose readings when the hypoglycemia symptoms occurred. Previously, a survey on the prevalence of hypoglycemia among insulin-treated patients with diabetes (The Colombian International Operations Hypoglycemia Assessment Tool (IO HAT)) reported the data from 451 patients with DM2; survey results demonstrated that 50% of these patients experienced a hypoglycemic event in the last 6 months, of whom 48% had severe episodes [10]. This finding highlighted the importance of using standardized instruments for detecting the population at risk for hypoglycemia.

The proportion of patients with hypoglycemia was relatively high in this study; however, the rates of hypoglycemia are consistently higher in real-world data studies than in controlled clinical trials [5]. Although the aforementioned Colombian study published in 2019 [10] included patients with DM1, the clinical characteristics of its population (age, body mass index, and disease duration) were similar to those of this study; moreover, the proportion of patients with at least one hypoglycemic event was lower, while the proportion of patients who presented with severe hypoglycemia was higher than that in this study (48% vs 25%). The lower incidence of severe hypoglycemia in this study may be related to the limited use of intensive insulin regimens (83.7% vs 49%). Additionally, in this study, approximately 40% of the patients used second-generation insulin analogs.

The analysis of the data on the implementation of behaviors that favor the reduction of hypoglycemia in real life by the patient and/or caregiver showed that only 50% of the patients perform capillary blood glucose readings when experiencing hypoglycemia symptoms, despite having access to a device for this purpose. Similar findings have been reported in previous study; that is, only 24% of the patients with DM2 more frequently performed self-monitoring as a strategy to reduce the risk of hypoglycemia, while 55% of the patients with DM1 less frequently performed self-monitoring [9]. The combination of patient education, pharmaceuticals, and technology significantly decreased the frequency of global and nocturnal hypoglycemia [18]. Therefore, the population that requires this type of intervention must be identified, in addition to optimizing the resources allocated for diabetes management.

Severe hypoglycemic events often result in emergency/ambulance calls and hospitalizations, thus increasing the healthcare burden [19]. The percentage of patients requiring hospitalization for severe hypoglycemia found in this study is similar to that reported in previous studies (7.8% vs 7.5%); however, the proportion of patients seeking medical advice or requiring hospitalization as a result of hypoglycemia during the prospective period of the IO-HAT was lower, which is attributed to the increased patient knowledge on hypoglycemia management after receiving an educational

intervention [10]. These findings suggest the importance of establishing structured education programs for diabetes educators focused on detecting hypoglycemia symptoms and increasing the frequency of self-monitoring capillary glucose readings, which have been a widely applied strategy to reduce hypoglycemic events in patients with DM1 [20]. These strategies reduce the frequency of hypoglycemia and improve the detection and perception of hypoglycemia symptoms, thus reducing the frequency of severe hypoglycemia [20] in addition to improving adherence to self-monitoring through capillary blood glucose readings.

This study has some strengths. A considerable number of patients from several institutions with sociodemographic diversity were identified using an instrument for evaluating different dimensions of hypoglycemia awareness, thus allowing the characterization of its behavior based on real-life data, including information on nocturnal episodes, which is one of the advantages of this instrument; the inclusion of a "real-life" population in the follow-up at different levels of complexity reflects the usual clinical practice and expands the information about the frequency of hypoglycemia and its severity in the population with DM2.

One of the limitations of the study is the lack of follow-up and memory bias in recalling previous episodes of hypoglycemia. Because it is a cross-sectional study, we do not have data on weight change or other long-term factors that may be related to hypoglycemic events. Although this was proposed as a cross-sectional study, the level of hypoglycemia awareness can vary depending on the educational intervention received by the patient; therefore, its evaluation over time enables the collection of data that contribute to the clinical decision making. In relation to memory bias, the information collected was compared with the data obtained through the review of clinical history; moreover, the inclusion of patients treated following a structured diabetes program with a quarterly follow-up increases the patients' awareness of their symptoms and therefore encourages them to report more objective data. Moreover, the results are consistent with those reported in other previous studies.

Conclusion

Hypoglycemia in patients with DM2 treated with insulin is commonly observed in clinical practice. Unlike other instruments used to detect patients with hypoglycemia awareness, the HypoA-Q questionnaire provides additional information on the characteristics of global and nocturnal hypoglycemic episodes, determines their severity and frequency, as well as the use of health services. This information could help healthcare professionals detect patients at a higher risk and implement strategies that can effectively reduce the frequency of hypoglycemic events.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethical clearance This manuscript is related to a protocol titled Correlation between the HypoA-Q instrument and clinically significant hypoglycemia measured by continuous glucose monitoring in patients diagnosed with type 2 diabetes, which has been approved by the Clinical Ethics and Research Committee of the Pontificia Universidad Javeriana FM-CIE-0108-19 and Research Committee of the Faculty of Nursing of the Fundación Universitaria de Ciencias de la Salud - FUCS EPR-DEC-I-0276-17.

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ORIGINAL ARTICLE

Predictors of glycemic and weight responses to exenatide in patients with type 2 diabetes mellitus

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Abstract

Objective This study aimed to identify reliable predictors of haemoglobin A1c (HbA1c) reduction and weight loss within 6 months after treatment with exenatide.

Methods A total of 343 patients with type 2 diabetes mellitus were examined and followed up for 12 months. The study patients were divided into two groups: responders and non-responders, which were defined based on their glycemic control (responders: HbA1c reduction of ≥ 1.0%) and weight response (responders: weight loss of ≥ 3%) within 6 months after exenatide administration. Binary logistic regression analysis was performed to identify the predictors associated with exenatide response, and a receiver operating characteristic (ROC) curve was plotted to assess the predictive ability of the identified factors.

Results Of the 148 patients who met the inclusion criteria, 53 (35.81%) were responders and 95 (64.19%) were non-responders. Binary logistic regression analysis revealed that baseline HbA1c, baseline weight, and duration of diabetes were significant predictors of glycemic and weight responses to exenatide. The area under the curve of the ROC for the predictors of HbA1c and weight responses within 6 months after exenatide initiation was 0.765 (95% confidence interval: 0.686–0.845).

Conclusion Baseline HbA1c, baseline weight, and duration of diabetes may serve as predictors for glycemic and weight responses to exenatide.

Keywords Exenatide · Type 2 diabetes mellitus · Glycemic response · Weight response · Predictor

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterised by hyperglycaemia. Without timely intervention and treatment, it may eventually bring about life-threatening microvascular and macrovascular complications [1]. Maintaining tight glycemic control in patients with T2DM may prevent or reduce the risk of progression to some complications [2]. Until now, people have been trying to determine and maintain the appropriate glucose levels; however, nearly 50% of patients with T2DM still fail to achieve the target haemoglobin A1c (HbA1c) level of below 7%, which is extremely detrimental to the stability of the disease [3]. In addition, some patients may try to lose 5–10% of their body weight to lower their blood glucose levels, which in turn may reduce the risk of cardiovascular diseases (CVDs) to some extent [4]. However, only a few anti-hyperglycemic agents are effective in controlling weight gain [5]. Therefore, finding an appropriate anti-hyperglycemic agent is an urgent concern in clinical practice, which can not only lower HbA1c level, but also reduce weight gain.

At present, glucagon-like peptide-1 receptor agonist (GLP-1 RA) is a novel therapeutic strategy that stimulates insulin production to lower blood glucose level in a glucose-dependent manner [6]. GLP-1 RA can also indirectly play roles in various glucose regulation via the inhibition of glucagon secretion. This increases satiety which in turn

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reduces food intake and slows gastric emptying [7–10]. Clinical studies have shown that HbA1c levels and body weight were significantly reduced in patients who were administered GLP-1 RA, which is undoubtedly a good treatment option for patients with T2DM [11–13]. Yet, owing to individual differences, only 50–70% of patients had distinct therapeutic efficacy when GLP-1 RA was administered [14]. In addition, the high cost of GLP-1 RA remains a significant challenge. Physicians are aware of this issue and are cautious when prescribing GLP-1 RA to their patients. Identifying factors that can predict responses to GLP-1 RA therapy is critical in clinics as it helps physicians personalise and optimise drug utilisation in patients with T2DM with poor glycemic control.

According to the literature and our previous research, the initial high levels of HbA1c is one of the most significant predictors of the glucose-lowering effect of GLP-1 RA [15]. However, the role of weight loss in predicting the efficacy of GLP-1 RA therapy has not been fully explored. T2DM and obesity are well-known risk factors for CVDs and mortality, and substantial evidence has shown the effects of body weight reduction in preventing CVDs in patients with T2DM [16]. However, whether these factors can predict the GLP-1 RA-induced glycemic and weight responses simultaneously was not observed in patients with T2DM. Therefore, we aimed to identify the predictors of glycemic and weight responses to exenatide treatment in patients with T2DM.

Materials and methods

Study participants

Patients with T2DM admitted to in the Endocrinology Department of the Affiliated Hospital of Xuzhou Medical University in China between January 2017 and September 2019 were recruited in this retrospective study. Patients who were diagnosed with T2DM, aged > 18 years, and received exenatide treatment twice a day as part of their diabetes treatment for at least 12 months prior to data collection were included in this study. In contrast, patients who developed other subtypes of diabetes (such as type 1 diabetes and gestational diabetes), discontinued exenatide treatment within 12 months, were lost to follow-up, and were previously treated with another GLP-1 analogue were excluded.

Treatment with exenatide at a dose of 5 μg administered twice daily was initiated. After 1 month of treatment, the exenatide dosage was increased to 10 μg twice daily. The patients were followed up at 3, 6, and 12 months after treatment initiation. A total of 343 exenatide-treated patients with T2DM met the inclusion criteria. Meanwhile, 29 participants who developed adverse reactions, 101 participants who had incomplete data, and 65 participants who were lost to follow-up were excluded; hence, only 148 participants were included in the final analyses. The detailed flow chart of the participant selection process was described in our previous study [15]. The research was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University.

Measurement of the anthropometric and biochemical parameters

Data on anthropometric measurements, clinical history, and blood analyses were obtained from medical records at baseline and at each follow-up visit. Anthropometric measurements included age, weight, height, and body mass index (BMI). BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2). The duration of diabetes and use of concurrent diabetic medications were recorded. Biochemical data including HbA1c, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting serum insulin (FINS), postprandial serum insulin (PINS), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were also retrieved. Insulin resistance and β-cell function were evaluated using the following formulas: homeostasis model insulin resistance assessment (HOMA-IR) = FINS (mU/L) × FPG (mmol/L)/22.5, and homeostasis model β-cell functional assessment (HOMA-B) = 20 × FINS/(FPG-3.5) [17].

Study cohorts

According to the National Institute for Health and Care Excellence guidelines regarding the use of GLP-1 RA in the treatment of patients with T2DM, glycemic response was defined as an HbA1c reduction of ≥ 1.0% and weight responses as a weight loss of ≥ 3% within 6 months after GLP-1 RA treatment [18, 19]. Therefore, patients with T2DM were categorised into two cohorts in terms of glycemic and weight responses to exenatide: responders and non-responders. Responders were defined as patients who achieved an HbA1c reduction of ≥ 1% and a weight loss of ≥ 3%, whereas non-responders referred to patients who failed to achieve these decreases within 6 months after exenatide administration.

Statistical analysis

Statistical analyses were performed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA). Variables with a normal distribution were expressed as mean ± standard error, while those with a non-normal distribution are expressed as median (interquartile range) or percentages as appropriate. The baseline characteristics

between responders and non-responders were compared using the independent Student's *t*-test for continuous data and the chi-square test or Fisher's exact test for categorical data. Repeated measures analysis of variance was used to determine changes in clinical parameters at certain time points for responders group and non-responders group. Linear regression was utilised to evaluate the relationship between weight change from baseline and HbA1c change from baseline after 6 months of exenatide therapy, while binary logistic regression analysis was utilised to identify the independent predictors of glycemic and weight responses to exenatide. The odds ratio (OR) values were presented along with 95% confidence intervals (CIs). The area under the curve (AUC) of the receiver operating characteristic (ROC) curve and the 95% CI were calculated to compare the predictive power of the predictors. A p value of < 0.05 was considered significant.

Results

Patient disposition and baseline characteristics

Patient selection and disposition for the individual studies were described previously [15]. The final analysis included 148 patients (90 men and 58 women), who were divided into responders and non-responders. Of the 148 patients, 53 were responders (35.81%) and 95 were non-responders (64.19%). After 6 months of exenatide treatment, only 35.81% of the patients with T2DM achieved the composite end point (HbA1c reduction of $\geq 1\%$ and weight loss of $\geq 3\%$). The baseline characteristics of the patients are summarised in Table 1. Patients in the responder group had higher mean values for HbA1c ($p < 0.05$), weight ($p < 0.001$), and BMI ($p < 0.01$) but had a lower mean value for duration of diabetes ($p < 0.001$) than those in the non-responder group.

Effects of exenatide on clinical parameters at each time point

Supplementary material Tables S1 show the changes in clinical parameters from baseline to 12 months after exenatide treatment by repeated measures ANOVA on clinical data. Exenatide treatment led to significant improvements in HbA1c, body weight, BMI, FPG, PPG, HOMA-IR, HOMA-B, and blood lipids levels in each group. Furthermore, the clinical parameters were also compared between responders and non-responders. The baseline HbA1c level was higher among responders, while the HbA1c levels at 3, 6, and 12 months were higher among non-responders (Fig. 1A). Weight was, on average, greater in responders than in non-responders during the entire study period (Fig. 1B). The BMI at baseline was greater in responders than in non-responders (Fig. 1C). The patterns of FPG and PPG changes were similar between responders and non-responders (Fig. 1D, E). In addition, no significant difference was observed between the two groups in terms of HOMA-B at baseline and 3 months; in contrast, the responders had higher levels of HOMA-B at 6 months and 12 months than the non-responders (Fig. 1F).

Table 1 Comparison of baseline characteristics between responders ($n = 53$) and non-responders ($n = 95$) in patients with T2DM after exenatide initiation

Parameters	Group		p value
	Responders	Non-responders	
N (male/female)	53 (34/19)	95 (56/39)	0.534
Age (years)	47.92 ± 1.30	50.03 ± 0.91	0.180
HbA1c (%)	9.51 ± 0.20	8.98 ± 0.11	0.033
Weight (kg)	89.31 ± 1.73	82.12 ± 1.26	<0.001
BMI (kg/m^2)	30.88 ± 0.59	28.82 ± 0.34	0.006
Duration of diabetes (years)	1.57 ± 0.35	5.09 ± 0.57	<0.001
Exenatide only (%)	11 (20.75)	21 (22.11)	0.848
Exenatide + OHAs (%)	21 (39.62)	32 (33.68)	0.470
Exenatide+ insulin (%)	8 (15.09)	14 (14.74)	0.953
Exenatide+ OHAs + insulin (%)	13 (24.53)	28 (29.47)	0.519

Data are provided as mean ± standard error or percentages unless otherwise noted

HbA1c haemoglobin A1c, *BMI* body mass index, *OHA* oral anti-hyperglycemic agent

Predictors of glycemic and weight responses within 6 months after exenatide initiation

Linear regression analysis was used to assess the possible predictors of glycemic and weight responses within 6 months after exenatide initiation. Baseline HbA1c (OR = 1.664, CI: 1.059–2.616, $p = 0.027$), baseline weight (OR = 1.064, CI: 1.022–1.109, $p = 0.003$), and duration of diabetes (OR = 0.757, CI: 0.647–0.886, $p = 0.001$) were independent predictors of response to exenatide treatment (Table 2). Patients with a higher baseline HbA1c, a higher baseline weight, and a shorter duration of diabetes were more likely to be classified as responders to exenatide. Furthermore, the association between changes in HbA1c level and weight, which were closely related to the response to exenatide within 6 months after treatment, was further investigated using linear regression analysis. The results showed that the variation in weight was consistent with that of HbA1c level (Fig. 2). That is to say, as the weight loss increased, the HbA1c reduction also increased, which may have strengthened the glycemic and weight control within the 6-month period.

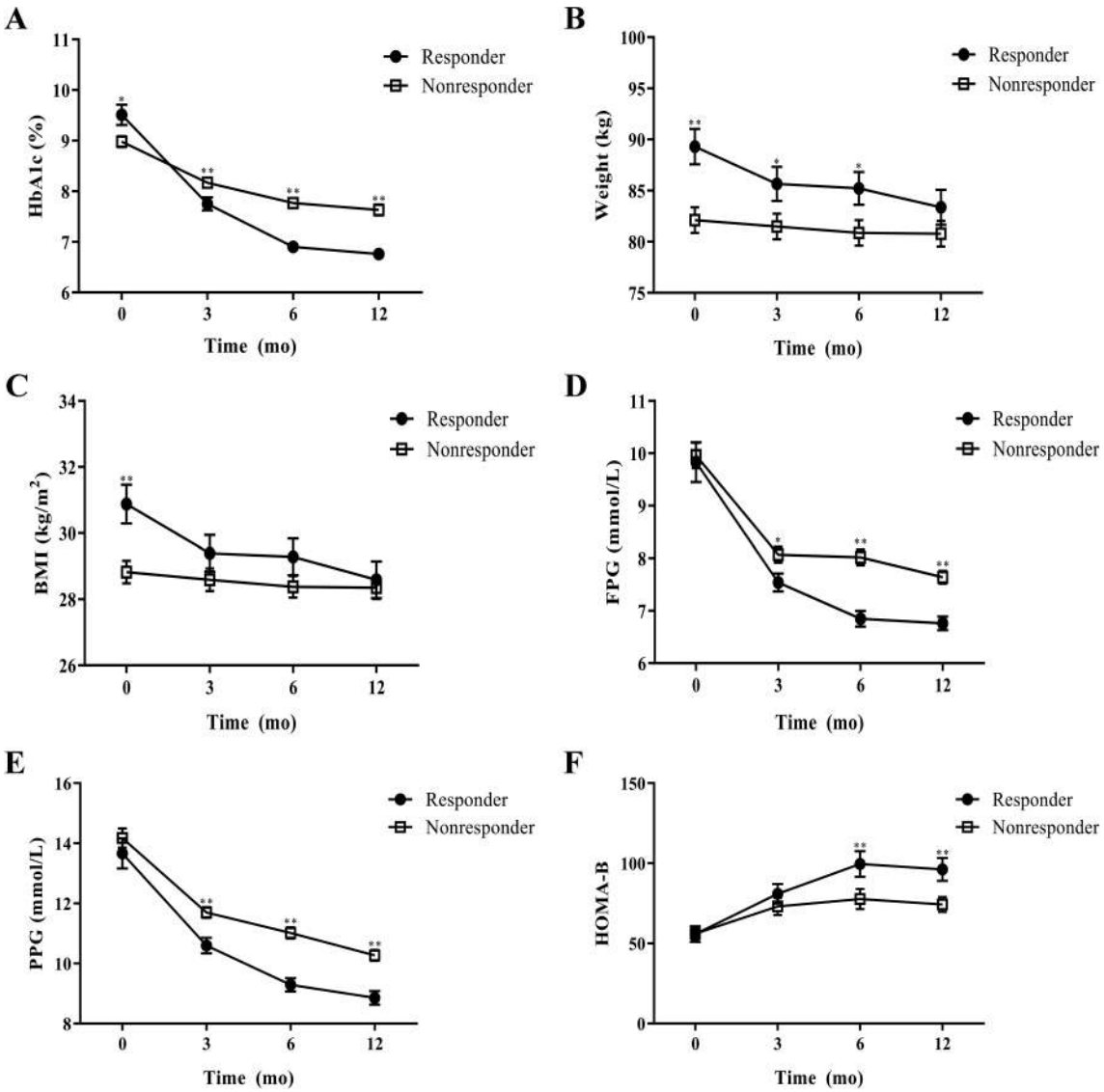

Fig. 1 Comparison of HbA1c (**A**), weight (**B**), BMI (**C**), FPG (**D**), PPG (**E**), and HOMA-B (**F**) between responders (n = 53) and non-responders (n = 95) after treatment with exenatide at baseline, 3 months, 6 months, and 12 months. *$p < 0.05$, **$p < 0.01$ compared with non-responders. Abbreviations: HbA1c, haemoglobin A1c; BMI, body mass index; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; HOMA-B, homeostasis model assessment for beta cell function

ROC curves for the predictors of response to exenatide

The ROC curves of the response to exenatide for HbA1c, weight, and duration of diabetes are shown in Fig. 3. The area under the ROC curve for the predictors of HbA1c and weight reduction within 6 months of exenatide initiation was 0.765 (95% CI: 0.686–0.845). Furthermore, the area under the ROC curve was used to determine the extent of the predictors of response to exenatide therapy (Supplementary material Fig. S1). The areas under the ROC curves were 0.704 (95% CI: 0.621–0.787) for diabetes duration, 0.667 (95% CI: 0.576–0.757) for baseline weight, and 0.606 (95% CI: 0.500–0.712) for baseline HbA1c. However, no significant difference was observed in the AUC for all three predictors ($p \geq 0.05$).

Discussion

Findings from the present study confirmed the potential predictors of glycemic and weight responses to exenatide in patients with T2DM. Our data showed that baseline HbA1c, baseline weight, and duration of diabetes were independent predictors of response to exenatide treatment. Patients with higher baseline HbA1c, higher baseline weight, and shorter

Table 2 Binary logistic regression analysis of the potential variables for predicting response on HbA1c level and weight changes

Variables	Odds Ratio	95% CI	*p* value
Age (years)	1.020	0.968-1.075	0.454
Sex[a]	1.396	0.529-3.679	0.500
Duration of diabetes (years)	0.757	0.647-0.886	0.001
Baseline Weight (kg/m^2)	1.064	1.022-1.109	0.003
Baseline HbA1c (%)	1.664	1.059-2.616	0.027
Baseline FPG (mmol/L)	1.048	0.761-1.443	0.773
Baseline PPG (mmol/L)	0.815	0.627-1.060	0.127
Baseline FINS (mU/L)	0.963	0.904-1.026	0.246
Baseline PINS (mU/L)	1.004	0.984-1.024	0.720
Baseline TC (mmol/L)	0.845	0.450-1.584	0.599
Baseline TG (mmol/L)	0.779	0.523-1.159	0.217
Baseline HDL-C (mmol/L)	1.610	0.235-11.050	0.628
Baseline LDL-C (mmol/L)	0.816	0.436-1.528	0.526
OHA only[b]	1.955	0.644-5.931	0.237
Insulin only[b]	1.873	0.444-7.896	0.393
OHAs and Insulin[b]	0.864	0.242-3.081	0.822

BMI, body mass index; HbA1c, haemoglobin A1c; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; FINS, fasting serum insulin; PINS, postprandial serum insulin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OHA, oral anti-hyperglycemic agent; CI, confidence interval

[a]Denotes a variable that was compared against males

[b]Denotes a variable that was compared against exenatide as a monotherapy

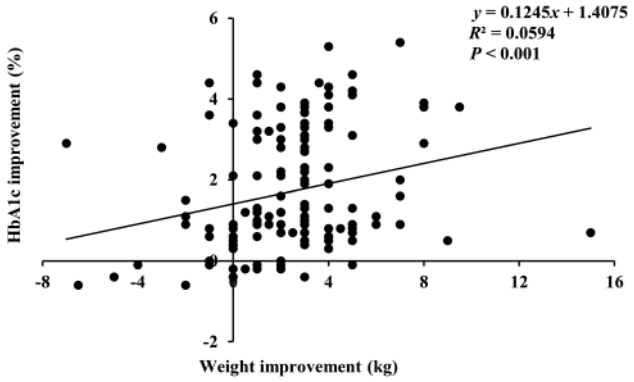

Fig. 2 Linear relationship between weight change from baseline and HbA1c change from baseline after 6 months of exenatide therapy. Results were shown using the equation for the line of best fit. Abbreviation: HbA1c, haemoglobin A1c

duration of diabetes may be more responsive to exenatide treatment, which suggests that these factors can be valuable for predicting the effect of exenatide therapy.

Accumulating evidence has reported that exenatide improved not only glycemic control, but also weight loss

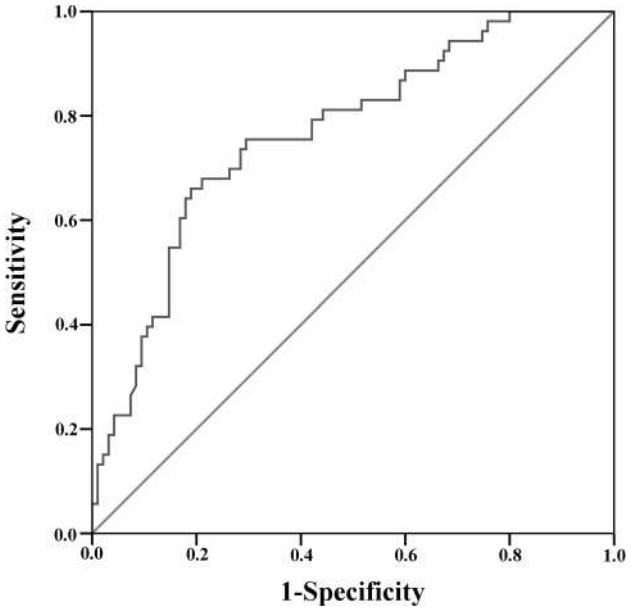

Fig. 3 ROC curve for the predictors of glycemic and weight responses to exenatide in patients with T2DM

[20, 21]. However, only 35.81% of the study patients treated with exenatide for 6 months achieved an HbA1c reduction of ≥ 1% and a weight loss of ≥ 3%. Based on the marked interindividual differences in therapeutic response of patients with T2DM treated with exenatide, further studies are needed to identify the novel predictors for individualised treatment [22]. To the best of our knowledge, this study was the first to identify the factors that can predict the glycemic and weight responses to exenatide simultaneously in patients with T2DM. The predictors of glycemic and weight responses are baseline HbA1c, baseline weight, and duration of diabetes in patients on exenatide therapy. Several studies have reported that the baseline HbA1c level appears to be an independent predictor of glycemic response to exenatide, as observed in our previous studies [11, 15, 18]. In a 24-month retrospective study of once-weekly exenatide in Spain, HbA1c was found to be a unique independent predictor of glycemic response; higher BMI and previous treatment with DPP-4i could also predict weight response [11, 15]. However, these studies did not agree with our results. Furthermore, these inconsistencies are likely due to the different grouping schemes and timing of subcutaneous injections.

The reason that patients gradually lose weight after taking exenatide may be delayed gastric emptying and enhanced satiety induced by the hypothalamus which control caloric intake [23, 24]. Additionally, the responder group had a more significant trend in weight loss with a higher baseline BMI, which was broadly in line with previous findings [25, 26]. These data provided strong evidence to allay the physicians' concerns that exenatide-treated

patients with T2DM may experience excessive weight loss when compared with the upper baseline weight. Futhermore, patients with a history of ≥ 5 years of diabetes were less likely to achieve good glycemic control than those with a history of < 5 years of diabetes [27]. Another drug for GLP-1 RA, liraglutide, was also more effective in patients with a shorter duration of diabetes [28]. However, it is important to note that the risk of hypoglycemia increases by 19% per decade of duration on the premise of longer duration of diabetes, meaning that without intervention, patients could be subjected to lifelong insulin therapy [29]. Hence, the duration of diabetes was considered a valuable predictor of response to exenatide treatment.

With regard to the discriminatory powers of predictive ability, our ROC curve analysis showed that the AUC was attributed to predicting HbA1c and weight reduction within 6 months after exenatide treatment. The AUC values for the duration of diabetes, baseline weight, and baseline HbA1c were 0.704, 0.667 and 0.606, respectively. Although many studies have evaluated the predictors of response to exenatide, only a few have attempted to plot an ROC curve to assess the predictive ability of these factors, which were identified by binary logistic regression analysis [11, 14, 15, 18, 21]. To date, only one study found that the mean preprandial blood glucose level had the highest AUC (0.72) for the prediction of glycemic response to GLP-1 RA, and the results of this study were similar to those of our study [30].

Although we have identified the predictive factors, there are still some inherent problems in this study that need to be improved. First, owing to the number of patients who did not meet the inclusion criteria, the number of patients included for the final analysis was small, resulting in a wide range in CI after the analysis. It is necessary to expand the sample size; in this case, other races can also be included to increase the richness of the samples, and the generalisability of the results could be determined subsequently. Furthermore, after exenatide administration, if the patients had other treatments or strictly controlled diet and rest, it would have been difficult to clearly judge whether the significant decreases in HbA1c levels and body weight were simply a direct effect of exenatide. This suggests that we should pay attention to changes in medication categories and lifestyles during patient follow-up and assess their impact on the efficacy of exenatide. Finally, this study only tentatively identified baseline HbA1c, baseline weight, and duration of diabetes as predictors of responses to exenatide and their relationships to other indicators. However, the scopes of the three predictors have not been further refined; thus the clinical reference of the results could not be highlighted. The determination of the applicable scopes of predictors still requires the support of a larger sample size. Therefore, a prospective clinical study with a stricter protocol and a greater number of patients using each formulation is necessary to further evaluate the effectiveness of GLP-1 RA therapy.

Conclusion

Baseline HbA1c, baseline weight, and duration of diabetes could be independently used to predict glycemic and weight responses to exenatide in patients with T2DM. The scopes of each indicator has not been determined; thus, further explorations are needed to provide more valuable references for the applicable conditions of exenatide and selections for patients with T2DM. Significant predictors of exenatide and reliable clinical data can contribute to individualised treatment, which is more conducive to achieving better glycemic and weight gain control in patients with T2DM.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-023-01239-8.

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Authors Contribution Yuhan Huang: Conceptualization, Methodology, Writing-Original Draft, Writing-Reviewing and Editing. Yanan Yu: Formal Analysis, Methodology, Data Curation, Investigation. Ruonan Hu: Data Curation, Validation, Writing-Editing. Ke Xu: Data curation, Writing-Editing. Tao Wang: Methodology, Resources, Supervision, Funding Acquisition, Project Administration, Writing-Reviewing and Editing. Hongwei Ling: Data Curation, Investigation, Supervision. Jia Han: Data Curation, Methodology, Investigation. Dongmei Lv: Resources, Conceptualization, Supervision, Project Administration.

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Data availability The data underlying this article are available in the article and its online supplemental material.

Declarations

Ethical approval The study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (Xuzhou, China) and was performed in accordance with the Declaration of Helsinki. The ethical approval number was XYFY 2018-KL085 on 31 December 2018.

Conflicts of interest The authors declared no other competing financial interests or disclosures relevant to this manuscript exist for all authors.

Consent of participation Written informed consent was obtained from all participants prior to enrollment.

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ORIGINAL ARTICLE

Effect of dapagliflozin on the triglyceride-glucose index and the atherogenic index of plasma used as markers of atherosclerosis in patients with type 2 diabetes mellitus

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Abstract

Objective Atherogenic index of plasma (AIP) and triglyceride-glucose (TyG) index are inexpensive and non-invasive markers with high predictive value for early detection of cardiovascular disease in DM patients. Herein, dapagliflozin reduced the AIP and TyG and caused positive cardiovascular effects in patients with type 2 diabetes mellitus (T2DM).

Methods We retrospectively evaluated the data of patients aged >18 years with T2DM ($n = 348$; 210 [60.3%] women and 138 [37.7%] men; mean age = 59.24, standard deviation [SD] = ±10.44 years) who presented to a single-center internal medicine outpatient clinic between June 01, 2017, and December 30, 2020, and who were started on dapagliflozin as part of their treatment. Demographic data and clinical data of the patients at 0, 6, 12, and 24 months were retrieved from the electronic medical records of the hospital.

Results Hypertension was the most common comorbidity ($n = 155$ [48.9%] patients). AIP values measured before dapagliflozin initiation (mean = 0.68; SD, 0.33) and at 6 months (mean = 0.62; SD, 0.30) were significantly different ($p < 0.00$). Furthermore, TyG index values measured before initiation of medication (mean = 9.98; SD, 0.76) and at 6 months (mean = 9.73; SD, 0.71) were significantly different ($p < 0.00$). These differences persisted until 12 and 24 months after treatment initiation.

Conclusions Dapagliflozin administration lowered the AIP and TyG index in patients with T2DM; this may slow the atherosclerotic process and prevent the associated macrovascular complications.

Keywords Type 2 diabetes mellitus · Dapagliflozin · AIP · TyG · Atherosclerosis

Introduction

Diabetes mellitus (DM) is a major public health problem with an increasing prevalence worldwide [1]. Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of increased morbidity and mortality in patients with diabetes. The risk of cardiovascular death (CVD) events is approximately twice as high in patients with type 2 diabetes mellitus (T2DM) compared with those without T2DM, which has made cardiovascular prevention an important goal in the treatment of T2DM [2, 3].

Dapagliflozin is a selective sodium-glucose co-transporter 2 inhibitor (SGLT2i) that induces glycosuria and lowers plasma glucose by preventing glucose reuptake via proximal renal tubules. Animal experiments have suggested that dapagliflozin exerts an antiatherogenic effect through SGLT2 inhibition which reduces intestinal cholesterol absorption, thereby increasing fecal excretion of LDL and macrophage-derived cholesterol, potentially reducing LDL retention in the arterial intima [4].

In cardiovascular safety studies, SGLT2 inhibitors reduced plasma glucose while reducing systolic blood pressure, diabetic kidney disease, and heart failure and facilitating weight loss [5, 6]. This drug reduces mortality in patients with chronic heart failure and reduced ejection fraction and in patients with chronic renal failure, regardless of the presence or absence of T2DM [7–9].

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Current guidelines strongly recommend SGLT2i therapy in patients with chronic heart failure and reduced ejection fraction [10]. The atherogenic index of plasma (AIP) and the triglyceride-glucose (TyG) index are markers with high predictive value for the early detection of cardiovascular disease in patients with DM [11, 12]. The TyG index is an independent risk factor for DM and the incidence of coronary atherosclerosis [13]. AIP was reported to be a reliable indicator for cardiovascular disease [14].

The purpose of the present study was to investigate whether dapagliflozin can serve as an acceptable treatment option in preventing the progression of atherosclerosis. To this end, we evaluated the long-term—at 0, 6, 12, and 24 months—effects of dapagliflozin on the AIP and the TyG index in T2DM patients who presented to Mersin Training and Research City Hospital Internal Medicine outpatient clinic between June 01, 2017, and December 30, 2020.

Methods

This study retrospectively assessed patients aged >18 years who presented to the internal medicine outpatient clinic of Mersin Training and Research City Hospital between June 01, 2017, and December 30, 2020, who were diagnosed with T2DM and were started on dapagliflozin as part of their treatment. Patients were included in the study if they had T2DM and were started on dapagliflozin; they were excluded if they had type 1 diabetes mellitus (T1DM) or did not attend follow-up checks. Patients' data related to age, sex, comorbidities, medications, time of diagnosis, and laboratory test results at 6, 12, and 24 months were retrospectively retrieved from electronic medical records.

Study data were processed using the SPSS 20·0 software suite. Data analysis was preceded by missing data analysis, as suggested by Bennet (2001); the missing data were distributed in the form of missing completely at random (MCAR) (Little's MCAR test: $p > 0.05$). Therefore, the missing data were completed through group averaging. Data were analyzed using descriptive and inferential statistics.

Before the analyses, data were checked for normality of distribution using Skewness–Kurtosis, histograms, and Kolmogorov–Smirno tests. Normally distributed data for continuous variables were reported in mean and standard deviation, whereas non-normally distributed data were reported in median and interquartile range. Categorical variables were presented using frequencies and percentages. AIP was calculated using the formula log10(TG/HDL-C) [13]. TyG index was calculated using the formula Ln[TG × FBG/2] [15]. Differences between time-dependent repeated measurements of AIP and TyG indices were determined using repeated measures ANOVA analysis. Intergroup differences of categorical variables were compared using the chi-square test or Fisher's exact test depending on the parametric data relating to the variables. Statistical significance was set at 0.05.

The study received approval from the Non-Invasive Clinical Research Ethics Committee of the Mersin University (2023/291).

Results

For the period between June 01, 2017, and December 30, 2020, there were 745 patients who had T2DM and who were started on dapagliflozin in addition to their existing therapies. Patients were excluded if they were pregnant or lactating, were started on hyperlipidemia medications during follow-up, consumed alcohol, and had incomplete follow-up data. Therefore, the analysis finally included 348 patients. Of the patients included in the study, 210 (60.3%) were women and 138 (37.7%) were men, with a mean age of 59.24 (standard deviation [SD], 10.44) years and average diabetes duration was 10.36 ± 5.32 years. The most common comorbidity was hypertension, seen in 155 (48.9%) patients. The patients were started on dapagliflozin in addition to the medications they had been prescribed for existing diseases. The demographic data of the patients are presented in Table 1.

Laboratory test results of the patients before initiation and at 6, 12, and 24 months after initiation of dapagliflozin are presented in Table 2.

Table 1 Demographic data of the patients

Feature	All patiens (*n*=348)
Gender	
Female, *n* (%)	210 (60.3)
Male, *n* (%)	138 (39.7)
Age, $\bar{x}$ (ss)	59.24 (10.44)
Additional drugs	
Calcium channel blocker, *n* (%)	38 (10.9)
Beta blocker, *n* (%)	42 (12.1)
Statin/fenofibrate, *n* (%)	107 (30.7)
Metformin, *n* (%)	229 (65.8)
Dpp-4 inhibitor, *n*(%)	134 (38.5)
Sulfonylurea, *n* (%)	50 (14.4)
Insulin users, *n* (%)	80 (23)
Thiazolidinedione, *n* (%)	55 (15.8)
Glinid, *n* (%)	8 (2.3)
ARB_ACE inhibitor, *n* (%)	115 (33)
Additional diseases	
Cancer, *n* (%)	4 (1.3)
Coronary artery disease, *n* (%)	30 (9.5)
Hyperlipidemia, *n* (%)	110 (34.7)
Hypertension, *n* (%)	155 (48.9)
Cerebrovascular ischemia, *n* (%)	1 (0.02)
Congestive heart failure, *n* (%)	4 (1.3)
Chronic obstructive pulmonary disease, *n* (%)	13 (4.1)

Table 2 Laboratory test results before and after initiation of medication

Laboratory test results	Before initiation of medication	After initiation of medication		
	1. Measurement	2. Measurement (6 months)	3. Measurement (1 year)	4. Measurement (2 years)
Fasting plasma glucose (mg/dL)	216 (113)[a]	180.50 (91.75)[a]	193.50 (79.8)[a]	179 (96)[a]
HBA1C (%)	9.2 (2.8)[a]	8.77 (1.50)[a]	8.75 (1.8)[a]	8.85 (1.9)[a]
Creatinine (mg/dL)	0.8 (0.3)[a]	0.8 (0.3)[a]	0.8 (0.2)[a]	0.8 (0.3)[a]
ALT (U/L)	21 (13)[a]	22 (10.75)[a]	21 (10)[a]	21 (10)[a]
AST (U/L)	18 (9.75)[a]	20 (8)[a]	21 (8)[a]	20 (9)[a]
Triglyceride (mg/dL)	199 (132.7)[a]	209 (95)[a]	209 (88.8)[a]	210.5 (110.8)[a]
LDL cholesterol (mg/dL)	111.61 (36.4)[b]	108.82 (32.75)[a]	105.07 (31)[b]	105.79 (32.79)[b]
HDL cholesterol (mg/dL)	43 (13)[a]	46 (11.34)[a]	46.28 (10)[a]	47 (12)[a]
Total cholesterol (mg/dL)	197.50 (53.5)[a]	95.46 (41)[a]	189.34 (33.8)[a]	195.94 (42.13)[b]
Na (mEq/L)	139 (3)[a]	139 (3)[a]	138.7 (2)[a]	139 (3.5)[a]
K (meq/L)	4.7 (0.5)[a]	4.7 (0.4)[a]	4.7 (0.8)[a]	4.63 (0.45)[b]
Mg (mg/dL)	1.96 (0.1)[a]	1.98 (0.01)[a]	1.95 (0)[a]	2.1 (0.1)[a]
Hematocrit (%)	41 (6.68)[a]	41.09 (6.18)[a]	40.99 (5.3)[a]	41 (5.9)[a]
Hemoglobin (g/dL)	13.65 (2.2)[a]	13.74 (2.28)[a]	13.61 (1.6)[b]	13.95 (2.3)[a]

[a]Non-normally distributed data in median and interquartile range in parentheses; [b]normally distributed data in mean and standard deviation in parentheses

The time-dependent effect of dapagliflozin on the AIP was evaluated using repeated measures ANOVA. The analysis detected a significant difference between at least two groups in terms of AIP. Furthermore, Mauchly's test of sphericity—one of the assumptions of the analysis—having yielded a significant result, the Greenhouse–Geisser value was reported, $F(3.1041) = 6.624$, $p = 0{\cdot}00$. Post hoc analysis with Bonferroni correction was used to determine which measurements led to significant differences. The results showed a significant difference between the first and second measurement and between the first and third measurement. Therefore, a significant difference ($p < 0.00$) was noted between the AIP values before initiation of dapagliflozin (at baseline) (mean, 0.68; SD, 0.33) and at the second measurement (mean, 0.62; SD, 0.30). Additionally, a significant difference was observed between the AIP values at baseline (mean, 0.68; SD, 0.33) and at the third measurement (mean, 0.63; SD, 0.26) ($p < 0.00$). Data on these findings are shown in Table 3.

The time-dependent effect of dapagliflozin on TyG was evaluated using repeated measures ANOVA test. The analysis revealed a significant difference between at least two groups in terms of TyG, $F(3.345) = 12.95$, $p = 0.00$. Post hoc analysis with Bonferroni correction was used to determine which measurements were responsible for the significant differences. The findings showed significant differences between the first measurement and the next three measurements. Thus, there was a significant difference ($p < 0.00$) between the TyG values at baseline (mean, 9.98; SD, 0.76) and at the second measurement (mean, 9.73; SD, 0.71). Additionally, a significant difference ($p < 0.00$) was observed between the TyG values at baseline and at the third measurement (mean, 9.78; SD, 0.65). Likewise, a significant difference ($p < 0.00$) was noted between the TyG values at baseline and at the fourth measurement (mean, 9.78; SD, 0.65). Data on these findings are given in Table 4.

Use of medication for hyperlipidemia

To determine the effect of hyperlipidemia medications (statin and fenofibrate) on the analysis results, the tests for the AIP and TyG were repeated among those who did not use medication and ANOVA findings showed that the significant relationship persisted at a level of 0.03.

Table 3 Time-dependent changes in AIP in patients receiving dapagliflozin

AIP value	Average	Standard deviation	*F*	*p*	Post hoc
1. Measurement, $\bar{x}$ (ss)	0.686	0.33	*F*=5.96	<0.00	1>2**
2. Measurement (6 months), $\bar{x}$ (ss)	0.622	0.30			1>3**
3. Measurement (1 year), $\bar{x}$ (ss)	0.629	0.26			
4. Measurement (2 years), $\bar{x}$ (ss)	0.643	0.31			

Table 4 Time-dependent changes in the triglyceride-glucose index

TyG value	Average	Standard deviation	*F*	*p*	Post hoc
1. Measurement, $\bar{x}$ (ss)	9.98	0.76	13.01	<0.00	1>2**
2. Measurement (6 months), $\bar{x}$ (ss)	9.73	0.71			1>3**
3. Measurement (1 year), $\bar{x}$ (ss)	9.78	0.65			1>4**
4. Measurement (2 years), $\bar{x}$ (ss)	9.79	0.77			

Discussion

Studies have shown that the atherogenic index of plasma (AIP) and triglyceride-glucose (TyG) index are new markers for atherosclerosis, insulin resistance, and inflammation, respectively [12, 16]. The TyG index can predict CVD risk in the general population [17]. An elevated TyG index is significantly associated with a higher risk of arterial stiffness [3].

To the best of our knowledge, this is the first study on such a large number of patients with a follow-up period of 2 years to investigate the effects of dapagliflozin, an SGLT2i agent, on the AIP and TyG index, predictors of cardiovascular risks. The results showed a significant difference between the AIP values measured before initiation of dapagliflozin (mean, 0.68; SD, 0.33) and the AIP values measured at month 6 (mean, 0.62; SD, 0.30) ($p < 0.00$). Additionally, we noted a significant difference between the TyG values at baseline (mean, 9.98; SD, 0.76) and the TyG values measured at month 6 (mean, 9.73; SD, 0.71) ($p < 0.00$). These differences lasted and remained significant at 12 and 24 months.

In an animal study, B. Ganbaatar et al. treated diabetic mice using SGLT2i (empagliflozin) for 12 weeks. The drug was shown to lower blood glucose ($p < 0.001$) and lipid levels (triglyceride $p = 0.005$). It significantly reduced atherosclerotic lesion size in the aortic arch through reduction of lipid accumulation ($p < 0.05$), macrophage accumulation ($p < 0.001$), and inflammatory molecule release ($p < 0.01$) in the treatment group compared to the control group [18].

In animal experiments conducted by Y. Liu et al., mice with induced diabetes were given empagliflozin for 12 weeks. The results showed that the drug reduced atherosclerotic lesion burden in the aortic arch (−8.6%, $p = 0.004$). In addition, empagliflozin also led to reductions in body weight (−3.27 g, $p = 0.002$) and lipid profiles (TC: [−15.3 mmol/L, $p = 0.011$]; TG: [−2.4 mmol/L, $p < 0.001$]; LDL: [−2.9 mmol/L, $p = 0.010$]) [19].

A meta-analysis conducted by Ghosh-Swaby et al. with a total of 38,723 participants, including four cardiovascular outcome studies, EMPA-REG OUTCOME, CANVAS, DECLARE-TIMI 58, and CREDENCE, showed that patients who received SGLT2i had 12% lower risk of atherosclerotic MACE (cardiovascular mortality, non-fatal MI, and non-fatal stroke) compared to patients on placebo [hazard ratio, 0.88; 95% confidence interval, 0.82–0.94] [20].

T. Hayashi et al. compared sitagliptin and dapagliflozin and showed that dapagliflozin significantly reduced body weight, systolic blood pressure, plasma triglycerides, and liver transaminases and increased adiponectin. Dapagliflozin did not lead to a change in LDL-C concentrations, but lowered sdLDL-C by 20% and increased lbLDL-C by 18%. It also increased HDL2-C by 18% without affecting HDL3-C. Dapagliflozin decreases sdLDL-C, which has a strong atherogenic potential, and increases HDL2-C, a favorable cardiometabolic marker [19]. In our study, LDL cholesterol decreased by 5% and HDL cholesterol increased by 7%. LDL cholesterol continued to decrease and HDL cholesterol continued to rise at months 12 and 24.

To determine how analysis results were affected by hyperlipidemia medications (statin and/or fenofibrate) which were started before initiation of dapagliflozin, tests for AIP and TyG were repeated among those who did not receive hyperlipidemia treatment; ANOVA findings revealed that the significant correlation persisted at a level of 0·03.

A study from Turkey showed that administration of SGLT2i agents dapagliflozin and empagliflozin resulted in a significant reduction in AIP and TyG indexes at month 6 ($p < 0.01$ with dapagliflozin and $p < 0.05$ with empagliflozin) [21]. These results are in line with our study, which showed that AIP and TyG indexes continued to decrease at months 12 and 24.

In the present study, dapagliflozin was also analyzed for its effects on glycemic control and was found to improve HbA1c and FBG for 24 months; it reduced HbA1c by 0.43% at month 6 and by 0.35% at month 24. It decreased FBG levels by 36 mg/dl at month 6 and by 35 mg/dl at month 24. These results are in line with previous studies from Turkey and other countries [21, 22].

The present study has some limitations: it was a single-center cross-sectional study and thus its results cannot be generalized to the entire population. The study's retrospective nature, single-center setting, and absence of a control group should be considered when interpreting the results. Further research with controlled designs and longer follow-up periods is warranted to confirm the observed effects and their clinical implications. The retrospective design limits the control over variables, potential biases, and the ability to establish causality. Unmeasured confounders could impact the observed effects. Also, as it was a retrospective study, it could not access patient outcomes

for weight and blood pressure before and after treatment. While AIP and TyG indices are relevant markers, the study does not directly assess clinical cardiovascular outcomes, such as myocardial infarction or stroke. The strength of our study is that it is the first real-life study conducted with such a large number of patients, over a long follow-up period covering 24 months.

DM is an increasingly important public health problem that affects quality of life and leads to economic consequences. A number of treatments are being developed to improve quality of life and prevent complications among patients. SGLT2i agents are one of these agents and have been found to have positive effects through reduction of MACEs, CV mortality, HF, and CKD, which are largely of atherosclerotic origin. However, the role of SGLT2i in different ASCVD events remains to be explored more extensively. The precise mechanisms linking SGLT2 to atherosclerotic processes have not yet been fully elucidated. Further high-quality experimental and observational studies with sufficient numbers of patients and follow-up periods are needed to investigate the potential role of SGLT2-i in subclinical atherosclerosis and ASCVD events.

Conclusion

In conclusion, dapagliflozin initiated at the time of diagnosis in patients with type 2 diabetes mellitus lowers the AIP and TyG index, which may help slow down the atherosclerotic process and prevent associated microvascular and macrovascular complications.

Author contribution DENİZ G, SEMRA ÖÖ, and ZEHRA K were involved in original all articles, applying eligibility criteria, and identifying original articles. All authors contributed extensively to manuscript writing, figure and table design, and revision.

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

Declarations

Ethical clearance This study was approved by Mersin University Non-Interventional Clinical Research Ethics Committee (Decision date/No: 2023/291) and conducted in accordance with the Declaration of Helsinki and Human Rights. Before the survey, participants provided electronic informed consent and were informed of their right to withdraw without explanation.

Informed consent Informed consent was obtained from all the subjects online.

Conflict of interest The authors declare no competing interests.

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ORIGINAL ARTICLE

A study on clinical profile of diabetes mellitus in COVID-19 patients, hyperglycemia management, and risk assessment for mortality

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Abstract

Background Diabetes mellitus is an immune compromised state and Covid-19 an infection associated with immune dysregulation. This study was conducted to appreciate the factors which may contribute to increased morbidity and mortality in people with Diabetes.

Objective To understand the profile of diabetes mellitus in COVID-19 patients and to ascertain their risk factors for mortality.

Methods This was a single-center, retrospective observational study conducted in a tertiary care hospital. The data of adults with established or newly diagnosed diabetes mellitus admitted with COVID-19 between April 2020 and January 2021 was analyzed in relation to their age, sex, duration of hospitalization, systolic blood pressure (SBP) at admission, presence of other comorbidities, initial fasting plasma glucose, oxygen therapy, CT severity, biochemical parameters, inflammatory markers, and hyperglycemia management, and compared between survivors and non-survivors, to ascertain the risk factors for mortality.

Results A total of 2640 adults, above 18 years of age with diabetes mellitus and COVID-19, were included. Among them, 2229 (84.4%) survived, and 411 (15.6%) died. Preexisting diabetes mellitus was recorded in 2246 patients (85.1%) and newly diagnosed in 394 patients (14.9%) with mortality of 16.8% and 8.4% respectively. Multivariate logistic regression analysis showed odds ratio (OR) of 4.33 (95% CI= 2.533–7.402) for severity in CT chest and 3.9 (95% CI= 3.108–4.895) for use of oxygen therapy, which were independently associated with in-hospital mortality in the study population and the risk was higher in adults aged more than 45 years, OR 2.035 (95% CI = 1.379–3.003). The subgroup of patients with risk factors like multiple comorbidities, fasting plasma glucose >140 mg/dl, and abnormal SBP also had higher levels of inflammatory markers and poorer outcomes.

Conclusion Based on our study, advanced age, extremes of SBP, uncontrolled fasting plasma glucose, and presence of other comorbidities like hypertension, coronary artery disease, chronic kidney disease, thyroid disorders, bronchial asthma, or COPD at admission appeared as risk factors for mortality in people with diabetes and COVID-19. Such patients had higher likelihood of elevated renal parameters, liver enzymes, C reactive protein, and more severe lung involvement, necessitating supplemental oxygen therapy. Interaction of all these parameters leads to higher morbidity and mortality in this population.

Keywords Diabetes mellitus · COVID-19 · Risk factors · Hyperglycemia management · Mortality

Introduction

It has been over 3 years since the COVID-19 pandemic hit the world by storm. Wave after wave, more and more people got infected throughout the world and the associated morbidity and mortality have taken a severe toll on the healthcare system of almost all nations with enormous economic implications. Though the infection rate and severity appear to have reduced now, especially after vaccination, the viral mutations are unpredictable.

Elderly people and people with comorbidities like obesity, diabetes mellitus, systemic hypertension, coronary artery disease, pulmonary disease, chronic kidney disease, chronic hepatic disease, malignancy, and other immunosuppressive disorders are reported to be highly susceptible to the various adverse outcomes and complications of COVID-19 including death [1, 2].

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Diabetes mellitus is a life style disease of epidemic proportions with India having more than 74 million people affected by it as per International Diabetes Federation 2021 Report [3]. It is an immune compromised state [4] and COVID-19 is a peculiar infection with immune dysregulation [5] rendering people with diabetes mellitus more prone to worse prognosis. Longer duration of diabetes mellitus is associated with higher frequency of micro- and macro-vascular complications which in turn leads to a higher risk of morbidity and premature mortality compared to non-diabetic counterparts.

When we consider the number of people infected with the novel coronavirus in India, those with both diabetes mellitus and COVID-19 form a large proportion. Moreover, COVID-19 has also been associated with new onset diabetes [6] and the steroids used in the treatment protocols of COVID-19 are commonly implicated in drug-induced diabetes. This has also led to a surge of patients being newly detected with diabetes mellitus at the time of hospitalization for COVID-19 [7]. The infection appears to exacerbate the already dysregulated pathophysiology in people with diabetes. Not many studies are available in the literature which have been conducted exclusively in diabetes patients to analyze the risk factors and the existing studies mostly include limited parameters for analysis. To understand the profile of COVID-19 patients with diabetes and to ascertain the risk factors for mortality in this population, we propounded to study the impact of diabetes mellitus and hyperglycemia on COVID-19.

Methods

Study design

This retrospective observational study was conducted at Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, India, which is a tertiary care hospital designated for treatment of patients with COVID-19 infection in the state of Tamil Nadu. Data of patients admitted from April 2020 to January 2021 was retrieved. A total of 2640 COVID-19 patients over 18 years of age with either established or newly detected diabetes were included in the study. Patients who left against medical advice and records with incomplete data were excluded from this study.

Data collection

All the details related to history, clinical status, progression of illness, laboratory results, radiological reports, treatment, and outcome of the patients who fulfilled the inclusion criteria were retrieved from the case records of the Medical Records Department and the data was collected using WHO/ICMR forms.

Age, gender, duration of hospitalization, blood pressure at admission, diabetes history, presence or absence of other comorbidities, symptoms of COVID-19, initial fasting plasma glucose (FPG), and mode of oxygen therapy (if given) were recorded. We also noted the chest CT changes, C-reactive protein (CRP) levels, renal parameters, serum electrolytes, liver function test, lactate dehydrogenase (LDH), and creatine kinase (CK) levels. Treatment administered for diabetes was also recorded.

Patients' data was categorized according to their age, sex, and diabetic history. Patients with no prior history of diabetes but fulfilling the criteria for diagnosis of diabetes (FPG $\geq$ 126 mg/dl and 2-h post-prandial plasma glucose $\geq$ 200 mg/dl or random plasma glucose $\geq$ 200 mg/dl with symptoms of diabetes) after admission but before initiating steroid therapy were categorized as newly detected diabetes mellitus. Mode of presentation was categorized into classical COVID-19, atypical COVID-19, and asymptomatic COVID-19. Previous treatment history in established diabetes and presence of other comorbidities was noted. Admission blood pressure (BP) was noted and systolic BP (SBP) was categorized into <90 mm of Hg, 90 to 139, and $\geq$ 140 mm of Hg. Oxygen saturation and mode of oxygen therapy were analyzed in three categories: no oxygen requirements, non-invasive oxygen therapy like oxygen delivered by nasal prongs or masks, high flow nasal oxygen therapy and continuous positive airway pressure (CPAP), and invasive oxygen therapy (mechanical ventilation). CT chest results were categorized based on percentage of lung involvement into normal, mild (1–25), moderate (25–75), and severe (>75). Venous FPG soon after admission was categorized into controlled ($\leq$ 140 mg/dl) and uncontrolled (>140 mg/dl). CRP (in mg/dl) was categorized as <10, 11 to 50, 51 to 100, and >100.

Treatment for hyperglycemia was categorized based on whether the patient was treated with meal plan, oral glucose lowering agents, subcutaneous insulin, or intravenous insulin infusion either individually or in combination. Hyperglycemia was managed based on Institute of Diabetology (IOD) Protocol (Table 1) according to plasma glucose levels and severity of COVID-19 illness. When fasting plasma glucose was less than 126 mg/dl and random glucose was less than 200 mg/dl, medical nutrition therapy alone was advised. Asymptomatic patients and those with mild illness were treated with oral antidiabetic agents like sulfonylureas, metformin, and DPP-4 inhibitors. Subcutaneous insulin was added if CBG (capillary blood glucose) was more than 250 mg/dl. Patients with moderate to severe illness were treated with multiple daily subcutaneous insulin and intravenous insulin infusion, wherever indicated. All patients were monitored for their fasting blood glucose and premeal capillary blood glucose. Total duration of hospital stay was divided into <2 days, 2 days to 2 weeks, and >2 weeks. Patient outcome was categorized as improved and discharged, or death.

Table 1 IOD protocol for management of diabetes in COVID-19 patients at RGGGH

Category	Mild	Moderate	Severe
	Patients on OGLA/insulin	Patients on OGLA/insulin	Patients with complications (like DKA/HHS) or with other comorbidities
Condition of the patient	Mild symptoms of COVID + hemodynamically stable +	Classical symptoms of COVID + hemodynamically stable	Hemodynamically unstable/altered sensorium
	Good glycemic control No ketosis	Uncontrolled hyperglycemia	Uncontrolled hyperglycemia
	Not on respiratory support	Not on respiratory support	Patient on respiratory support
Diet	Oral feeds	Oral feeds	On Ryle's tube feeds/NPO/oral feeds
Others		Patient on methyl prednisolone/HCQS	On methyl prednisolone/dexamethasone/HCQ/toclizumab
Treatment	Continue OHAS/insulin	Check urine ketones/ABG	Check urine ketones/ABG
	Monitor blood glucose every 2–3rd day	Monitor blood glucose daily (2–3 times)	Check CBG hourly
	If patient condition deteriorates, check blood glucose SOS and escalate treatment as applicable	If patient's condition deteriorates, check blood glucose SOS and start on insulin infusion if CBG > 300mg/dl	If fall in blood glucose not satisfactory increase infusion rate by 2–4 units/h
Aim	PG(F) < 110 mg/dl	PG(F) < 140mg/dl	PG(F) < 180mg/dl
	PG(PP)/PG(R)/< 140 mg/dl	PG(PP)/PG(R)<180mg/dl	PG(PP)/PG(R)<250mg/dl

Abbreviations: *IOD*, Institute of Diabetology; *RGGGH*, Rajiv Gandhi Govt. General Hospital; *OGLA*, oral glucose lowering agents; *DKA*, diabetic ketoacidosis; *HHS*, hyperglycemic hyperosmolar state; *NPO*, nil per oral; *HCQS*, hydroxy chloroquine sulfate; *ABG*, arterial blood gas; *CBG*, capillary blood glucose; *PG(F)*, plasma glucose fasting; *PG(PP)*, post-prandial plasma glucose; *PG(R)*, random plasma glucose

Laboratory methods

COVID-19 was diagnosed by real time–reverse transcription polymerase chain reaction (RT-PCR) of nasopharyngeal swab. The plasma glucose value was estimated by hexokinase method, blood urea was measured by urease method, and serum creatinine was measured by Jaffe (kinetic) method using spectrophotometry. CRP was measured by immunoturbidimetric method, CK and LDH by UV assay recommended by IFCC, CK-MB was measured by UV assay-immunoinhibition method, liver function tests by spectrophotometric method, and serum electrolytes by potentiometry method.

Data Analysis The data were entered and the results were tabulated, cleaned, and systematically analyzed by the research scientist of Multi-Disciplinary Research Unit (MMC). The analysis was performed using SPSS version 15.0. Descriptive statistics was used and clinical variables were expressed as median and interquartile range. Since the data was not normally distributed as per the normality test, mean ranks were compared using Mann-Whitney test for continuous variables. Categorical data was expressed as frequencies and percentages. Multivariate logistic regression analysis was used to compare odds ratio (OR) with 95% confidence interval (C*I*) for analysis of potential risk factors for non-survival in COVID-19 patients with diabetes. A two-sided $p < 0.05$ was defined as statistically significant.

Results (Tables 2 and 3)

A total of 2640 subjects fulfilled the inclusion criteria of which 2229 (84.4%) improved and were discharged after treatment whereas 411 (15.6%) succumbed to their illness. Of all the deaths in people with diabetes, 31.4% occurred within 48 h of admission, 62.3% died between 2 days and 2 weeks, and 6.3% died after 2 weeks of hospital stay.

Gender and age distribution

61.5% (1623) of the study population were males and 38.5% (1017) were females. Mortality appeared higher among males than females (68.9% vs. 31.1%). The mortality rate was 7.5% in patients aged ≤ 45 years and increased to 16.8% in those > 45 years (p value = 0.001).

Preexisting vs. newly diagnosed diabetes

85.1% of patients already had diabetes and 14.9% were diagnosed with diabetes at or after admission. Among patients with preexisting diabetes, 83.2% (1868) improved and 16.8% (378) did not survive. Among those who were diagnosed with diabetes after admission, the outcomes

Table 2 Clinical profile and laboratory parameters of survivors and non-survivors

	Characteristics	Total cases *n*=2640 (%)	Survivors *n*=2229 (%)	Non-survivors *n*=411 (%)	*p* value
Sex	Male	1623 (61.5)	1340 (82.6)	283 (17.4)	0.001
	Female	1017 (38.5)	889 (87.4)	128 (12.6)	
Age group (years)	≤45	441 (16.7)	408 (92.5)	33 (7.5)	0.001
	>45	2199 (83.3)	1821 (82.8)	378 (17.2)	
Diabetes status	Preexisting	2246 (85.1)	1868 (83.2)	378 (16.8)	0.001
	Newly detected	394 (14.9)	361 (91.6)	33 (8.4)	
Mode of presentation	Classical COVID symptoms	2167 (82.1)	1803 (80.9)	364 (88.6)	0.001
	Asymptomatic	336 (12.7)	311 (14.0)	22 (5.4)	
	Atypical presentation	137 (5.2)	115 (5.1)	25 (6.0)	
Other comorbidities	Yes	1578 (59.8)	1264 (80.1)	314 (19.9)	0.001
	No	1062 (40.2)	965 (90.9)	97 (9.1)	
Duration of hospital stay	<48 h	345 (13.1)	216 (62.6)	129 (31.4)	0.001
	2 days to 2 weeks	1891 (71.6)	1635 (86.5)	256 (62.3)	
	>2 weeks	404 (15.3)	378 (93.6)	26 (6.3)	
Systolic blood pressure (mm of Hg)	< 90	37 (1.4)	17 (45.9)	20 (54.1)	0.001
	90 to 139	1928 (73.0)	1671 (86.7)	257 (13.3)	
	≥140	675 (25.6)	541 (80.1)	134 (19.9)	
CT chest	Not suggestive	456 (17.3)	441 (96.7)	15 (3.3)	0.001
	Mild	1237 (46.9)	1191 (96.3)	46 (3.7)	
	Moderate	556 (21.1)	461 (82.9)	95 (17.1)	
	Severe	391 (14.8)	136 (34.8)	255 (65.2)	
FPG (mg/dl)	≤140	1927 (73)	1760 (91.3)	167 (8.7)	0.001
	>140	713 (27)	469 (65.8)	244 (34.2)	
CRP (mg/dl)	<10 11–100 >100	519 (19.7) 1763 (66.9) 354 (13.4)	508 (97.9) 1552 (88.0) 167 (47.2)	11 (12.1) 211 (12.0) 187 (52.8)	0.001
Hyperglycemia management	Medical nutrition therapy ± OGLA	520 (19.7)	497 (95.6)	23 (4.4)	0.0001
	SC insulin + OGLA	1916 (72.6)	1600 (83.5)	316 (16.5)	
	SC insulin + insulin Infusion ± OGLA	204 (7.7)	132 (64.7)	72 (35.3)	
Medical oxygen therapy	No requirements	1717 (65.0)	1572 (91.6)	145 (8.4)	0.0001
	Non-invasive oxygen therapy	820 (31.1)	638 (77.8)	182 (22.2)	
	Invasive oxygen therapy	103 (3.9)	19 (18.4)	84 (81.6)	

Data presented as number (percentage)

Abbreviations: *SPB*, systolic blood pressure; *FPG*, fasting plasma glucose; *CRP*, C-reactive protein; *OGLA*, oral glucose lowering agents; *SC*, subcutaneous

were significantly better with improvement in 91.6% (361) and death in 8.4% (33) (p value = 0.001).

Other comorbidities

When we analyzed the association of diabetes with other comorbidities like hypertension, CAD, CKD, liver disease, malignancy, and thyroid dysfunction, it was observed that 59.8% had one or more additional comorbid illness along with diabetes and 40.2% had only diabetes. Among the multiple comorbid illness cohort, 80.1% recovered and 19.9% died and in those with diabetes alone, 90.9% recovered while 9.1% succumbed to their illness.

Mode of presentation

The symptoms at the time of admission were divided into asymptomatic, classical, and atypical COVID-19. Among those who were discharged, 80.9% were admitted with classical symptoms of COVID-19 like fever, cough, breathlessness, and sore throat; 5.1% had atypical symptoms like loss of appetite, nausea, vomiting, and vascular

Table 3 Laboratory parameters of survivors and non-survivors

	Survivors Median (IQR)	Non-survivors Median (IQR)	*p* value
Blood urea (mg/dl)	37 (25–43)	49 (42–78)	0.0001
Serum creatinine (mg/dl)	0.9 (0.7–1.2)	1.2 (0.9–1.7)	0.0001
Serum sodium (meq/l)	134 (132–138)	134 (132–139)	0.353
Serum potassium (meq/l)	4.1 (4–4.7)	4.4 (4–5)	0.0001
SGPT (units/l)	24 (24–25)	24 (22–36)	0.004
ALP (units/l)	63 (63–76.5)	78 (63–113)	0.0001
Total protein (g/dl)	6.9 (6.3–7)	6.8 (6.2–7)	0.0001
Albumin (g/dl)	3.4 (3.4–3.8)	3.4 (3.1–3.5)	0.035
LDH (IU/l)	384 (270–450)	452 (443–657)	0.0001
CRP (mg/l)	34 (11–38.5)	88.7 (34–201)	0.0001
Fasting plasma glucose (mg/dl)	124 (114–135.5)	230 (124–292)	0.0001
SpO2 (%)	97 (96–98)	94 (85–97)	0.0001

Data presented as median (IQR)

Abbreviations: *IQR*, interquartile range; *SGPT*, serum glutamic pyruvic transaminase; *ALP*, alkaline phosphatase; *LDH*, lactate dehydrogenates; *CRP*, C-reactive protein; *SpO2*, oxygen saturation

complications; and 14% were asymptomatic. Among the 411 patients who died 88.6% presented with classical COVID-19 symptoms, 5.4% had atypical COVID-19 and 6.1% were asymptomatic at the time of admission.

Systolic blood pressure

SBP at the time of admission was noted and its analysis revealed that 86.7% (1671) with SBP between 90 and 139 mmHg recovered, whereas 13.3% died. The percentage of recovery and death was 80.1% and 19.9% in SBP more than 140 mmHg subgroup and 45.9% and 54.1% in SBP less than 90 mmHg subgroup respectively.

Other biochemical parameters

Blood urea, serum creatinine, liver enzymes, serum potassium, total protein, albumin, lactate dehydrogenase, creatine kinase, and C-reactive protein levels were significantly abnormal in non-survivors, whereas the difference in levels of sodium was not statistically significant. CRP (in mg/dl) levels were analyzed and revealed that 97.9% of diabetes patients with CRP less than 10 recovered and 2.1% died. When CRP was between 11 and 100 mg/dl, recovery rate was 88.0% and mortality rate was 12.0% and in those with CRP more than 100mg/dl, recovery was seen in 47.2% and death in 52.8%.

CT chest

CT chest was normal in 17.3%, mild changes in 46.9%, moderate involvement in 21.1%, and severe in 14.8% of diabetes patients. 96.7% of patients with normal CT chest and 96.3% with mild changes in CT chest recovered and mortality was 3.3% and 3.7% respectively in each subgroup. Recovery rate was 82.9% in those with moderate lung involvement and mortality rate was 17.1% whereas those with severe lung involvement showed 34.8% recovery and 65.2% mortality.

Hyperglycemia management

Of the total 2640 patients, 520 were controlled by either medical nutrition therapy alone or required oral glucose lowering agents. 95.4% of them recovered and 4.4% died. Another 1916 patients were treated with both oral glucose lowering agents and subcutaneous insulin. Among them, 83.5% recovered and 16.5% died. A total of 204 patients were treated with oral agents, subcutaneous insulin, and insulin infusion and among them, 64.7% recovered while 35.3% died.

Status of FPG

The median FPG among the survivors was 124 with interquartile ratio (IQR) from 114 to 135.5 mg/dl and among non-survivors, it was 230 (IQR 124–292) mg/dl. Analysis of the initial FPG value recorded on admission showed that 91.3% of patients with FPG $\leq$ 140 mg/dl survived and 8.7% died whereas in those with FPG >140 mg/dl, 65.8% survived and 34.2% died.

Medical oxygen therapy

Median SpO2 at admission was 97% (IQR 96–98) among the survivors and 94% (IQR 85–97) among the non-survivors.

A total of 1717 (65.03%) patients did not require any kind of oxygen therapy and among this cohort, 91.6% survived and 8.4% died. Eight hundred twenty (31.06%) patients were treated with non-invasive oxygen therapy, 18.4% of these recovered and mortality was 22.8% in this group. A total of 103 (3.9%) patients were escalated to invasive oxygen therapy, among which 21.2% recovered and 81.6% died.

Multivariate logistic regression analysis (Table 4) was conducted for variables like age, sex, status of diabetes, presence of comorbidities, CT chest abnormality, and necessity of oxygen therapy and it was noted that in adults more than 45 years of age, OR was 2.035 (95% CI 1.379–3.003) and OR with presence of multiple comorbidities was 1.341 (95% CI 1.082–1.662). These patients had higher severity in CT chest with an OR of 4.33 (95% CI 2.533–7.402) and more frequent use of oxygen therapy, OR 3.9 (95% CI 3.108–4.895).

Discussion

Diabetes and COVID-19 to a large extent appear to share a common ground for pathogenesis, with COVID-19 inflammation being superposed on that of diabetes. Preexisting tissue damage from long standing hyperglycemia may worsen the outcome among people with diabetes infected with COVID-19. The analysis of this retrospective observational cohort was instrumental in identifying a variety of risk factors associated with the clinical course, severity, and mortality in patients with diabetes infected with COVID-19 who were hospitalized at Rajiv Gandhi Government General Hospital, which served as a tertiary referral center for patients with COVID-19 during the pandemic. An analysis of the duration of hospital stay revealed that most of the deaths (93.7%) occurred within the first 2 weeks of hospitalization, of which one-third occurred within the first 48 h of admission. On further analysis, it was observed that a significant number of patients requiring critical care with higher oxygen demands had extensive lung involvement, elevated inflammatory markers, or had multiple comorbidities and were aged more than 45 years. Over 92% of the patients less than 45 years of age recovered and the mortality rate showed a gradual rise with increasing age. The higher mortality among elderly was consistent with the data from a New York study where more men over 60 years of age with diabetes and hypertension succumbed to COVID-19 [2].

When genders alone are compared, the results show higher mortality in males than females but when we consider other factors like age, status of diabetes, presence of comorbidities, severity in CT chest, and necessity of oxygen therapy which were included in multivariate analysis, in the scenario of COVID-19, male gender appears protective against adverse outcomes. It is a well-established fact that ACE-2 receptor is an important receptor involved in binding of SARS-CoV-2 in human respiratory epithelium and males not only express higher levels of ACE-2 receptors in lungs, but also do so in more cell types of lungs compared to females. The difference in expression of ACE-2 with age and between genders may be associated with an altered risk of COVID-19 infection [8, 9]. In a study on type2 diabetes and COVID-19 in a critical care setting in England, their subgroup analysis suggested an excess mortality risk in females [10] whereas the Open SAFELY [1] study which analyzed the factors associated with COVID-19 death showed higher mortality in males.

Patients with preexisting diabetes had higher mortality rate when compared to patients with newly detected diabetes. This was concurrent with a study published from Delhi, India, which also reported more severe illness and greater mortality in people with diabetes [11]. This can be explained by the associated comorbidities, longer duration of diabetes, and its complications and impairment of immune response to combat the infection [12].

In our study, presence of multiple comorbidities like hypertension, coronary artery disease, kidney or liver disease, chronic pulmonary disease, and thyroid dysfunction in patients with diabetes was identified to contribute significantly towards a poorer prognosis. Presence of comorbidities correlating with higher mortality was also observed

Table 4 Multivariate logistic regression analysis for COVID-19 mortality in people with diabetes mellitus

Variables	OR	CI 95%	*p* value
1. Sex — male	0.694	0.546–.88	0.003
2. Age >45 years	2.035	1.379–3.003	0.0001
3. Status of DM (preexisting/newly detected)	0.216	0.105–0.444	0.0001
4. Presence of comorbidities (multiple vs. none)	1.341	1.082–1.662	0.007
5. CT chest abnormality (none vs. any)	4.33	2.533–7.402	0.0001
6. Oxygen requirement (none vs. any)	3.9	3.108–4.895	0.0001

Study population (*N*) — 2640

Abbreviations: *OR*, odds ratio; *CI*, confidence interval; *DM*, diabetes mellitus

B for constant

in another study published from our center but that cohort included patients with moderate to severe illness [13]. Similar pattern was also observed in various small and large studies conducted worldwide where advanced age, male gender, preexisting cardiovascular, respiratory, and metabolic disease were independently associated with higher morbidity and in-hospital mortality in patients with COVID-19 [2, 6]. Antecedent tissue damage and hyperinflammatory phenotype in diabetes patients may tend to induce acute organ damage due to COVID-19 and an overall poorer outcome [14].

Blood pressure at the time of admission was identified as an important marker of prognosis. Extremes of systolic BP were associated with negative outcomes, with hypotension being the more significant parameter. The study from Delhi, India, had higher mortality in people with hypertension [11]. A study from England noted that a systolic BP of 140 mm Hg or more was associated with lower mortality rate in COVID-19 patients with type 2 diabetes [15] which is not consistent with our findings.

CRP levels revealed a strong positive correlation with mortality in our study and more than half of the cohort had CRP between 11 and 100 mg/dl. Other biochemical parameters like blood urea, serum creatinine, liver enzymes, lactate dehydrogenase, and creatine kinase were also significantly elevated in non-survivors and revealed a strong correlation with severity of COVID-19 and mortality risk. However, levels of serum sodium had no statistically significant difference between the survivors and non-survivors.

Hyperglycemia increases the risk of serious infection owing to impaired host defenses. In our study, the level of FPG at the time of admission appeared to have a major impact on patient outcome. Better overall glycemic control improved patient outcomes by a considerable margin when affected with COVID-19 just like other infections. The study from England on COVID-19-related mortality was significantly and independently related to the preceding level of hyperglycemia, which correlates with the result of our study [6]. It was also observed that predinner CBG was the first to elevate in patients after initiation of steroid therapy. This pattern was observed both among patients with preexisting diabetes and newly detected diabetes.

Higher grades of CT chest were associated with longer duration of hospitalization, increased necessity for critical care, and higher mortality. Patients with normal CT chest or mild changes had a very good recovery. Almost half of the patients with diabetes had mild lung involvement. Recovery rate was inversely proportional to the severity of CT chest involvement which was consistent with earlier studies [16].

Majority of patients with diabetes presented with classical symptoms of COVID-19 like fever, cough, breathlessness, and sore throat. Contact tracing, mandatory RTPCR testing for travelers, and preoperative patients all helped to detect asymptomatic COVID-19 patients and patients with atypical presentation like loss of appetite, nausea, vomiting, diarrhea, conjunctivitis, and vascular complications [17]. Among these patients, some deteriorated over the course of hospital stay and expired. The importance of contact tracing and testing specially the elderly with diabetes and other comorbidities appears essential so that they can be hospitalized and treated early and aggressively to reduce the morbidity and mortality.

Hypoxia necessitated supplemental oxygen therapy and was associated with more severe illness. About 2/3 of the patients did not require oxygen therapy and recovery was good in this cohort. Mortality increased with increasing oxygen requirement and survival rate (18.4%) was very poor among patients who needed invasive modes of oxygen therapy.

IOD protocol for management of hyperglycemia in COVID-19 patients was included early in the first COVID-19 wave which helped doctors without prior experience in diabetes management to initiate appropriate treatment. Those on invasive ventilation, under intensive care, with hyperglycemia and ketosis, diabetic ketoacidosis, and poor oral intake with CBG more than 400 mg/dl were initiated on intravenous insulin infusion. Outcome was poorer in patients who required insulin infusion for glycemic control at any point of time during hospital stay. The authors do not intend to imply that insulin infusion therapy is detrimental for managing diabetes in these patients but rather intend to point out the fact that poor glycemic control due to severe insulin resistance induced by various factors including steroid therapy, stress, and florid inflammation necessitated high requirements of insulin as infusion to achieve an optimum control of the plasma glucose. This pattern was observed more frequently in those with multiple comorbidities and more severe illness. Aggressive insulin therapy with multiple daily insulin and continuous intravenous insulin is the recommended therapy in ICU setting [18, 19]. In a small study on 59 patients with moderate COVID-19 infection, protective effect of tight glycemic control was shown and morbidity and mortality outcomes were substantially lower in those treated with insulin infusion than in those without [20].

Conclusions

The study suggests that factors like advanced age, extremes of SBP, uncontrolled fasting plasma glucose, and presence of other comorbidities like hypertension, coronary artery disease, chronic kidney disease, thyroid disorders, bronchial asthma, or COPD at admission appeared as risk factors for mortality in people with diabetes and COVID-19. Such patients had higher likelihood of elevated renal parameters, liver enzymes, C-reactive protein, and more severe lung involvement, necessitating

supplemental oxygen therapy. Interaction of all these parameters contributed towards higher morbidity and mortality in this population. Hence, this study highlights the importance of early admission and aggressive management of both hyperglycemia and inflammation in patients with diabetes and COVID-19 infection before inflammation and lung involvement advances. This significantly contributes to our understanding and knowledge regarding prevention of morbidity and mortality in COVID-19 patients with diabetes in the future.

Strengths

To the knowledge of the authors, this study is one of the largest single-center studies to analyze risk factors associated with severity of COVID-19 and its outcomes in patients with diabetes mellitus. A vast array of clinical, biochemical, and radiological parameters have been analyzed which have not been done in any other study. Our study included a whole spectrum of patients affected from mild to severe infection with varying degrees of hyperglycemia and multiple comorbidities.

Limitations

Being a single-center study from a tertiary care center, the results may not have universal representation and may not extrapolate to the general population and may even be variable between institutions. HbA1c was not performed in all patients and hence we could not confirm if the new onset diabetes was undiagnosed diabetes unmasked during hospitalization for COVID-19 or was COVID-19-induced diabetes or stress hyperglycemia. No data on overweight or obesity was included in our study which has been discussed as an important determinant in patient outcome in various studies. Complications in diabetes are associated with longer duration of the disease but we could not determine the duration; hence, data could not be analyzed in relation to duration of diabetes. Severity of illness at presentation, ICU requirement, steroid, and other antiviral/anti-inflammatory drug use which are important players in the prognosis of COVID-19 have not been analyzed.

Future research

The observation that predinner blood glucose is the first to elevate after starting steroid therapy needs to be further studied and analyzed. When multiple parameters are involved which relate to increased morbidity and mortality, a scoring system may be created for COVID-19 which will be helpful in triaging patients, identifying those at higher risk of complications, and guiding in initiating appropriate treatment, thus helping to improve life of patients with diabetes and COVID-19.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Pushpa Saravanan, Ganesan Rajkamal, Ravindra Saravanan, and Rajednran Karthick. The first draft of the manuscript was written by Pushpa Saravanan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Conceptualization: Panneerselvam Dharmarajan, Iyakannu periyandavar. Methodology: Panneerselvam Dharmarajan, Iyakannu periyandavar. Formal analysis and investigation: Ganesan Rajkamal, Rajendran Karthick. Writing the original draft preparation: Pushpa Saravanan, Ganesan Rajkamal, Ravindra Saravanan, Rajendran Karthick, Raman Venkateshwaran, Ammapalayam Kulandasamy Porkodi, Abhideep Saravanan. Data interpretation: Pushpa Saravanan, Ravindra Saravanan, Ranganathan Vasuki, Thayanithi Jayapackiam, Shanmugam Govarthanan, Abhideep Saravanan. Writing review and editing: Panneerselvam Dharmarajan, Iyakannu periyandavar, Ranganathan Vasuki, Ellappan Dhanasekar, Thayanithi Jayapackiam. Data acquisition: Ganesan Rajkamal, Ellappan Dhanasekar, Ammapalayam Kulandasamy Porkodi, Raman Venkateshwaran. Literature search: Ganesan Rajkamal, Shanmugam Govarthanan, Raman Venkateshwaran, Ammapalayam Kulandasamy Porkodi, Abhideep Saravanan. Critical revision: Ravindra Saravanan, Ranganathan Vasuki, Thayanithi Jayapackiam. Review for final approval: Pushpa Saravanan, Panneerselvam Dharmarajan, Iyakannu Periyandavar, Ellappan Dhanasekar. Statistical analysis: Rajendran Karthick.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Institutional Ethical Committee Approval was obtained. IEC, Madras Medical College, Chennai. IEC No. 030420.

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ORIGINAL ARTICLE

The role of serum level of irisin in diabetic retinopathy

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Abstract

Objective Diabetes retinopathy (DR) is one of the top listed world health issues. Screening is usually advised for diabetic cases on a routine basis. However, there is only partial access to ophthalmologists in all parts of the world. Irisin is a myokine that is being investigated for this reason. Our study aims to assess the role of irisin in detecting DR.

Methods and materials In a cross-sectional study, 140 diabetes cases, including 70 patients with diabetic retinopathy and 70 patients free from diabetic retinopathy, were enrolled. Demographic and anthropometric data were gathered, and the patients underwent physical examination. Also, microalbuminuria and serum irisin levels were assessed in both groups. The results were compared between the two study groups.

Results Among the enrolled cases, 55 (39.28%) were men, and 85 (60.72%) were women. The disease duration ($p=0.016$) and the serum level of HBA1c ($p=0.014$) were significantly higher in the diabetic retinopathy group compared to the group free from diabetic retinopathy. Moreover, irisin was significantly higher in the patients free from diabetic retinopathy ($p=0.007$).

Conclusion Irisin may have value in screening diabetic retinopathy; however, further studies are needed for this proposal.

Keywords Irisin · Diabetes retinopathy · Diabetes type 2 · Clinical laboratory tests

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Introduction

According to the newest proposed statistics of the International Diabetes Federation (IDF), in 2021, 537 million adults deal with diabetes around the world. This means around 10% of the world population is affected by this disease, and the number will increase to 643 million in 2030. In this regard, 796,000 deaths due to diabetes were recorded in 2021. Furthermore, 75% of diabetes cases are reported in low- and middle-income countries [1].

Besides the high burden of the disease, many macrovascular and microvascular complications are related to diabetes [2]. Diabetic retinopathy is the most common microvascular disorder in diabetic patients [3], and many diabetic individuals suffer from diabetic retinopathy, which is a major cause of blindness during working age, particularly in low- and middle-income countries. According to the latest epidemiologic reports, the prevalence of diabetic retinopathy among diabetes patients in the Middle East and North Africa (MENA) is 32.9%, which is among the regions with the highest prevalence of this problem [4].

Part of this high burden is due to the lack of screening methods for diabetic retinopathy. It is advised that type 2 diabetic patients should undergo a routine retinal examination anually. However, not all parts of the world have full access to ophthalmologists for this reason, and many investigations have found the lack of cost-effectiveness of this type of screening [5–7].

The proposal of new biomarkers has paved the way toward easy and accessible screening methods for diabetic retinopathy. Irisin is a recently discovered myokine that may be useful in this regard. This hormone was first discovered in an animal model and was proposed to be secreted from mouse and human skeletal muscles. It is reportedly responsible for the white fat's browning and thermogenesis during exercise [8, 9]. It is also reported that there may be some link between low levels of irisin and the development of glucose intolerance [10, 11]. Recently, a study [12] proposed that diabetic retinopathy cases have a significantly higher level of irisin than healthy controls. Controversially, Hu et al. [26] proposed that both serum and vitreous irisin levels were lower in type 2 diabetes with diabetic retinopathy compared to those diabetes cases without diabetic retinopathy and even normal controls. This shows that the evidence level is insufficient for a final conclusion, and controversies have remained. In this cross-sectional study, we assessed serum irisin levels in diabetic patients with and without diabetic retinopathy and evaluated the possible association between the presence of diabetic retinopathy and serum irisin levels.

Methods and materials

Study design

This cross-sectional study was conducted on 140 confirmed type 2 diabetes cases referred to Ghaem Hospital and Hazrat-e-Rasoul outpatient clinic. The sample size was calculated according to the prevalence of retinopathy in Rasoulinejad et al.'s [13] study, considering an alpha error of 5% and a power of 90%. We included patients aged between 30 and 65 years. Those who had level 3 or 4 heart failure, cerebrovascular patients, dialysis cases, sepsis cases, patients with history of retinopathy treatment including laser therapy, intraocular injection, or ocular surgery, patients with previous drug history of glucocorticoid, pioglitazone, or orlistat therapy, patients with systemic inflammatory diseases including rheumatologic disorders, and those who had glycated hemoglobin (HBA1c) levels of > than 10% were excluded. Recent research has revealed that HbA1c levels greater than 10% are a significant and strong predictor of diabetic retinopathy [14]. Therefore, we considered this group of patients as very high-risk patients who should undergo retinal examination periodically. Pregnancy was an additional exclusion criterion in our study.

The patients were examined by an expert retinal ophthalmologist and were classified into two groups of 70, including those who had confirmed retinopathy and those without retinopathy. The case and control groups were age- and sex-matched. To confirm retinopathy, the retinal blood flow and retinal artery diameter were assessed using a laser Doppler velocimeter (LDV).

Data gathering

Using a predesigned checklist, data including age, gender, diabetes family history, age at the diagnosis of diabetes, duration of the disease, underlying diseases, and other chronic diabetes complications were extracted. Then, patients underwent physical examination; weight, height, body mass index (BMI), waist circumference, and systolic and diastolic blood pressure were measured. To measure fasting blood sugar, creatinine, hemoglobin A1c, triglyceride, HDL, LDL, and insulin serum levels, 5 cc of brachial vein blood was obtained. Moreover, the presence of microalbuminuria was assessed using a random urine sample. Furthermore, serum irisin levels were assessed using an adequate blood sample.

Ethics

All the patients were provided with written informed consent. They were free to discontinue the study whenever they wanted to do so. Furthermore, the patients' names and identity factors were removed from the dataset, and the data were coded to ensure confidentiality. All the steps of the study were in accordance with Helsinki's declaration and were approved by the ethics committee of Mashhad University of Medical Sciences (Ethic Code: IR.MUMS.MEDICAL.REC.1397.599).

Statistical analysis

The data were entered in SPSS software version 20 for further analysis. The frequency and percent of the qualitative data and the mean and standard deviation of qualitative data were measured. Qualitative data were compared between the two groups using chi-square and the exact Fisher's test. Quantitative data were compared between the two study groups using an independent sample *t*-test. The correlation between age, BMI, and different laboratory findings and the irisin level was assessed using Pearson's test. Eventually, to adjust for possible confounders, a multivariate logistic regression model adjusting for age, gender, HbA1C, and disease duration was assembled to evaluate the association of serum irisin levels with the presence of diabetic retinopathy (dependent variable). A *p*-value less than 0.05 was considered significant.

Results

Among the 140 type-2 diabetes patients enrolled in this study, 55 (39.28%) were male, and 85 (60.72%) were female. Table 1 shows the comparison between diabetic retinopathy and non-diabetic retinopathy groups. As evident, the disease duration ($p=0.016$) and HBA1c ($p=0.014$) were significantly higher in patients with diabetic retinopathy than those without it. Irisin serum levels were significantly higher in patients free from diabetic retinopathy compared to those with diabetic retinopathy($p=0.007$). Figure 1 demonstrates the comparison of irisin levels between the two study groups.

In a multivariable regression model, serum irisin levels were inversely associated with the presence of diabetic retinopathy (OR = 0.893 95% CI; 0.673–0.984), suggesting its protective role against the occurrence of diabetic retinopathy (data not shown in table).

Table 2 assesses the correlation between different laboratory parameters and irisin levels. A positive correlation between irisin level and HDL level was identified, while LDL was negatively correlated with irisin levels in all assessment groups.

Table 1 Comparison of the data between diabetic retinopathy and non-diabetic retinopathy groups

Features	Patients with diabetic retinopathy	Patients free from diabetic retinopathy	*p*-value
Age	55.21 ± 7.58	54.81 ± 8.24	0.765
Body mass index	28.15 ± 4.06	29.34 ± 4.37	0.116
Waist circumference	100.62 ± 8.75	101.60 ± 10.77	0.577
Height	158.19 ± 25.46	161.32 ± 9.48	0.412
Weight	73.26 ± 10.35	75.59 ± 59	0.169
Disease duration	11.39 ± 6.01	8.77 ± 6.62	0.016
Fast blood sugar	162.99 ± 66.67	168.97 ± 56.49	0.569
Hemoglobin A1c	8.07 ± 1.56	7.48 ± 1.20	0.014
HDL	43.47 ± 8.72	45.96 ± 10.78	0.137
LDL	96.56 ± 33.43	98.84 ± 28.32	0.665
Triglyceride	143.82 ± 75.45	158.10 ± 67.41	0.241
Creatinine	1.03 ± 0.28	1.06 ± 0.30	0.514
Microalbuminuria	67.11 ± 224.06	45.17 ± 77.30	0.449
Insulin	12.22 ± 8.49	9.60 ± 4.66	0.15
Systolic blood pressure	124.19 ± 15.89	123.38 ± 17.11	0.781
Diastolic blood pressure	77.26 ± 11.76	74.56 ± 9.61	0.153
Irisin	10.10 ± 7.08	14.32 ± 10.76	0.007

Discussion

It is only a decade since the introduction of irisin; however, several roles have been proposed for this hormone. The name and the origin were first proposed by Boström et al. [9], who named the peptide according to the good news goddess in ancient Greece, whose name was Iris. This 12 kDa peptide is produced from the cleavage of fibronectin III domain-containing protein 5 (FNDC5) during physical activity or muscle shivering. Animal studies have revealed that irisin increases O_2 consumption, CO_2 production, and heat production [15]. Furthermore, irisin promotes energy expenditure by means of white adipose tissue browning [16]. In this regard, it brings good news in the case of metabolic hemostasis [17].

This adipo-myokine increases the uptake of glucose by skeletal muscle and has a positive effect on glucose and lipid metabolism. In this regard, adiponectin lowers insulin resistance [17, 18]. The production of adiponectin is regulated by a cascade that is initiated with peroxisome proliferator–activated receptor (PPAR)-γ coactivator 1α (PGC1α) secretion and further triggers fibronectin type III domain-containing 5 (FNDC5). In fact, it is the cleavage of the FNDC5 that results in the production of irisin. The released irisin further upregulates uncoupling protein 1 (UCP1) expression and

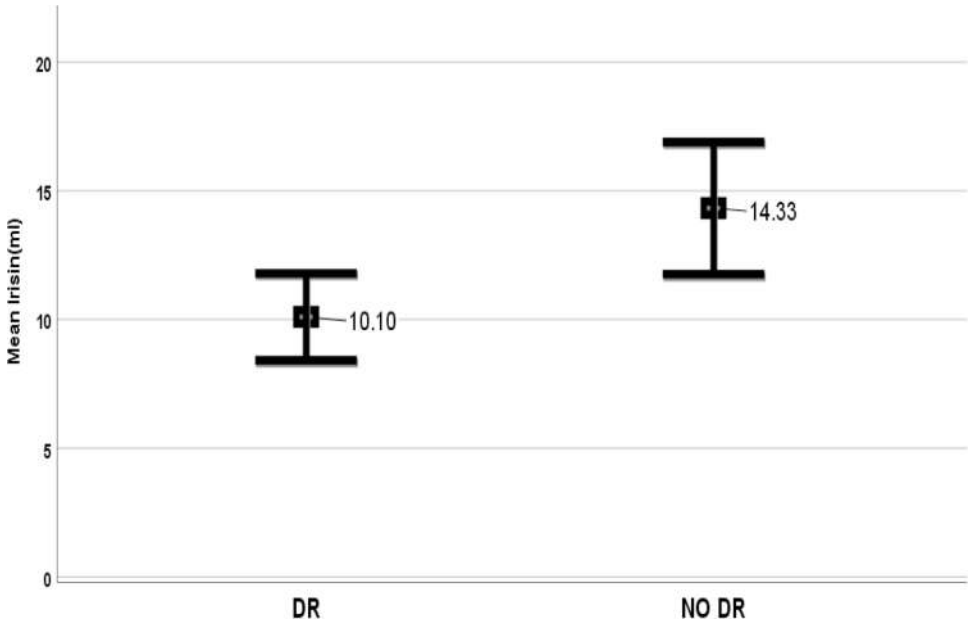

Fig. 1 Comparing the mean irisin level between diabetic retinopathy (DR) cases and patients without retinopathy (NO DR)

thus increases fatty acid oxidation, which in turn results in lower body weight and a lower rate of insulin resistance [19].

Aside from the benefits listed above, irisin may be useful as a diagnostic or predictive marker in diabetes complications. A negative association between irisin level and serum creatinine has been proposed among studies [20, 21]. Even so, there is an inverse association between irisin and the stage of nephropathy [22, 23]. A recent meta-analysis in 2021 reported that type 2 diabetes patients with microalbuminuria and macroalbuminuria have significantly lower levels of irisin compared to normoalbuminuric diabetic cases. Moreover, the irisin level in macroalbuminuria cases was significantly lower compared to the microalbuminuria cases [24].

The role of irisin in diabetic retinopathy is also being investigated. Our study revealed that patients without diabetic retinopathy had a higher irisin level compared to diabetic retinopathy cases. Moreover, there was a negative association between irisin level and LDL and a positive association between irisin and HDL. Similar findings are proposed by Tarboush et al. [25]. They conducted a case–control study that showed a higher level of irisin in the non-diabetic retinopathy group compared to those with retinopathy. Moreover, irisin was negatively correlated with hemoglobin A1c and LDL levels and was positively correlated with HDL levels. In contrast with our findings, Hu et al. [26] proposed that non-diabetic retinopathy diabetic patients have lower serum and vitreous levels of irisin compared to diabetic retinopathy cases.

Irisin levels did not correlate with age in the total population study or in gender-specific evaluations (gender-specific data not shown). Similarly, Sanchis-Gomar et al. [27] did not observe any correlation between age and serum irisin levels, in either diabetics or morbidly obese patients

Table 2 Correlation between different laboratory parameters and irisin level

Features	Diabetic retinopathy group		Non-diabetic retinopathy group		Total	
	R	*p*	*R*	*p*	*R*	***p***
Age	0.046	0.705	0.01	0.932	0.017	0.841
Body mass index	0.11	0.39	0.05	0.697	0.101	0.259
Disease duration	−0.129	0.252	0.28	0.02	−0.21	0.521
Fast blood sugar	0.135	0.266	0.049	0.686	0.092	0.281
Hemoglobin A1c	−0.092	0.449	−0.079	0.513	−0.124	0.145
HDL	0.38	0.001	0.31	0.01	0.33	0.001
LDL	−0.41	0.001	−0.28	0.01	−0.31	0.001
Triglyceride	0.013	0.913	−0.057	0.642	−0.002	0.978
Total cholesterol	−0.042	0.835	−0.046	0.851	−0.043	0.779
Creatinine	−0.091	0.454	−0.031	0.803	−0.038	0.653

aged between 50 to 70 years. In contrast, Ruan et al. [28] reported a negative correlation between serum irisin levels and age in subjects aged between 17 and 82.

Our study population had an age range of 30–65 years, which is similar to the one of Sanchis-Gomar et al.'s [27] study and is notably narrower than Ruan et al.'s [28]. It is possible that these correlations are more observable in populations comprising individuals with a broader age range, encompassing both younger and older individuals, as seen in Ruan et al.'s [28] study. Further research is thus suggested for the evaluation of irisin serum levels' correlation with age.

The role of irisin in diabetic nephropathy is not fully investigated, and our study can add to the present findings. Wang et al. [29] tried to investigate the role of irisin in diabetic retinopathy by assessing different factors. They proposed that through investigating this role in the early stage of retinopathy, using partial correlation and regression analysis. IL-17A was proposed as a risk factor for diabetic retinopathy, and irisin may pose some anti-IL-17A effect. These anti-inflammatory features may be a key to the protection against progressive retinopathy.

It seems a long way toward the proposal of irisin as a marker of retinopathy. Moreover, its role in the pathophysiology of diabetic retinopathy is not fully understood. Our study added some evidence to this role, but it has some limitations. First of all, the study was conducted in only one ethnicity, and secondly, we could not assess the level of other markers that may play a part in diabetic retinopathy. However, our study is among the few studies conducted in this field and has a good sample size, too.

Conclusion

There may be a protective role for irisin in cases of preventing diabetic retinopathy, as the level of irisin in retinopathy cases was higher in our findings. Another finding of our study was the improvement of lipid profile, with decreasing LDL levels and increasing HDL. All these findings may be a part of the anti-inflammatory effects of this hormone. However, further studies are needed to prove these findings and find the best-proposed pathophysiology.

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Declarations

Ethical Clearance The Mashhad University of Medical Sciences ethics committee fully approved the current research under the code: IR.MUMS.MEDICAL.REC.1397.599.

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

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ORIGINAL ARTICLE

Isocaloric diet is as effective as the hypocaloric diet in ameliorating symptoms in PCOS patients

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Abstract

Background There is no randomized study comparing the isocaloric (ICHD) and hypocaloric diet (HCD) in PCOS subjects.
Objective To compare ICHD and HCD in weight-matched PCOS subjects.
Methods PCOS subjects were randomized to receive either a HCD or ICHD. Clinical, biochemical, hormonal and dietary assessments were performed at baseline, 3 months and at one year.
Results There were 168 PCOS subjects randomized to receive either ICHD ($n = 84$) or HCD ($n = 84$). We observed that while the hypocaloric diet was more effective in reducing weight, both the diets were effective in improving clinical symptoms. Around 50% patients showed improvement in clinical symptoms at 3 months and 30% were on diet alone therapy at one year in both the groups. The effect on hirsuitism was modest by both the diets at one year. The effectiveness of both the diets was similar with both the per protocol and the Intention to treat analysis; nevertheless, there was a greater loss to follow-up in patients on ICHD with high baseline caloric intakes.
Conclusion HCD and ICHD are equally effective in as many as 30% patients with mild symptoms over long term. Patients having higher caloric intakes at baseline should not be offered isocaloric diets.

Keywords Dietary pattern · Meal timing · Isocaloric diet · Hypocaloric diet · PCOS · Total caloric intake

Introduction

PCOS is a common disorder of the reproductive age group presenting with varying degrees of hirsuitism and oligoanovulation. Obesity can exacerbate the metabolic and reproductive abnormalities that are associated with the disorder. Weight loss in obese PCOS patients reduces circulating androgens and raises SHBG, enhances insulin sensitivity, and improves menstrual cyclicity and fertility rates [1–3].

There is no standardized prescribed diet for PCOS owing to the regional and cultural differences in the meal composition and diet preferences. The short-term beneficial effects of various diets including hypocaloric, isocaloric, low carbohydrate, and high protein diets have been studied [4–8]. However, there is paucity of randomized long-term studies comparing the different forms of diet.

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As compared to the Western cohort, we observed that Indian patients with PCOS were younger and had a lower BMI [9]. Indian diet differs from the West in having a greater proportion of carbohydrates and less proteins. In our previous study, we observed that our PCOS patients had comparable caloric and macronutrient distribution; however, they differed in food quality and meal timings in comparison to weight-matched controls [10]. It is not known if just a modification of relative macronutrient proportion is sufficient for these patients or a reduction of calories is also additionally needed. The present study aims to study the effect of a hypocaloric diet(HCD) compared to isocaloric healthy diet (ICHD) over a period of one year on different components of polycystic ovarian syndrome, i.e., obesity, menstrual cycles, hirsuitism, and biochemical parameters.

Materials and methods

Patients with PCOS diagnosed as per Rotterdam Criteria were included. Exclusion criteria were as follows [1]: endocrinological problems including hypothyroidism, androgen secreting tumors, Cushing's, prolactinoma, or CAH [2]; pregnant or lactating women [3]; those on oral

contraceptives or antiandrogens or insulin sensitizers within the past 6 months [4]; and use of antidepressants or lipid lowering medications. Blood pressure, height, weight, BMI, waist–hip ratio, hirsuitism score (by Ferriman Gallaway scoring), and acneform scoring as previously described [10, 11].

Blood was drawn at 0 h and 2 h after the oral administration of 75 gm glucose for both the subjects and controls. Glucose and lipid profiles were analyzed the same day and samples for insulin and other hormones (LH, FSH, testosterone, free testosterone, DHEAS, androstenedione and SHBG,TFT, Cortisol, ACTH, prolactin, and 17 OHP) were stored at – 20 °C and analyzed later.

Making a dietary record

PCOS subjects were trained by two dedicated dieticians to make a record of their eating patterns as previously described [10]. This included showing them models and pictures representing sample items viz chapattis, bowls, and glasses of different sizes commonly used in Indian households. The subjects were also asked to record the timings of the three major meals, tea, and mid meal snacks taken throughout the day. The mean of dietary recall over the past two days was noted. Dieticians spent additional 45 min to an hour on each patient enquiring about their dietary patterns, probing junk intake, physical activity, cross checking timings, and quantity of the meals taken. The total caloric intake along with differential carbohydrate, protein, fat, and fiber intake was calculated as per Indian standards established by NIN (National Institute of Nutrition) [12, 13]. Since NIN provides the nutritive data for raw foods, the relative proportion of various ingredients used in recipes for cooked foods was taken from recipe book published by the Institute of Home Economics [14]. The nutritional content of packaged foods was taken from the information provided overleaf.

Patients and controls were categorized as having late breakfast if they were taking breakfast after 10:00 AM, late lunch if they were having lunch after 3:00 PM, and late dinner if they were having dinner after 10:00 PM [15]. Physical activity in terms of number of days of regular physical exercise per week was categorized as previously described [16].

Dietary intervention

Subjects were randomized to receive either a hypocaloric diet (HCD) or an isocaloric healthy diet (ICHD) based on computer generated random number table. The ICHD diet

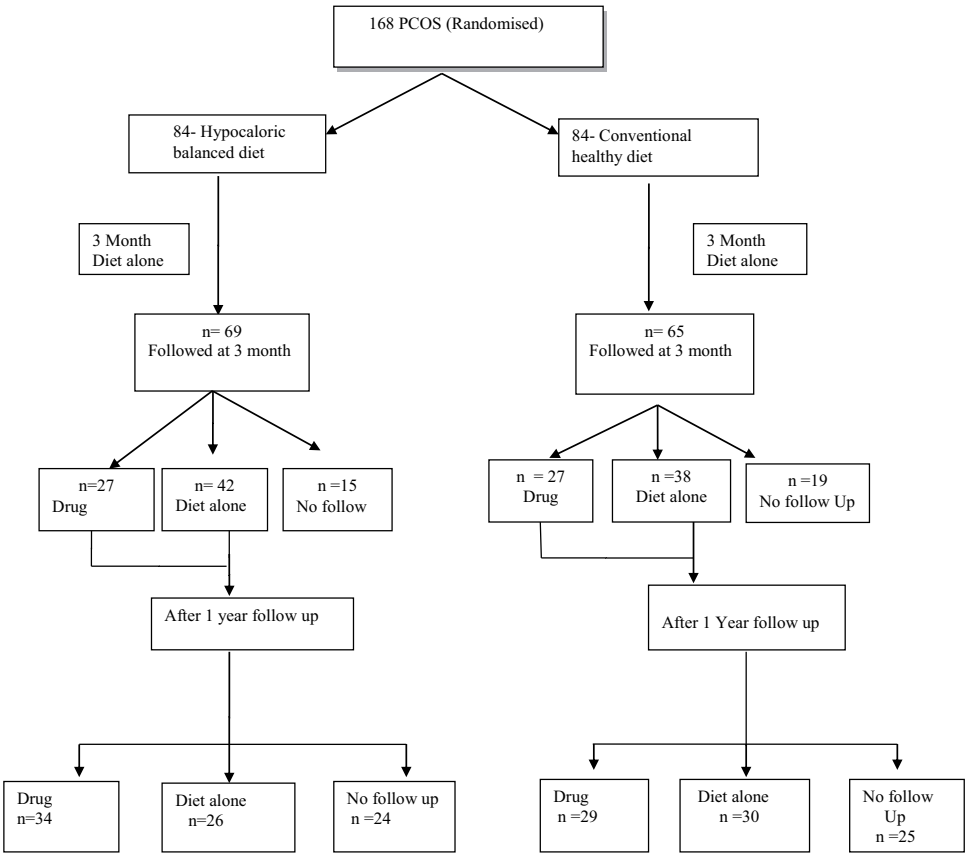

Fig. 1 Outline of PCOS patients

Table 1 Comparative clinical and biochemical parameters among PCOS patients assigned isocaloric diet versus hypocaloric diet

	Hypocaloric diet ($n = 84$)	Isocaloric diet ($n = 84$)	p-value
Age (years)	22.88 ± 5.23	24.74 ± 5.54	0.024*
BMI (kg/m^2)	26.38 ± 6.73	25.87 ± 6.14	0.64
Hirsuitism	56 (66.7%)	57 (67.9%)	0.50
Irreguiar periods	56 (66.7%)	54 (64.3%)	0.44
Acneform eruptions	52 (61.9%)	48 (57.1%)	0.31
Hirsuitism score	4.9 ± 3.19	7.6 ± 5.9	0.051
Menstrual cyclicity	63.58 ± 42.9 (45)	64.6 ± 45.27 (50)	0.76
WHR	0.86 ± 0.07	0.86 ± 0.08	0.86
RtOvvol (ml)	9.33 ± 3.83	10.36 ± 5.16	0.47
LOV	9.26 ± 4.62	9.29 ± 4.21	0.88
BP systolic (mm Hg)	117.58 ± 7.46	117.99 ± 7.95	0.79
BP diastolic (mm Hg)	72.13 ± 7.27	70.33 ± 6.33	0.06
GTT 0hour (mg/dl)	81.24 ± 11.02	78.33 ± 12.21	0.07
GTT 2hour (mg/dl)	103.00 ± 22.49	110.33 ± 29.48	0.08
Total cholesterol (mg/dl)	161.88 ± 35.05	158.56 ± 28.19	0.76
HDL cholesterol (mg/dl)	42.95 ± 9.13	45.96 ± 9.18	0.04*
LDL cholesterol (mg/dl)	96.80 ± 28.67	92.76 ± 23.22	0.32
VLDL cholesterol (mg/dl)	20.67 ± 8.23	19.76 ± 8.57	0.37
TRIGLYCERIDE (mg/dl)	103.87 ± 41.16	98.99± 42.64	0.36
TSH (UU/ml)	3.53 ± 6.51	2.93± 1.72	0.85
FSH (mIU/ml)	5.66 ± 1.91	5.48 ± 1.86	0.18
LH (mIU/ml)	10.88 ± 12.18	8.14 ± 12.44	0.01*
Prolactin (ng/ml)	16.56 ± 10.01	17.90 ± 12.58	0.97
Testosterone (nmol/l)	1.37 ±.72	1.32 ±.71	0.50
DHEAS (µg/ml)	2.29 ± 1.00	2.10 ± 1.19	0.06
Cortisol (nmol/l)	257.99±117.26	241.08 ± 99.09	0.59
Insulin 0H (µIU/ml)	14.33 ± 11.08	13.12 ± 8.88	0.59
Insulin 2H (µIU/ml)	54.81 ± 32.41	57.65 ± 41.00	0.85
17OHP (ng/ml)	2.69 ± 2.58	2.59 ± 2.28	0.32
Free testosterone (pg/ml)	3.17 ± 1.71	2.84 ± 1.70	0.13
SHBG (nmol/l)	83.58 ± 52.19	82.89 ± 54.82	0.92
Androstenedione (ng/ml)	3.60 ± 2.23	3.34 ± 2.28	0.16
Exercise >3 days/ week	22(26.9%)	15(17.8%)	0.30

*Denotes p value less than 0.05

was isocaloric to the calories being consumed by the subject prior to intervention. HCD diet was prescribed as either an energy reduction of 500 kcal/day or intake of 20 kcal /kg for overweight and obese PCOS subjects and 25 kcal/kg for normal weight individuals whichever was lower [7]. The macronutrient contents of both the diets were similar (60% carbohydrates, 15% proteins, and remaining by fat). A schedule of three main meals and at least 2 snacks was introduced for both the groups. They were advised to refrain from high glycemic foods and incorporate salads and fruits in their diet. A sample menu plan was provided for each patient, and the participants were encouraged to select foodstuffs suited to their preferences. Subjects were also encouraged to not miss their meals and take their major meals at appropriate timings (breakfast before 10:00 AM, lunch before 3:00 PM, and dinner before 9:00 PM). Participants in both the groups were encouraged to do regular physical activity of 20 min of walking for at least 3 days a week [16].

PCOS subjects were asked to make a hospital visit at 3 months, 6 months, and one year. During these visits, clinical symptoms, physical examination, and two-day dietary recall were performed. A verbal reinforcement for dietary compliance was done. Samples for biochemical and hormonal assessment were taken at three months and one year. Patients unsatisfied with the clinical response to diet therapy were put on medications (antiandrogens or insulin sensitizers or OCP).

Statistical analysis

Data was entered in excel sheet and analyzed in SPSS (Version 20 Chicago, IL, USA). The baseline mean values and delta changes in parameters from baseline in the two groups were compared with the independent sample Student's t test and the Mann–Whitney U test. Chi square test was used for non-parametric variables. Paired t test was used to compare the changes in mean values in parameters at three months. One-way repeated measure analysis of variance was used to compare the parameters at multiple timepoints. Multiple comparisons using Bonferroni corrections were performed to identify which pair showed significance. Further, the variables, failing normality assumption, were treated with Friedman's one-way repeated measures ANOVA. Both the groups were also compared by the intention to treat or per protocol analysis. p value less than 0.05 was considered as significant.

Assays

DHEAS, prolactin, and thyroid function tests were performed by chemiluminescence (Beckman CoulterDxI, 600). Insulin and testosterone were analysed by chemiluminescence (Vitros ECI, Johnson and Johnson). Plasma androstenedione, free testosterone, and SHBG were performed by ELISA (on Bio-Rad Evolis, Twin Plus)

Table 2 Mean changes in the clinical, biochemical, and hormonal parameters in PCOS subjects on isocaloric and hypocaloric diet at 0 and 3 months

PCOS patients on hypocaloric diet ($n = 69$)				PCOS patients on isocaloric diet ($n = 65$)		
Parameters	0 month	3 months	p value*	0 month	3 months	p value*
Weight (kg)	66.06 ± 18.09	64.71 ± 18.83	0.004*	62.39 ± 13.20	63.06 ± 13.57	.74
Cyclicity	67.47. ± 46.01(45)	53.76 ± .40.64(35)	0.00*	66.26 ± 47.38(47.5)	48.15 ± 27.53(35)	.00*
MODHIRSCO	5.33 ± 3.12	4.82 ± 3.07	0.002*	7.48 ± 6.04	7.43 ± 5.91	.28
Acne code	1.33 ± 0.47	0.61 ± 0.64	0.00*	1.21 ± 0.41	0.52 ± 0.50	.00*
GTT 0hour (mg/dl)	81.88 ± 10.95	81.55 ± 11.70	0.99	78.62 ± 12.27	79.17 ± 10.76	.78
GTT 2hour (mg/dl)	102.73 ± 22.52	108.00 ± 28.53	0.14	108.81 ± 30.49	108.28 ± 30.60	.88
Insulin 0H (μIU/ml)	13.67 ± 9.53	10.32 ± 5.25	0.01*	13.63 ± 9.41	10.37 ± 6.17	.01*
Insulin 2H (μIU/ml)	56.22 ± 31.58	55.60 ± 33.47	0.96	56.78+ 34.50	61.01 ± 46.63	.45
Total cholesterol (mg/dl)	162.33 ± 36.88	163.46 ± 34.83	0.61	155.88 ± 26.31	156.68 ± 29.04	.78
Triglyceride (mg/dl)	106.09 ± 41.66	107.96 ± 68.33	0.61	97.65 ± 41.61	96.97 ± 40.05	.90
TSH (UU/ml)	2.78 ± 1.64	2.89 ± 1.92	0.61	2.81 ± 171	3.45 ± 2.21	.004*
FSH (mIU/ml)	5.62 ± 1.61	5.61 ± 2.48	0.87	5.47 ± 1.85	5.49 ± 1.63	.08
LH (mIU/ml)	10.17 ± 10.46	9.12 ± 5.98	0.93	6.98 ± 5.07	7.71+6.01	.31
Prolactin (ng/ml)	17.18 ± 10.84	19.23 ± 13.50	0.51	17.29 ± 10.09	17.55 ± 9.33	.88
Testosterone (nmol/l)	1.35 ± .66	1.32 ± .43	0.98	1.29 ± .65	1.34 ± .74	.64
Free testosterone (pg/ml)	3.25 ± 1.78	3.19 ± 1.601	0.72	2.91 ± 1.83	2.56 ± 1.34	.23
SHBG (nmol/l)	86.98 ± 54.42	84.19 ± 50.88	0.73	92.09 ± 55.73	98.72 ± 63.77	.84
Androstenedione (ng/ml)	3.69 ± 2.36	3.68 ± 2.20	0.92	3.33 ± 2.29	3.29 ± 1.76	.72
AVR. energy (kcal)	1870.92 ± 453.77(1801.67)	1400.30 ± 310.89(1417.80)	0.00*	1510.85 ± 301.05(1563.36)	1650.39 ± 376.89(1618.98)	.00*
CHO(%energy	57.77 ± 5.00 (58.42%)	59.98 ± 15.86 (59.17%)	0.23	58.37 ± 7.7 (57.76%)	58.63 ± 5.87 (59.16%)	.80
AVR. protein (gm)	60.09 ± 19.43(54.50)	50.27 ± 13.45(47.83)	0.00*	48.57 ± 13.25(47.93)	55.26 ± 13.65(52.71)	.00*
PRO(%energy)	12.68 ± 2.18 (12.07%)	14.44 ± 2.72 (13.79%)	0.00*	12.91 ± 2.80 (12.28%)	13.39 ± 1.75(13.15%)	.13
FAT(%energy)	29.75 ± 5.51 (29.01%)	27.42 ± 4.50 (26.86%)	0.002*	29.92 ± 5.20 (30.18%)	28.27 ± 6.33 (26.79%)	.04*
Total fiber (gm)	32.77 ± 11.41(32.07)	34.35 ± 8.72(34.58)	0.19	29.23 ± 7.13(29.84)	38.63 ± 11.55(36.60)	.00*
Soluble fiber (gm)	6.29 ± 2.37(5.80)	7.15 ± 2.15(6.92)	0.00*	5.62 ± 1.52(5.88)	7.71 ± 2.76(7.34)	.00*
Pulse intake (gm)	126.74 ± 85.15(103.29)	143.26 ± 94.37(139.67)	0.22	112.90 ± 88.46(92.10)	168.72 ± 101.54(162.50)	.00*
Fruit intake (gm)	46.88 ± 62.52(17.90)	73.01 ± 56.33(67.20)	0.004*	25.89 ± 46.93(.00)	80.47 ± 86.20(51.55)	.00*
Dairy intake (gm)	188.14 ± 151.19(138.10)	162.01 ± 117.46 (145.37)	0.10	131.24 ± 140.22(108.75)	176.36 ± 135.82(138.1)	.02*
Junk intake (gm)	340.51 ± 201.89(305.28)	99.33 ± 105.11(67.46)	0.00*	273.89 ± 196.43(283.47)	156.46 ± 135.48(141.16)	.00*
Late/missed breakfast	24 (34.7%)	14 (20.2%)	0.026	33 (50.7%)	10 (15.3%)	.001*
Late/missed lunch	21 (30.4%)	10(14.4%)	0.011	20 (30.7%)	5 (7.69%)	.001*
Late/missed dinner	8 (11.5%)	3 (4.3%)	0.052	10 (15.3%)	4 (6.1%)	.042*

*Denotes p value less than 0.05

Results

Figure 1 gives the outline of PCOS patients randomized into two diet groups and their follow-up at 3 months and at one year. 168 patients were randomized to receive either HCD or ICHD ($n = 84$ in each group). Of 168 PCOS subjects, 134 followed at 3 months (69 in the HCD and 65 in the ICHD). 42(50%) patients in the HCD and 38(45%) in the ICHD continued with diet therapy at 3 months, while 27 patients in both the groups required drug therapy. 119 patients followed at one year, 60 in the HCD, and 59 in the ICHD diet subgroup. Of 49 subjects who did not follow at one year, 17 refused to come, 8 were transferred out of Delhi, 4 became pregnant, and 20 patients did not pick up telephone calls.

Table 3 Clinical, biochemical, and dietary profile of PCOS patients for PCOS subjects on hypocaloric diet only for one year

Parameters	0 month ($n = 26$)	3 months ($n = 26$)	1 year ($n = 26$)	p value*
Weight (kg)	64.31 ± 13.16	62.83 ± 14.37	63.52 ± 14.62	0.18
Cyclicity (months)	55.15 ± 32.87(50)	38.21 ± 10.68(35)	39.04 ± 14.19(35)	0.18[a,b]
MODHIRSCO	5.21 ± 2.74	4.47 ± 2.71	4.00 ± 1.987	0.004*[a]
Acne code	1.20 ± 0.41	0.57 ± 0.64	0.40 ± 0.63	0.00*[a,b]
GTT 0hour (mg/dl)	78.38 ± 9.54	80.79 ± 8.49	83.50 ± 13.31	0.025
GTT 2hour (mg/dl)	103.67 ± 21.00	100.54 ± 20.63	110.75 ± 23.80	0.64
Insulin 0H (μIU/ml)	12.15 ± 9.01	8.98 ± 4.38	8.25 ± 6.99	0.81
Insulin 2H (μIU/ml)	48.70 ± 28.75	53.48 ± 31.84	44.30 ± 35.05	0.44
Total cholesterol (mg/dl)	160.08 ± 40.63	160.08 ± 34.31	165.60 ± 45.74	0.44
Triglyceride (mg/dl)	95.54 ± 33.06	88.58 ± 52.73	111.80 ± 56.90	0.10
TSH (UU/ml)	2.88 ± 1.80	2.56 ± 1.21	2.54 ± 0.91	0.56
FSH (mIU/ml)	5.78 ± 2.17	6.36 ± 3.25	7.60 ± 3.25	0.02*[b]
LH (mIU/ml)	9.24 ± 9.36	9.21 ± 6.66	10.73 ± 12.06	0.94
Testosterone (nmol/l)	1.16 ± 0.51	1.25 ± 0.50	1.39 ± 0.79	0.37
DHEAS (μg/ml)	2.25 ± 0.95	2.83 ± 1.35	2.24 ± 0.84	0.18[c]
Free testosterone (pg/ml)	2.82 ± 1.19	3.39 ± 2.10	2.65 ± 1.56	0.44
AVR. energy (kcal)	1864.35 ± 526.25	1476.81 ± 358.73	1530.58 ± 384.51	0.00*[a,b]
CHO (%energy)	58.24 ± 5.24	57.99 ± 3.77	57.66 ± 6.02	0.88
PRO (%energy)	12.06 ± 1.25	14.01 ± 2.52	12.85 ± 1.59	0.004*[a]
FAT (%energy)	29.28 ± 5.20	27.48 ± 4.20	29.50 ± 6.08	0.42
Total fiber (gm)	34.11 ± 13.26	35.55 ± 9.47	31.13 ± 8.18	0.42
Soluble fiber (gm)	6.38 ± 2.57	7.18 ± 2.25	6.28 ± 2.17	0.44
Fruit intake (gm)	56.09 ± 68.09	86.65 ± 59.24	58.36 ± 94.06	0.06
Junk intake (gm)	307.02 ± 192.65	103.58 ± 116.50	208.57 ± 168.23	0.00*[a,b,c]
Late/missed breakfast	5 (19.2%)	4 (15.3%)	4 (15.3%)	0.747
Late/missed lunch	8 (30.27%)	5 (19.2%)	2 (7.69%)	0.111[b]
Late/missed dinner	3 (11.5%)	2 (7.69%)	1 (3.8%)	0.350

Parameters given as mean ± SD

*p value significant by repeated measures ANOVA or $a = 0$ m vs. 3 m; $b = 0$ m vs. 1 yr; $c = 3$ m vs. 1 y (post hoc analysis)

26 (31%) in the HCD and 30 (34%) in the ICHD group were satisfied with diet alone therapy at one year, while 34 patients in HCD group and 29 patients in the ICHD group required drug therapy at one year. While there was no difference in the effectiveness of the two diets either by the intention to treat or per protocol analysis at one year, around 30% patients in each group did not follow-up at one year.

Table 1 gives the comparative clinical and hormonal data of the HCD and ICHD subgroups at baseline. Patients in the ICHD group were slightly older; however, both the groups had comparable BMI and waist–hip ratios. The glucose, insulin, lipids, and physical activity were comparable in the two groups. Patients on ICHD had higher hirsutism score; however, menstrual cyclicity and androgen levels were comparable in the two groups. Patients on HCD had slightly higher total energy intake compared to the ICHD subgroup (mean 1750 kcal/day versus 1600 kcal/day, $p = 0.03$).

Table 2 gives the baseline and 3-month follow-up of PCOS patients on the HCD and ICHD groups. Only 50% patients (11/23) with caloric intakes (> 1800 kcal) followed in the ICHD group, whereas all 24 subjects in the HCD group with higher caloric intake followed at three months. There was a significant reduction in body weight in the HCD group while there was no change in weight in the ICHD group. Acne and menstrual cyclicity significantly improved in both the groups at 3 months (median 45 days vs. 35 days). While there was a mild reduction in hirsutism score with HCD, no change in hirsutism was observed with ICHD. There was no change in glucose or lipids or androgens; however, there was significant decrease in 0-h insulin levels with both HCD and ICHD. While the total energy reduced significantly with HCD, there was a slight increase in energy intake (100 kcal) with ICHD. This occurred as 24 out of 28 patients in ICHD group, who were missing any one major meal at baseline,

Table 4 Clinical, biochemical, and dietary profile of PCOS patients for PCOS subjects on isocaloric diet only for one year

Parameters	0 month ($n = 30$)	3 months ($n = 30$)	I year ($n = 30$)	p value*
Weight (kg)	58.15 ± 12.92	58.67 ± 13.38	58. 36 ± 12.35	0.25
Cyclicity (months)	50.90 ± 26.44(45)	41.63 ± 16.51(35)	38.37 ± 9.23(35)	0.27*[b]
MODHIRSCO	5.29 ± 4.81	5.13 ± 4.89	4.88 ± 4.54	0.04*
Acne code	1.22 ± 0.42	0.44 ± 0.51	0.11 ± 0.32	0.00*[a,b]
GTT 0hour (mg/dl)	79.33 ± 13.19	80.04 ± 11.30	89.55 ± 7.98	0.08*[c]
GTT 2hour (mg/dl)	110.74 ± 29.68	110.37 ± 27.90	114.50 ± 30.81	0.57
Insulin 0H (μIU/ml)	14.17 ± 7.44	10.35 ± 5.35	9.74 ± 4.09	0.51
Insulin 2H (μIU/ml)	60.94 ± 33.84	69.17 ± 47.14	56.19 ± 42.5	0.69
Total cholesterol (mg/dl) (TCH)	159.92 ± 30.11	157.92 ± 32.56	160.55 ± 28.2	086
Triglyceride (mg/dl)	97.88 ± 36.32	87.27 ± 30.93	90.68 ± 31.9	0.92
FSH (mIU/ml)	5.54 ± 1.62	5.48 ± 1.73	7.05 ± 2.55	0.008*[b,c]
LH (mIU/ml)	7.57 ± 5.41	10.11 ± 7.96	9.26 ± 7.94	0.58
Testosterone (nmol/l)(TESTO)	1.31 ± 0.65	1.42 ± 0.92	1.20 ± 0.51	0.48
DHEAS (μg/ml)	1.96 ± 0.92	2.04 ± 1.14	1.90 ± 0.87	0.20
Free testosterone (pg/ml)	2.71 ± 1.51	2.60 ± 1.51	3.05 ± 1.73	0.23
AVR. energy (kcal)	1554.26 ± 404.99	1704.46 ± 368.97	1539.25 ± 368.64	0.005*[a,c]
CHO (%energy)	59.03 ± 10.64	57.77 ± 6.34	57.86 ± 5.51	0.67
PRO (%energy)	13.40 ± 3.36	13.71 ± 1.80	13.31 ± 2.38	0.19[c]
FAT (%energy)	30.27 ± ± 6.52	28.60 ± 6.11	28.85 ± 5.25	0.48
Total fiber (gm)	30.09 ± 7.59	39.88 ± 12.43	33.20 ± 9.36	0.004*[a,c]
Soluble fiber (gm)	6.12 ± 2.05	7.88 ± 2.42	6.56 ± 1.71	0.00*[a,c]
Fruit intake (gm)	36.72 ± 57.19	86.13 ± 88.09	45.23 ± 52.19	0.006*[a]
Junk intake (gm)	311.95 ± 215.11	143.12 ± 123.42	148.84 ± 127.69	0.001*[a,b]
Late/missed breakfast	12 (40%)	4 (13.33%)	6 (20%)	0.031[a,b]
Late/missed lunch	7 (23.33%)	2 (6.66%)	6 (20%)	0.166[a]
Late/missed dinner	3 (10%)	2 (6.66%)	4 (13.33%)	0.391

Parameters given as mean ± SD

*p value significant by repeated measures ANOVA

a = 0 m vs. 3 m; b = 0 m vs. 1 yr; c = 3 m vs. 1 y (post hoc analysis)

now started taking all the three meals after dietary advice. There was a significant reduction in fat and junk intake whereas there was a significant increase in protein, fruits, and fiber in both the groups at three months. The meal timings significantly improved for breakfast, lunch, and dinner at three months for both the diet groups.

There was no change in the body weight, glucose, insulin, and androgens with both the diets at one year (Tables 3 and 4). There was a significant improvement in acne and menstrual cyclicity. There was a modest decrease in hirsutism score with HCD, while no change was observed with ICHD. There was a reduction in fat and junk intake and increase in fruit intake at one year compared to baseline; however, there was a deterioration compared to diet at three months in both the groups. There was an improvement in the meal timings compared to baseline diet (Fig. 2).

Table 5 compares the mean differences (delta change 0–3 months and 0-one year) in parameters between the two diets at three months and one year. Both the diets were similar in terms of changes in the clinical and biochemical parameters including menstrual cyclicity and hirsutism except weight which improved significantly with the HCD diet compared to the ICHD diet at three months.

Discussion

We studied the comparative effectiveness of hypocaloric versus isocaloric healthy diet (HCD vs. ICHD) in women with PCOS over a duration of one year. Isocaloric diet is a diet identical in total caloric intake but different in terms of macronutrient distribution. Previous studies have used different isocaloric diets (low-protein high-carbohydrate diet versus high-protein low-carbohydrate diet [17, 18] or low-carbohydrate high-fat diet versus high-carbohydrate low-fat diet) [19, 20]. Due to paucity of Indian studies on diet in PCOS patients, we have prescribed diet with macronutrient distribution as per diet for PCOS by Gower BA which is also are very close to the Indian recommendations [12,13,21]. On

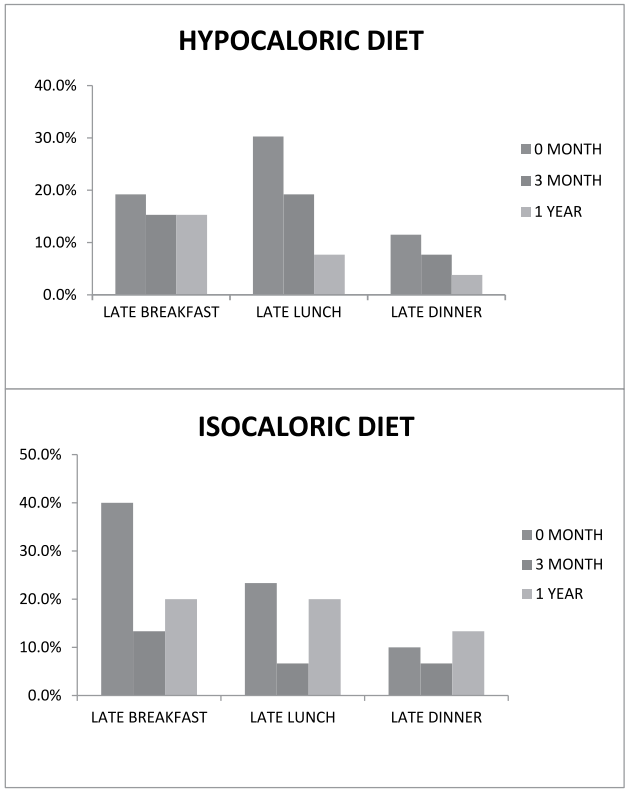

Fig. 2 Meal patterns of PCOS patients on two diets over a period of one year

the other hand, HCD is a diet low in calories aimed to sustain negative energy balance and, thereby, weight loss. The reduction in the calories consumed is variously described as absolute reduction from baseline (around 400–750 kcal/d) or a relative reduction from baseline (25%), or an intake below that required for weight maintenance [22, 23]. We have prescribed a hypocaloric diet with energy deficit of 500 kcal/day [22]. We added clause (20 kcal/kg for obese and 25 kcal/kg for lean whichever was lower) for patients who had baseline intake of more than 2500 kcal/day [7].

A systematic review by Moran et al. concluded that weight loss should be considered as the main approach to dealing with PCOS, regardless of dietary composition [24]. The short-term effectiveness of hypocaloric diets with varying macronutrient distribution has been shown earlier [7, 8, 22]. While there are more studies available on the hypocaloric diets, there is less data on isocaloric diets available in PCOS patients. Barr et al. for the first time demonstrated improvement in insulin sensitivity in 26 PCOS women on low glycemic index isocaloric diet in 2013 [25]. Subsequently, Goss et al., Gower et al., and Panico et al. also reported favorable metabolic effects and loss of fat mass with lower carbohydrate isocaloric diets [21, 26, 27]. However, to the best of our knowledge, we could not come across any randomized study comparing hypocaloric or isocaloric diets in PCOS subjects.

In the present study, both the HCD and ICHD groups were weight-matched and had comparable clinical and biochemical parameters except hirsutism at baseline. We observed that while the hypocaloric diet was more effective in reducing weight, both the diets were effective in improving menstrual cyclicity and acneform eruptions. While most of our patients had mild hirsutism (HS < 15), the effect on hirsutism was modest by both the diets at one year. The

Table 5 Mean difference in parameters between the isocaloric and hypocaloric diet at 0–3 months and 0–1 year

Mean difference in parameters from 0 to 3 months				Mean difference in parameters from 0 to one year		
Parameters	ICHD (66)	HCD (68)	*p* value	ICHD (30)	HCD (26)	*p* value*
Weight (kg)	− 0.66 ± 6.98	1.34 ± 4.60	0.011*	0.03 ± 3.09	− 2.02 ± 5.13	0.075
Cyclicity	18.10 ± 29.27	13.70 ± 31.46	0.31	12.53 ± 26.31	16.11 ± 28.90	0.43
MODHIRSCO	0.047 ± 1.12	0.51 ± 1.12	0.07	0.41 ± 0.87	1.21 ± 2.39	0.29
GTT 0hour (mg/dl)	− 0.54 ± 12.33	0.33 ± 12.79	0.80	− 12.90 ± 18.09	− 6.75 ± 13.92	0.33
GTT 2hour (mg/dl)	0.53 ± 28.50	− 5.27 ± 28.72	0.23	− 2.72 ± 24.32	− 8.93 ± 31.55	0.80
Insulin 0H (μIU/ml)	3.25 ± 11.01	3.35 ± 8.78	0.67	4.47 ± 8.51	1.49 ± 8.12	0.55
Insulin 2H (μIU/ml)	− 4.22 ± 55.08	0.62 ± 43.70	0.60	5.37 ± 51.26	1.82 ± 41.18	0.60
Total cholesterol (mg/dl)	− 0.80 ± 25.95	− 1.13 ± 19.01	0.90	− 1.29 ± 21.60	− 1.00 ± 20.68	1.00
Triglyceride (mg/dl)	0.67 ± 43.59	− 1.87 ± 49.89	0.61	9.23 ± 28.12	− 17.06 ± 50.79	0.14
FSH (mIU/ml)	− 0.31 ± 1.40	0.006 ± 2.51	0.24	− 1.54 ± 2.19	− 1.63 ± 2.83	0.90
LH (mIU/ml)	− 3.39 ± 11.97	1.05 ± 10.26	0.21	− 1.53 ± 10.23	− 2.15 ± 12.37	0.79
Testosterone (nmol/l)	− 0.05 ± 0.60	0.03 ± 0.58	0.59	0.11 ± 0.76	− 0.26 ± 0.93	0.16
Free testosterone (pg/ml)	0.34 ± 1.99	0.05 ± 2.16	0.44	− 0.54 ± 2.04	0.06 ± 1.75	0.17
SHBG (nmol/l)	− 6.63 ± 87.81	2.80 ± 67.59	0.70	40.62 ± 69.22	13.17 ± 54.50	0.26
Androstenedione (ng/ml)	0.03 ± 2.54	0.008 ± 2.38	0.79	− 0.90 ± 2.90	− 1.41 ± 1.44	0.42

p value* significant using Mann–Whitney *U* test

effectiveness of both the diets was similar (around 30% at one year) with both the per protocol and the intention to treat analysis. It is strange that in spite of no improvement in weight of the patients, the ICHD diet was as effective as the HCD diet in improving symptoms. The quality of food choices in those on ICHD improved with reduction in junk intake and increase of pulses, fruits, and fiber intake. The food choices given to our patients were closer to the DASH diet (rich in fruits, vegetables, fibers, legumes, low-fat dairy, and low in cholesterol, refined carbohydrates, and processed food), which has been shown to improve insulin sensitivity in PCOS subjects in a recent metanalysis [28]. Insulin reduced with ICHD at three months in our study. Apart from food choices, the meal timings also improved with significantly lesser number of patients taking late breakfast or late lunch at follow-up.

Late eating is associated with impaired glucose tolerance and carbohydrate oxidation, which may affect insulin action and thereby the PCOS phenotype [29, 30]. Jakubowicz et al. observed that increase in breakfast calories with reduction in caloric intake at dinner results in improved insulin sensitivity and decreased activity of cytochrome 17 hydroxylase, which improves hyperandrogenism and menstrual cycles in PCOS women [31]. We also observed for the first time that meal timings of PCOS patients were significantly different from weight-matched controls and that both the late breakfast and late lunch were significantly associated with hirsutism and irregular periods in subjects with PCOS [10]. We have randomized the same cohort published above into two groups on ICHD and HCD. It is possible that improved meal timings and improvement of food choices resulted in reduction of insulin levels with consequent improvement in clinical manifestations in our subjects on ICHD.

There were some limitations with the ICHD diet. Patients with baseline caloric intakes larger than 1800 kcal did not follow-up at three months thereby indicating ineffectiveness of ICHD with higher calorie intakes. Also, ICHD was ineffective in reducing weight over short term. While both the diets were equally effective, it should be noted that only 30% wanted to continue with diet alone at one year in both the groups. There was a deterioration of food habits at one year with subjects taking more fat and junk and lesser fruits, pulses, and fiber compared to diet at three months; nevertheless, food choices and meal timings were still better compared to their baseline diet. Such a non-compliance and lack of adherence to long-term diet therapy is well known [22–24]. A non-significant reduction in insulin levels with HCD over one year may be related to fewer numbers at follow-up, deterioration of food habits or other non-recorded stressful events in routine life.

We observed that HCD was effective in weight loss after 3 months; however, no significant change in weight was observed at one year. Long-term studies on low-calorie diets indicate that while the initial results may be impressive, around 75–80% of the people trying to reduce body weight fail in terms of long-term weight loss maintenance [23, 32]. The underlying mechanisms for weight regain or lack of effect over long-term hypocaloric diet are poorly understood. Adaptations in energy expenditure, increase in fractional energy absorption, and lack of compliance are mechanisms postulated in pathophysiology of less than expected weight loss with HCD [23]. Brain energy homeostasis is also suggested to play a role in failure of therapy over long term. Studies show that obese individuals display reduced levels of high energy phosphate and phosphocreatinine that predicts subsequent food consumption [32, 33]. NADH decrease induced by repetitive low caloric dieting could lead to boosted food craving and consumption to satisfy the cerebral energy needs [33]. This effect is known as weight cycling and describes repeated periods of initially successful weight loss followed by regain even beyond the initial body weight.

To the best of our knowledge, this is the third largest study with a long follow-up of one year [10]. Possibly, this is also the first comparative randomized assessment of hypocaloric and isocaloric diet in PCOS patients. Both the groups were weight-matched and had comparable waist–hip ratios, androgens, and glucose levels at baseline. Limitations include loss to follow-up, greater in the patients with high caloric intakes in the ICHD group. Nevertheless, we have tried to address this problem by the intention to treat analysis. Another limitation is a single blinded nature of the study, where dietician knew which study arm patients were allocated. While recall bias may be a limitation compared to weighted prospective methods, a recent review concluded that dietary recall is a convenient and valid method of dietary assessment that gives accurate dietary information when collected by a trained interviewer using standardized methods [34]. Previous studies have indicated that the recall bias can be reduced by multiple pass questioning by trained dieticians with special emphasis on forgotten foods and direct probing [35–37]. We believe that detailed interview by our experienced dieticians probing meal patterns and timings, direct probing of junk and snacks may have reduced the recall bias in the present study.

Conclusion

The present study demonstrated that isocaloric diets can be as effective in improving clinical symptoms as hypocaloric diets in about 30% of patients with mild symptoms over long term. However, hypocaloric diets cause a greater weight loss over short term. The improvement of clinical symptoms by eucaloric diets is related to the improvement of food choices and correction of meal timings. Isocaloric diets are not effective if patients have a high caloric intake at baseline.

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Author contribution BK conceptualized the study, was involved in all stages, and wrote the manuscript. SP, BP, and PS were involved in data collection, statistical analysis, and literature review including manuscript preparation. NS and LS were involved in data collection, laboratory data, and manuscript preparation. All the authors approved the final version of the manuscript.

Declarations

Ethics approval This study was approved by the Insti-tutional Ethics Committee (IRIS ID no. 2013-0976). Consenting subjects and controls underwent a detailed history and physical examination including anthropometry.

Conflict of interest The authors declare no competing interests.

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LETTER TO THE EDITOR

Oral semaglutide significantly reduces low-density lipoprotein cholesterol level

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To the Editor:

Oral semaglutide is a glucagon-like peptide-1 (GLP-1) receptor analog to control the plasma glucose level of patients with type 2 diabetes mellitus (T2DM) by increasing insulin secretion from pancreatic β cells and reducing glucagon secretion from the pancreatic α cell [1]. It has not been thoroughly studied yet whether oral semaglutide reduces low-density lipoprotein (LDL) cholesterol levels. As a result, we investigated whether oral semaglutide reduces LDL cholesterol levels in patients with T2DM.

Study participants included those who went to our hospital in 2022. Exclusion criteria included type 1 diabetes mellitus (T1DM) and patients with T2DM already treated with injection type of glucagon-like peptide-1 receptor agonist (GLP-1 RA). Furthermore, patients who could not keep taking oral semaglutide because of severe effect such as nausea were excluded. A total of 54 subjects were included in this study. However, 4 patients could not keep taking oral semagutide because of nausea. As an add-on, oral semaglutide was recommended. Initially, a one-month prescription for oral semaglutide 3 mg/day was written. Thereafter, 7 mg/day of oral semaglutide was prescribed. The final patient number was 50 (38 men and 12 women, mean age 55.9 ± 11.1 years). Dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter 2 inhibitors, sulfonylurea, glinido, α-glucosidase inhibitors, GLP-1 RA, biguanide, peroxisome proliferator-activated receptor-gamma agonist and insulin were prescribed 13%, 79%, 20%, 10%, 13%, 5%, 38%, 5%, and 6%, respectively. Drugs for hyperlipidemia were prescribed to 18% of patients and antihypertensive medications to 19% of patients. Those medications were not changed through this clinical study. Clinical data were compared before starting oral semaglutide and 6 months after the initiation of oral semaglutide either 7 mg/day.

The mean duration with T2DM was 13.2 ± 11.8 years. The baseline characteristics of the patients are summarized in Table 1. Oral semaglutide significantly reduced body weight (86.7 ± 17.7 *vs*. 84.8 ± 17.6 kg, $p = 1.2E-05$) and also significantly enhanced casual plasma glucose and HbA1c (183.5 ± 87.2 *vs*. 153.7 ± 76.0 mg/dL, $p = 0.023$, 7.9 ± 1.5 *vs*. 7.0 ± 1.2%, $p = 2.30E-06$). Oral semaglutide significantly reduced LDL cholesterol level which was measured by direct measurement (124.0 ± 33.6 *vs*. 108.3 ± 33.3 mg/dL, $p = 2.40E-06$) but did not change high-density lipoprotein (HDL) cholesterol (50.7 ± 26.5 vs. 51.2 ± 31.2 mg/dL, $p = 0.674$) and triglyceride levels (232.2 ± 158.5 vs. 220.4 ± 134.0 mg/dL, $p = 0.162$). Oral semaglutide did not affect AST, ALT, γ-GTP, eGFR, and UACR levels (Table 2).

We found that oral semaglutide decreased LDL cholesterol by ~ 13% in patients with T2DM. This finding agreed with the result of the PIONEER 6 and the study by Dahl et al. [2]. Reduction of body weight, improvements in postprandial glucose by oral semaglutide might have induced decrease in LDL cholesterol levels.

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Table 1 Baseline characteristics for subjects

Clinical test items	Measured value	Normal range
Gender (male:female)	77:23	
Age (years)	55.9 ± 11.1	
Disease duration (years)	13.2 ± 11.8	
Body height (cm)	167.3 ± 9.1	
Body weight (kg)	86.7 ± 17.7	
Body mass index (kg/m^2)	30.9 ± 5.4	
Systolic blood pressure (mmHg)	130.2 ± 18.2	
Diastolic blood pressure (mmHg)	76.3 ± 16.5	
Casual plasma glucose (mg/dL)	183.5 ± 87.2	
Glycated hemoglobin (%)	7.9 ± 1.5	
Aspartate aminotransferase (U/L)	35.1 ± 23.0	10–40
Alanine aminotransferase (U/L)	44.3 ± 37.1	5–45
γ-glutamyl transpeptidase (U/L)	46.1 ± 47.2	≤ 79
High-density lipoprotein cholesterol (mg/dL)	50.7 ± 26.5	40–80
Low-density lipoprotein cholesterol (mg/dL)	124.0 ± 33.6	70–139
Triglyceride (mg/dL)	232.2 ± 158.5	< 175
Serum creatinine (mg/dL)	0.88 ± 0.27	0.65–1.09
Estimated glomerular filtration rate (mL/min/1.73m^2)	69.5 ± 17.2	60 ≤
Urinary albumin/creatinine ratio (mg/g·Cr)	94.1 ± 22.6	< 30

The characteristics of the subjects are summarized

SD standard deviation

Table 2 Effect of oral semaglutide on clinical measurement values

	Baseline	After 6 months	*p* value
Body weight (kg)	86.7 ± 17.7	84.8 ± 17.6	1.2E-05
Systolic blood pressure (mmHg)	130.2 ± 18.2	129.2 ± 21.6	*N.S*
Diastolic blood pressure (mmHg)	76.3 ± 16.5	78.2 ± 30.5	*N.S*
Casual plasma glucose (mg/dL)	183.5 ± 87.2	153.7 ± 76.0	0.023
Glycated hemoglobin (%)	7.9 ± 1.5	7.0 ± 1.2	2.30E-06
Aspartate aminotransferase (U/L)	35.1 ± 23.0	30.3 ± 11.6	*N.S*
Alanine aminotransferase (U/L)	44.3 ± 37.1	42.6 ± 2.4	*N.S*
Gamma-glutamyl transferase (U/L)	46.1 ± 47.2	49.9 ± 58.0	*N.S*
Triglyceride (mg/dL)	232.2 ± 158.5	220.4 ± 134.0	*N.S*
High-density lipoprotein (mg/dL)	50.7 ± 26.5	51.2 ± 31.2	*N.S*
Low-density lipoprotein (mg/dL)	124.0 ± 33.6	108.3 ± 33.3	2.40E-06
Estimated glomerular filtration rate (mL/min/1.73m^2)	69.5 ± 17.2	64.7 ± 15.5	*N.S*
Urinary albumin to creatinine ratio (mg/g·Cr)	24.1 ± 22.6	25.8 ± 37.4	*N.S*

Effect of oral semaglutide on clinical measurement values is summarized. Clinical data was compared before starting oral semaglutide (baseline) and 6 months after the initiation of oral semaglutide (after 6 months). To calculate the impact of oral semaglutide on clinical measurement values, paired *t*-test was utilized. All tests for significance and the resulting *p* values were two-sided with a 5% level of significance

Values were depicted as mean ± SD

SD standard deviation, *N.S*. Not significant

Abbreviations AST: Aspartate aminotransferase; ALT: Aspartate aminotransferase; γ-GTP: γ-Glutamyl transpeptidase; eGFR: Estimated glomerular filtration rate; UACR: Urinary albumin/creatinine ratio

Author contributions Shuichi Okada and Kihachi Ohshim acared for the patient. Shuichi Okada, Kazuya Okada, Koji Kikkawa, Junichi Okada, Eijiro Yamada, Tsugumichi Saito, Tetsuro Andou, and Kihachi Ohshima attended the clinical conferences on this patient and made important suggestions for the differential diagnosis and therapeutic strategy. Shuichi Okada, and Junichi Okada prepared the manuscript.

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Data availability The data that support the findings of this study are not publicly available due to the confidentiality of the participants; for example, they contain information that could compromise the privacy of research participants, but are available from the corresponding author upon reasonable request.

Declarations

Statement of ethics The study protocol was reviewed and approved by the review board of Hidaka Hospital (approved reference: 336) on 17 July 2021. The research complies with the guidelines for human studies in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Conflict of interest None of the authors have any potential conflicts of interest associated with this case presentation.

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CASE REPORT

Severe hypoglycemia due to insulin auto-antibodies of newly diagnosed multiple myeloma: A case report

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Abstract

Objective Insulin autoimmune syndrome (IAS) is a rare condition that can be overlooked in the differential diagnosis of hypoglycemia. Hematologic malignancies such as multiple myeloma (MM) have been associated with IAS. Very few cases of IAS due to multiple myeloma have been reported in the literature. We wanted to present our case who applied to our clinic with severe hypoglycemia and was diagnosed with multiple myeloma-associated IAS.

Case presentation An 83-year-old male patient was admitted to our clinic with recurrent severe hypoglycemia episodes. When the patient's plasma glucose was 37 mg/dL, insulin level was high (2266 μIU/ml), C-peptide level was relatively normal (3.44 ng/ml); cortisol and ACTH levels were also normal. No pathologic finding was detected in the abdominal MRI and Ga-68 PET/CT. Anti-insulin antibody (IAA) was tested for IAS, and the antibody level was found to be 95.9%. The patient was evaluated in terms of possible conditions that may lead to IAS. Hematology consultation was made due to the fact that the patient had anemia, chronic renal failure, and albumin/globuline discordance, and he was diagnosed with IgG kappa multiple myeloma. Bortezomib and dexamethasone treatment was initiated. A decrease in hypoglycemic symptoms was observed with chemotherapy.

Conclusion IAS should be considered in the differential diagnosis of recurrent hypoglycemia cases of unknown cause. It should be kept in mind that IAS may develop due to multiple myeloma, especially in patients with advanced age and anemia.

Keywords Multiple myeloma · Hypoglycemia · Insulin autoimmune syndrome · Case report

Introduction

Hypoglycemia is a severe and sometimes life-threatening condition that is seen with the Whipple's triad (low blood glucose, signs and symptoms of hypoglycemia, and disappearance of these symptoms after glucose administration) [1].

Insulin autoimmune syndrome is a rare syndrome that can be missed in differential diagnosis. Hematologic malignancies such as multiple myeloma and monoclonal gammopathy of undetermined significance can also be associated with insulin autoimmune syndrome related hypoglycemia. The first case of multiple myeloma-related hypoglycemia was described in 1972; however, limited number of cases have been reported since then.

In this case report, we describe an 83-year-old male patient, who was referred to our endocrinology clinic with severe and undiagnosed symptomatic hypoglycemia and diagnosed with multiple myeloma and hypoglycemia due to multiple myeloma-related insulin autoantibodies.

Case presentation

An 83-year-old male patient was referred to Manisa Celal Bayar University Faculty of Medicine Endocrinology clinic with severe and undiagnosed symptomatic hypoglycemia in 2020. He presented the symptoms of trembling and shortness of breath, especially at night. He had applied to emergency service many times with same

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signs and symptoms. Plasma glucose of the patient with the symptoms of hypoglycemia was found to be around 30–40 mg/dL. He had a history of hypertension and type 2 diabetes mellitus. Four years ago, he also suffered from these kinds of hypoglycemia signs and symptoms and was evaluated in the same hospital in endocrinology clinic. But no etiology was found. Due to his high insulin and normal C-peptide levels and the fact that his wife was also diabetic and under intensive insulin treatment, "factitious hypoglycemia" was suspected. He avoided his regular visits. The patient applied to our clinic with an episode of hypoglycemia several times, and counter-regulatory hormones were analyzed during his visits. His physical examination revealed only pale conjunctivae and pallor. When blood glucose level was 37 mg/dL, insulin level was found to be high (2266 μIU/mL); however, C-peptide, cortisol, and ACTH levels were normal. Baseline laboratory parameters are shown in Table 1.

To exclude insulinoma, an abdominal magnetic resonance imaging scan (MRI) was performed and no sign of insulinoma was detected in arterial phase of contrast enhanced MRI. Due to the fact that laboratory findings revealed a high suspicion of insulinoma, Ga-68 DOTATATE PET/CT was also performed and no abnormality was found.

He was evaluated together with hematology clinic because he also had anemia. The patient was investigated for multiple myeloma due to accompanying chronic renal failure and discordance in albumin/globulin measurements. Bone marrow aspiration and biopsy were performed. He was diagnosed with IgG–kappa multiple myeloma.

The patient was transferred to hematology clinic and chemotherapy with bortezomib and dexamethasone regime was initiated. Due to advanced age and poor performance (ECOG-2-3), the patient received a reduced dose of chemotherapy. During the chemotherapy course, insulin, C-peptide, and anti-insulin antibody levels were evaluated. Anti-insulin antibody (IAA) level was found to be 95.9%, and the patient was diagnosed with IAS. A reduction in hypoglycemic symptoms was observed with chemotherapy. The correlation between anti-insulin antibody levels, serum IgG levels and HbA1c are given in Figs. 1 and 2. Although his hypoglycemia attacks subsided, he was switched to lenalidomide-dexamethasone as the second line treatment because his clinical response was not sufficient. During his chemotherapy course, the patient experienced various added/intervening conditions which did not require hospitalization such as neuropathy and gastrointestinal motility disorder due to bortezomib treatment and deep cytopenia. During these times of delay, hypoglycemia attacks were observed. The patient is still undergoing chemotherapy.

Table 1 Baseline laboratory parameters

Laboratory parameters	Results	Reference range
Fasting plasma glucose	37	(74–106 mg/dL)
HbA1c	7.1	(4–5.7%)
Fasting plasma insulin	2230	(1.9–23 μIU/mL)
C-peptide	3.44	(0.48–5.05 ng/mL)
Anti-GAD antibody	Negative	
Anti-insulin antibody	95.9%	(4–10%)
Cortisol (baseline)	13.8	(3.09–22.4 μg/dL)
Cortisol (at the time of hypoglycemia)	18.1	(3.09–22.4 μg/dL)
GH (baseline)	0.16	(0.01–1 ng/mL)
GH (at the time of hypoglycemia)	8.9	(0.01–1 ng/mL)
TSH	2.77	(0.38–5.33 uU/mL)
fT4	0.88	(0.61–1.3 ng/dL)
ALT	20	(0–50 U/L)
AST	23	(0–50 U/L)
ALP	62	(30–120 U/L)
Plasma urea	63	(17–43 mg/dL)
Plasma creatinine	1.38	(0.67–1.17 mg/dL)
eGFR	47	> 60 mL/min
Leucocyte	8.8	(4.5–10.3 10^3/μL)
Hemoglobin	8.8	(13.6–17.2 g/dL)
Thrombocyte	229	(156–373 10^3/μL)
LDH	290	(0–248 U/L)
Sedimentation rate	70	(0–20 mm/h)
Albumin	3.3	(3.5–5.2 g/dL)
Globulin	5.1	(2.5–4.5 g/dL)
Calcium	9.0	(8.8–10.6 mg/dL)
Urine ketone	Negative	
Serum IgG	3020	(700–1600 mg/dL)
Serum IgA	89.5	(70–400 mg/dL)
Serum IgM	18.8	(40–230 mg/dL)
Serum kappa light chain	488	(6.7–22.4 mg/L)
Serum lambda light chain	16.5	(8.3–27 mg/L)
Beta-2 microglobulin	5783	(609–2366 μg/L)
Plasma cell (bone marrow)	50%	(%)

Discussion

Insulin autoimmune syndrome (IAS), also known as "Hirata's disease," is a rare condition which consists of hyperinsulinemic hypoglycemia, elevated insulin autoantibody (IAA) levels, no prior exposure to exogenous insulin, and no pathological abnormalities of pancreatic islets. It was named after Yukimasa Hirata, the author who first described the syndrome in 1970 [2].

IAS is autoimmune in nature, and its connection with human leukocyte antigen DRB1*0406 may be the reason why it is seen more frequently in Japanese populations

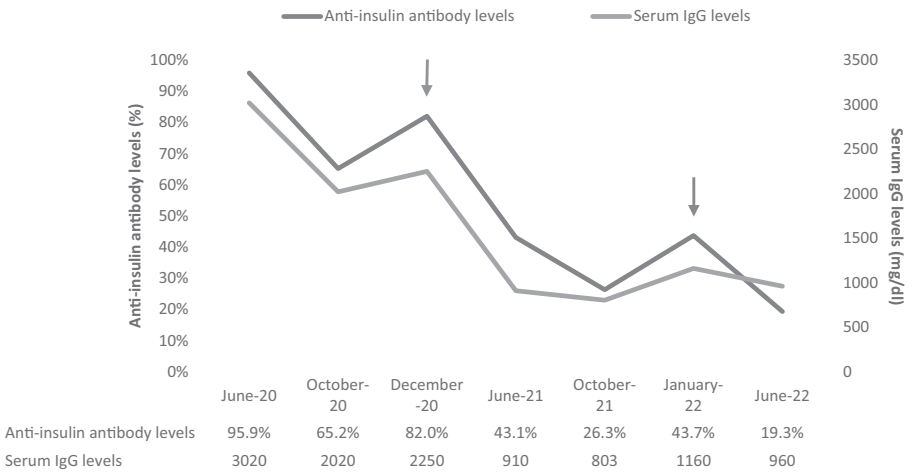

Fig. 1 The relationship between the patient's anti-insulin antibody and serum IgG levels. Gray arrow indicates the time of the first chemotherapy delay due to bortezomib-related motility disorder and neuropathy. Green arrow indicates the second chemotherapy delay due to cytopenia and poor general condition

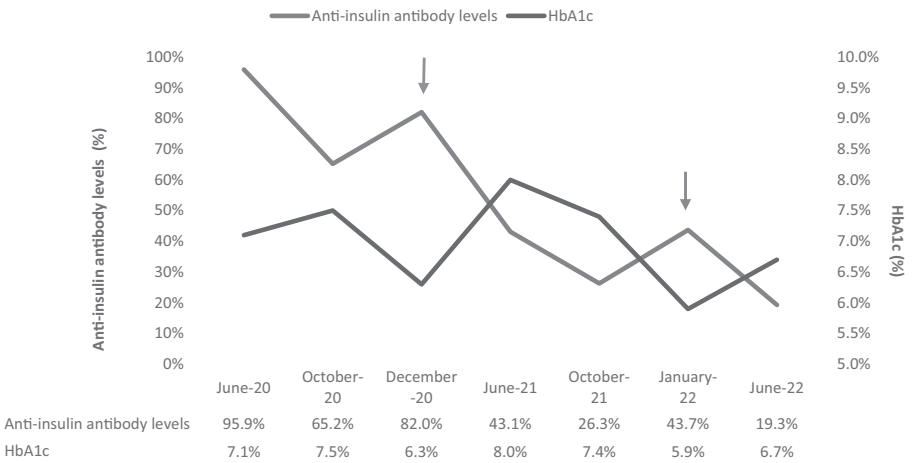

Fig. 2 The relationship between the patient's anti-insulin antibody and HbA1c levels. Gray arrow indicates the time of the first chemotherapy delay due to bortezomib-related motility disorder and neuropathy. Green arrow indicates the second chemotherapy delay due to cytopenias and poor general condition

[3]. In addition to autoimmunity, it has been speculated to involve drugs (especially containing sulfhydryl groups), and/or virus infections such as mumps, hepatitis C virus, rubella, chickenpox, and measles [4]. Viral infections, acting as super-antigens, may also activate the production of IAA, and thereby cause IAS [5].

The IAS accompanied by hematological diseases has a different mechanism from the classical one. Since the first case of multiple myeloma associated with IAS was reported in 1972, 10 other cases of MGUS/myeloma-related IAS have been defined [6–10]. In most of these cases, similar to our case, there was a correlation between the clinical course of multiple myeloma and the frequency of hypoglycemia. Nevertheless, in these cases, hypoglycemia had started at least 6 months prior to the diagnosis of myeloma. F. Waldron-Lynch et al. shared a case in which symptoms of hypoglycemia started 1 year before the diagnosis of multiple myeloma. Similar to our case, they stated that IAA levels decreased with the decrease of the M band during the remission period of multiple myeloma and increased again with relapse [9]. Similarly, in the case of Filho et al., symptoms of hypoglycemia started 2 years prior to the diagnosis of myeloma. After chemotherapy and autologous hematopoietic stem cell transplant, IAA levels decreased and hypoglycemia disappeared; however, it was stated that episodes of hypoglycemia were observed again with the relapse of the disease 6 months later [10]. While some of the cases reported in the literature passed due to multiple myeloma, some cases were found to be in remission like our case.

Hyperinsulinemic hypoglycemia in IAS due to multiple myeloma may be associated with anti-insulin antibodies binding to insulin and delaying its hepatic clearance. This may explain the high insulin levels and relatively low C-peptide levels without increased insulin production by the pancreas [11]. Spontaneous remission is not seen; severity of hypoglycemia episodes is associated with the prognosis of the underlying condition. Nutrition education, recognizing hypoglycemia episodes, and treatment of the underlying condition will help to resolve the situation [2]. Glucocorticoids, chemotherapeutic agents, and plasmapheresis may help to

decrease anti-insulin antibody levels in patients with severe hypoglycemic episodes.

In our case, following the initiation of chemotherapy, episodes of hypoglycemia disappeared and IAA levels significantly decreased. The patient's IAA level at the time of diagnosis was 95.9%. It was observed that 3 months after starting chemotherapy, IAA level decreased to 65.2%. At the end of the period when chemotherapy had to be interrupted due to neuropathy and gastroparesis, IAA level increased to 82% again. The patient experienced hypoglycemic attacks again during this period. After the alteration of the chemotherapy regime, IAA level rapidly decreased to 26.3%. It increased again to 43.7% following the period when chemotherapy had to be interrupted again due to cytopenia. The patient's treatment could be resumed later, and the final IAA level was 19.3%. It was noted that these antibody levels showed a positive correlation with serum IgG levels, which can be used as a marker for MM (Fig. 1).

The patient's HbA1c levels were negatively correlated with IAA levels. As a result of the cessation of hypoglycemia episodes due to the treatment of the patient, his HbA1c level increased to 7.5%. In the period when the treatment had to be interrupted, the HbA1c level decreased to 6.3% with the hypoglycemia attacks resuming. Subsequently, it was observed that HbA1c level increased to 8% with good hematological response. Linagliptin was added to the patient's diabetes treatment with the improvement of his general condition and the cessation of hypoglycemia attacks. When the chemotherapy was interrupted due to cytopenia that developed in the period shown with the green arrow in Fig. 2, the Hba1c value of the patient, who experienced a relapse of hypoglycemia attacks, was decreased to 5.9%. Linagliptin treatment was discontinued afterwards. The majority of the HbA1c and IAA levels in Fig. 2 seem to be negatively correlated. Nevertheless, the initiation of the diabetes treatment and the fact that HbA1c reflects the glycemic level of a wide period rather than an instant might be possible causes of some deflections regarding this data.

The patient is currently in remission regarding multiple myeloma, and his IAA levels are the lowest since the time of diagnosis; no episode of hypoglycemia is reported.

Conclusion

Given the rarity of hypoglycemia due to anti-insulin monoclonal antibodies and delayed diagnosis, we recommend testing for monoclonal gammopathies when evaluating patients with recurrent hypoglycemia of unknown origin, especially if the patient has anemia.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Code availability Not applicable.

Declarations

Ethics statement Written informed consent was obtained from the patient.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Association of adipokine levels and insulin resistance in prediabetes: hospital-based descriptive study in a tertiary care hospital in North Kerala

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Abstract

Objective Increasing evidence revealed the role of adipokines in carbohydrates and fat metabolism. The present study was designed to evaluate the adiponectin, leptin, and resistin levels in prediabetes subjects and evaluate the relationship between these adipokines and insulin resistance.

Methods A hospital-based descriptive study was conducted for one year. 200 individuals who met the inclusion criteria were enrolled in the study. Based on the oral glucose tolerance test, the study subjects were grouped into healthy controls ($n=100$), prediabetic non-obese ($n=61$), and prediabetic obese ($n=39$). Blood glucose estimation was done by the glucose oxidase peroxidase method. Chemiluminescent immunoassay was used for the measurement of insulin level and homeostasis model assessment-estimated insulin resistance was used for the assessment of insulin resistance. Serum adipokines levels were determined by ELISA. Statistical analysis was performed by using SPSS Software.

Results Serum adiponectin levels decreased significantly in obese prediabetes (7.21 ± 2.15 µg/ml) when compared to non-obese prediabetes (7.28 ± 2.41 µg/ml) and healthy control subjects (13.64 ± 2.88 µg/ml, $p < 0.001$). Serum leptin levels increased significantly in obese prediabetes (13.59 ± 2.59 ng/ml) when compared to non-obese prediabetes (9.84 ± 2.66 ng/ml) and healthy control subjects (12.28 ± 2.65 ng/ml, $p < 0.001$). Serum resistin levels increased significantly in obese prediabetes (17.67 ± 3.60 ng/ml) when compared to non-obese prediabetes (17.2 ± 3.93 ng/ml) and healthy control subjects (14.46 ± 4.16 ng/ml, $p < 0.001$). Adiponectin-leptin ratio decreased significantly in obese prediabetes (0.56 ± 0.22) when compared to non-obese prediabetes (0.79 ± 0.4) and healthy control subjects (1.15 ± 0.37; $p < 0.001$). Fasting insulin resistance was statistically significant ($p < 0.001$) in all groups.

Conclusion The present study strongly suggests that the adipokine profile is an ideal diagnostic tool to predict prediabetes and metabolic syndrome, especially among those with insulin resistance.

Keywords Prediabetes · Adipokines · ELISA · Insulin resistance · HOMA-IR

Introduction

Globally, Prediabetes is an emerging metabolic disorder, a condition characterized by slightly elevated blood glucose levels (140–199 mg/dL) regarded as indicating that the individual is at risk of progressing to type-2 diabetes (T2DM) (blood sugar level of 200 mg/dL or more). According to a report from the International Diabetes Federation, the worldwide prevalence of prediabetes will reach 471 million by 2035 [1].

In maintaining energy homeostasis, the adipose tissue plays a key role by communicating with the brain, muscle, liver, and pancreas which is mediated by adipokines such as adiponectin, leptin, and resistin, the bioactive peptides

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and proteins secreted by adipose tissue [2]. Adiponectin has anti-diabetic, anti-atherogenic, and anti-inflammatory properties. It promotes insulin sensitization by reducing hepatic glucose production and increasing insulin sensitivity in the liver [3, 4]. Leptin is primarily produced by adipose tissue in proportion to the amount of body fat stores involved in the regulation of food intake and energy homeostasis [5, 6]. Resistin, another pro-inflammatory cytokine has an association with insulin resistance [7]. Disturbance in the adipokine levels provides critical clues regarding the pathophysiological mechanism of T2DM, also their secretion contributes to insulin resistance and impairment of insulin production [8, 9]. Insulin resistance is a pathological condition in which insulin action is impaired in target tissues including the liver, skeletal muscle, and adipose tissue. Insulin resistance is the foremost characteristic of T2DM and assists multiple organ failure along with the resistance of insulin in skeletal muscle, and liver, adipose tissue. Obesity is another important phenomenon that has a link to T2DM, and it has been estimated that not less than 90% of T2DM are overweight or obese. Serum adiponectin level decreases in obesity [10]. The adiponectin/leptin ratio has been proposed as a marker of adipose tissue dysfunction [11, 12]. Evidence is still lacking regarding the effects of adipokines in the pathogenesis of insulin resistance. The different role of adiponectin and leptin in the pathophysiology of T2DM still needs to be studied [10]. With this view, the present study was designed to evaluate the adiponectin, leptin, and resistin levels in prediabetes subjects and assess the relationship between these adipokines and insulin resistance.

Materials and methods

Study design

After getting the necessary approval from the institutional ethical committee (EC/NEW/INST/2019/406 & ECR/301/Inst/KL/2013/RR), a descriptive hospital-based study was conducted for a period of one year from January 2021 to January 2022 in the Department of Endocrinology, Aster MIMS, a 950 bedded super specialty hospital in Calicut, Kerala, India. 200 individuals of both genders in the outpatient section, aged between 30 to 50 years, without diabetes, were enrolled in the study. Elderly subjects with diabetes and other serious physical or mental illnesses were excluded from the study.

The participants were informed about the study and their consent was received in the prescribed format. Based on the Glycated hemoglobin (HbA1c) and 75gm oral glucose tolerance test (OGTT) report, the study subjects were categorized into a normal control group and a pre-diabetes group, each had 100 participants. HPLC method was employed for HbA1c estimation and OGTT was carried out as per the WHO criteria. The participants with pre-diabetes were further grouped into obese and non-obese based on their Body mass index (BMI).

Study procedure

Initially, the demographic data was collected from all the participants in the prescribed proforma. After overnight fasting for 8-12 h, blood samples of the participants were collected in a set of evacuated tubes containing sodium fluoride and potassium oxalate which is used for blood glucose estimation. Another set of blood samples collected was centrifuged for 15 min at 4000 rpm and the serum obtained was stored at -80 °C for further evaluation.

Blood glucose estimation was done by the glucose oxidase peroxidase method. Chemiluminescent immunoassay was used for the measurement of insulin level and Homeostasis Model Assessment-Estimated Insulin Resistance (HOMA-IR) was used for the assessment of insulin resistance and was calculated by the formula:

$$\text{Homeostasis Model Assessment for Insulin Resistance} = \left(\frac{\text{glucose(mg/dl)x insulin}(\mu\text{U/ml})}{405}\right)$$

Biochemical parameters (Blood glucose and Plasma insulin) estimation was done on a fully automated biochemistry analyzer Cobas 6000. HbA1c estimation was done in a BIORAD-D10 analyzer (Hercules, California, United States). Adiponectin, Leptin, and Resistin levels were quantitatively assessed by Sandwich ELISA kits (Krishgen BioSystems, Mumbai).

Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by the Bonferroni test was applied to assess differences between the selected groups. The correlation between two variables was computed by Pearson's correlation coefficients (*r)* and graphically represented by scatter plots. The analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA). A *p*-value less than 0.05 was considered statistically significant.

Results

In this yearlong study, various significant results were obtained. Totally 200 patients (100 healthy controls, prediabetic non-obese 61, prediabetic obese 39) were enrolled

in the present study with a mean comparison age of 39.03 ± 5.725 in healthy controls, 38.39 ± 6.312 in the pre-diabetic non-obese group, and 39 ± 5.206 in the prediabetic obese group. However, the age was not significant with a *p*-value of 0.781. On comparison of other demographic and biochemical parameters such as BMI (Kg/m^2), SBP (mmHg), DBP (mmHg), HbA1C (%), FBS (mg/dl), PPBS (mg/dl), fasting insulin(μIU/ml) and fasting insulin resistance we observed a significant difference in the mean among the three groups with a *p*-value < 0.001. Based on the HbA1c and OGTT report, the study subjects were grouped into the normal control group (*N* = 100) and the pre-diabetes group (*N* = 100). Based on their BMI, the pre-diabetes group was further divided into obese (*N* = 39) and non-obese (*N* = 61). Initially, the socio-demographic data of study subjects were compared with biochemical parameters which is shown in Table 1.

The adiponectin, leptin, resistin, and the A/L ratio were compared among the three groups. The results of the mean comparison of adiponectin, leptin, resistin levels, and adiponectin-leptin (A/L) ratio between all three groups analyzed showed a statistically significant difference (*p*-value < 0.001). It was found that the serum adiponectin levels decreased significantly in obese prediabetes (7.21 ± 2.15 μg/ml) when compared to non-obese prediabetes (7.28 ± 2.41 μg/ml) and healthy control subjects (13.64 ± 2.88 μg/ml, *p* < 0.001). The results indicated that serum leptin levels increased significantly in obese prediabetes (13.59 ± 2.59 ng/ml) when compared to non-obese prediabetes (9.84 ± 2.66 ng/ml) and healthy control subjects (12.28 ± 2.65 ng/ml, *p* < 0.001). Serum resistin levels increased significantly in obese prediabetes (17.67 ± 3.60 ng/ml) when compared to non-obese prediabetes (17.2 ± 3.93 ng/ml) and healthy control subjects (14.46 ± 4.16 ng/ml, *p* < 0.001). The A/L ratio decreased significantly in obese prediabetes (0.56 ± 0.22) when compared to non-obese prediabetes (0.79 ± 0.4) and healthy control subjects (1.15 ± 0.37, *p* < 0.001) (Table 2).

A weak positive correlation in all three groups with no statistical significance was found on correlating the

Table 1 Comparison of socio-demographic data and biochemical parameters of study subjects

Parameters	Control (*N* = 100) Mean ± SD	Pre-diabetic; Non-obese (*N* = 61) Mean ± SD	Pre-diabetic: Obese (*N* = 39) Mean ± SD	*p*-Value
Age(years)	39.03 ± 5.72	38.39 ± 6.31	39 ± 5.20	0.78
BMI (Kg/m^2)	23.80 ± 2.08	24.15 ± 1.63	26.13 ± 0.54	< 0.001
Systolic BP	123.40 ± 7.41	131.80 ± 8.06	126.41 ± 7.77	< 0.001
Diastolic BP	81.10 ± 4.90	92.46 ± 12.86	84.10 ± 6.37	< 0.001
FBS (mg/dl)	86.83 ± 7.27	117.05 ± 4.04	118.51 ± 4.36	< 0.001
PPBS (mg/dl)	93.76 ± 12.5	152.64 ± 9.32	167.26 ± 14.33	< 0.001
HbA1c (%)	5.28 ± 0.37	6.03 ± 0.96	6.15 ± 0.20	< 0.001
Fasting insulin (μIU/ml)	7.01 ± 1.53	12.26 ± 3.83	18.42 ± 3.30	< 0.001
Fasting insulin resistance	1.49 ± 0.35	3.53 ± 1.12	5.38 ± 0.93	< 0.001

Mean comparison (Mean ± SD); *p*-value of < 0.05 considered to be significant

Table 2 Comparison of adipokines level and A/L ratio of study subjects

Parameters	Control (*N* = 100) Mean ± SD	Pre-diabetic: Non-obese (*N* = 61) Mean ± SD	Pre-diabetic: Obese (*N* = 39) Mean ± SD	*p*-Value
Adiponectin (μg/ml)	13.64 ± 2.88	7.28 ± 2.41	7.21 ± 2.15	< 0.001
Leptin (ng/ml)	12.28 ± 2.65	9.84 ± 2.66	13.59 ± 2.59	< 0.001
Resistin (μg/ml)	14.46 ± 4.16	17.2 ± 3.93	17.67 ± 3.60	< 0.001
A/L ratio	1.15 ± 0.37	0.79 ± 0.40	0.56 ± 0.22	< 0.001

Mean comparison (Mean ± SD); *p*-value of < 0.05 considered to be significant

Table 3 Correlation between adiponectin level and fasting insulin resistance in study subjects

Variable	Control (*N* = 100)		Pre-diabetic; Non-obese (*N* = 61)		Pre-diabetic; Obese (*N* = 39)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.148	0.143	0.031	0.119	0.471	0.812

adiponectin level and fasting insulin resistance with a -*p*-value of 0.143 in the control group, 0.119 in the prediabetic non-obese and 0.812 in the prediabetic obese group (Table 3).

In the case of leptin level and fasting insulin resistance, the results showed a weak positive correlation among the prediabetic obese group (*p*-value 0.299), and the prediabetic non-obese group (*p*-value 0.091) and an intermediate positive correlation among the control group (*p*-value 0.182). These results were statistically not significant (Table 4).

The Resistin levels and the fasting insulin resistance were correlated. On correlating resistin level with fasting insulin resistance also showed a positive weak correlation in control group (*p*-value 0.833) and positive intermediate correlation in the prediabetic-obese group (*p*-value 0.792) and a negative weak correlation in the prediabetic-non-obese group (*p*-value 0.167) (Table 5).

Discussion

Previous literature indicated that the fasting insulin level seems to be a reliable and promising tool for the diagnosis and management of prediabetes. Moreover, insulin resistance is the main determinant of developing prediabetes whereas beta cell function is the main determinant of T2DM [13, 14]. The results of the present study indicated that the state of insulin resistance may be a key point in the development of normal glucose tolerance in prediabetics. It was found that the mean fasting insulin resistance score among samples in experimental group (obese) (5.38 ± 0.93) was higher than the Fasting insulin resistance score among samples in experimental group (non-obese) (3.53 ± 1.12) and control group (1.49 ± 0.35). Fasting insulin resistance in all three groups was statistically significant ($p < 0.001$).

Adiponectin may be a useful marker in the identification of individuals with an elevated risk of prediabetes and coronary artery disease. Serum adiponectin concentration is inversely correlated with the severity of insulin resistance in patients with T2DM. A decreased level of serum adiponectin can be a risk factor for the progression of prediabetes and T2DM [15–17]. In the present study, serum adiponectin levels decreased significantly in obese prediabetes (7.21 ± 2.15 µg) when compared to non-obese prediabetes (7.28 ± 2.41 µg) and healthy subjects in the control group (13.64 ± 2.88 µg, $p < 0.001$). The adipose tissue is not only an inert storage depot for lipids, but also it secretes a variety of bioactive molecules, known as adipokines, which affect whole-body homeostasis. Adiponectin is the most abundant of these adipocytokines and is known to have a regulatory effect on the metabolism of glucose and lipids [14]. In the present study, the correlation of adiponectin level and fasting insulin resistance showed a weak positive correlation in all three groups with no statistical significance.

Leptin, another on adipokine may represent a predictor of obesity and T2DM [18]. Plasma leptin levels were associated with insulin resistance and prediabetes. Leptin may be an additional biomarker for screening individuals at high risk for prediabetes [19]. In the present study, serum leptin levels increased significantly in obese prediabetes (13.59 ± 2.59 ng) when compared to non-obese prediabetes (9.84 ± 2.66 ng) and healthy subjects in the control group (12.28 ± 2.65 ng, $p < 0.001$). The correlation between leptin level and fasting insulin resistance showed a positive weak correlation among prediabetic obese samples and prediabetic non-obese samples. However, a positive intermediate correlation among the control group was found.

In the present study, serum resistin levels increased significantly in obese prediabetes (17.67 ± 3.60 µg) when compared to non-obese prediabetes (17.2 ± 3.93 µg) and healthy subjects in the control group (14.46 ± 4.16 µg, $p < 0.001$). On correlating resistin level with fasting insulin resistance, they showed a positive weak correlation in control group and a positive intermediate correlation in the obese group i.e., as fasting insulin resistance increases, the resistin level increases and a negative weak correlation in the non-obese group i.e., as fasting insulin resistance increases, the resistin level decreases.

Table 4 Correlation between leptin level and fasting insulin resistance in study subjects

Variable	Control ($N=100$)		Pre-diabetic; Non-obese ($N=61$)		Pre-diabetic; Obese ($N=39$)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.173	0.182	0.275	0.091	0.105	0.299

Table 5 Correlation between resistin level and fasting insulin resistance in study subjects

Variable	Control ($N=100$)		Pre-diabetic; Non-obese ($N=61$)		Pre-diabetic; Obese ($N=39$)	
Fasting insulin resistance	R	*p*-value	R	*p*-value	R	*p*-value
	0.021	0.833	- 0.179	0.167	-0.044	0.792

According to previous data, it was considered that an Adiponectin-Leptin ratio equal or higher to 1.0 (with adiponectin concentrations expressed in μg/mL and leptin levels in ng/mL) can be considered normal, a ratio between 0.5 and 1.0 can indicate moderate-medium increased risk, and a ratio below 0.5 suggests a severe increase in cardiometabolic risk [12]. The findings of the present study indicated that the adiponectin-leptin ratio decreased significantly in obese prediabetes (0.56 ± 0.22) when compared to non-obese prediabetes (0.79 ± 0.4) and healthy subjects in the control group (1.15 ± 0.37, $p < 0.001$). The correlation between adiponectin-leptin ratio and fasting insulin resistance showed a negative weak correlation in both obese and non-obese groups and a positive weak correlation in the control group. The *p* values of all correlations appeared as not statistically significant.

Conclusion

Our study suggests that the adipokine profile is an ideal diagnostic tool to predict the underlying prediabetes and metabolic syndrome, especially in individuals with insulin resistance. Our findings suggested that there is a link between adipokines and insulin resistance in patients with prediabetes, adipocytokine (leptin, resistin, and adiponectin) concentration differed between patients who had normal BMI and those who were obese. Individuals with prediabetes who were obese also exhibited a disturbed adipocytokine profile in the form of a significantly increased leptin concentration and reduced adiponectin level, compared with prediabetic individuals with normal BMI. However, further studies are needed to identify the causal relationships involved and to determine whether treatment regulating adipocytokine levels could aid in personalized approaches for the management of diabetes and its prevention.

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Author contribution Study design (A), Data collection (B), Statistical analysis (C), Data interpretation (D), Manuscript preparation (E), Literature search (F), Fund collection. (ABCDEFG)- 1 Bineesh C P, (ACDEF)- 2 Pranav Kumar Prabhakar, (ACD)- 3 M. V. Vimal and (BEF)- 4 Vipin Viswanath.

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Declarations

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

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

Conflict of interest Authors declare no conflict of interests.

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ORIGINAL ARTICLE

Glycated albumin as a surrogate marker for prediabetes: a cross-sectional study

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Abstract

Objective Oral glucose tolerance test (OGTT) and glycated haemoglobin (HbA1c) have many limitations in diagnosing prediabetes. Glycated albumin (GA) estimation can be a potential tool for its early diagnosis. The present study aims to analyze the diagnostic efficacy of GA to identify prediabetes.

Methods Prediabetics (n = 406) and healthy (n = 406) subjects were included. OGTT was used as the diagnostic standard for identifying prediabetes. HbA1c was estimated in a Bio-Rad D-10 analyzer based on the High-Performance Liquid Chromatography (HPLC) method. GA was measured using the enzyme-linked immunosorbent assay (ELISA) technique and was expressed as a percent of total albumin. Total albumin was measured by the modified bromocresol Purple (BCP) dye-binding method in Siemen's autoanalyzer.

Results HbA1c ($5.83 \pm 0.57\%$) and GA ($14.43 \pm 1.92\%$) were significantly higher ($p < 0.05$) in the prediabetics as compared to healthy individuals. Both HbA1c and GA showed a significantly positive correlation with fasting plasma glucose (FPG) and 2-h plasma glucose. However, the correlation was stronger with 2-h plasma glucose for both parameters. GA and HbA1c also showed a significant positive correlation with each other. HbA1c, at 5.7% cut-off, predicted prediabetes with 74% sensitivity and 90% specificity. At the cut-off of 13.5%, GA showed 66% sensitivity and 85% specificity to identify pre-diabetes. The sensitivity of the combined tests was significantly greater than that for HbA1c alone (84% combined versus 74% HbA1c).

Conclusion GA, combined with HbA1c, can be used as a screening test for identifying pre-diabetes. Early diagnosis and interventions could prevent disease progression and limit dreadful complications.

Keywords Prediabetes · Glycated albumin · Diabetes mellitus · Marker · HbA1c

Introduction

Pre-diabetes is a state of hyperglycemia with raised blood glucose levels. It is not high enough to be considered type 2 diabetes mellitus (T2DM) yet, but persons with pre-diabetes are more likely to develop T2DM [1].

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The burden of diabetes continues to rise without effective prevention and control programs and it has been approaching epidemic proportions globally [2]. The prevalence of diabetes is increasing at a rapid rate, such that by 2045, there would be almost 629 million diabetic adults present in the world. Therefore, these alarming levels raise a matter of concern. Therefore, to reduce the chances of diabetes and also the complications associated with it, diabetes should be screened at the stage of prediabetes and an attempt should be made to delay or prevent the transition from prediabetes to diabetes [3, 4].

The Oral glucose tolerance test (OGTT) has been the diagnostic standard since 1979 to evaluate the ability to regulate glucose metabolism. However, this test is associated with many limitations. Within the past 10 years, for making the diagnosis of prediabetes or diabetes, American Diabetes Association (ADA) has adopted HbA1c in its diagnostic criteria. Although, HbA1c is a very simple and inexpensive test

it also has many disadvantages. HbA1c is associated with several limitations including poor specificity in pregnant women,, elderly population, and non-Hispanic blacks along with the risk of over-diagnosis of diabetes mellitus in patients with iron deficiency, RBCs loss, and its related anomalies. Moreover, alcohol consumption, chronic-end stage renal disease, and haemoglobin variants including genetic variants such as haemoglobin S and C traits can lead to an abnormal interpretation of HbA1c levels. These limitations can be overcome by implementing glycated albumin (GA) as a diagnostic marker. GA can demonstrate the glycemic status in a time period of approximately 14–21 days due to the short half-life of GA protein. GA was reported to show9-to-tenfold higher rate of non-enzymatic glycation compared to HbA1c.. Haemoglobin and iron-related anomalies do not affect the glycated albumin and hence can be used as reliable markers in such conditions [5–7]. Therefore, markers such as glycated albumin (GA), fructosamine, etc. could overcome these challenges. [5–7]. Thus, for the patients in whom the measurement of HbA1c may be unreliable, GA could be an attractive alternative option [8]. Therefore, the urgent need arises to introduce a surrogate marker such as GA which may be superior to or complement the existing glycemic control markers [9]. Therefore, we have taken this study to analyze the diagnostic efficacy of GA and correlate it with HbA1c in diagnosing prediabetes (Flowchart).

Materials and methods

Study design

Hospital-based observational, cross-sectional study. The present study was conducted at the HAHC Hospital, Jamia Hamdard University, New Delhi and Maharani Laxmi Bai Medical College, Jhansi, Uttar Pradesh.

Study inclusion criteria

Prediabetic subjects attended the outpatients' department (OPD) of a tertiary hospital of Hakeem Abdul Hameed Centenary Hospital (HAHC) and Maharani Laxmi Bai Medical College, Jhansi aged between 25–60 years, of either sex with a known history of prediabetes (based on the screening recommendation of the American Diabetes Association) along with healthy controls were chosen of the same age and sex-matched population among the escorts coming with patients attending OPD.

Study exclusion criteria

Diabetic patients who wereon anti-diabetic medication, presented albumin level ≤ 3.0 g/dL, anaemic patients, pregnant females, persons with, haemoglobinopathy rheumatic disorder, hepatic cirrhosis, chronic kidney disease, nephrotic syndrome, hypertension, hemodialysis, Cushing syndrome, and untreated thyroid dysfunction were excluded from the study as these disorders are known to influence the GA levels.

Study population

The study population consisted of 812 study subjects including 406 pre-diabetics and 406 healthy in the age group of 25–60 years (Fig. 1). Both males and females fulfilled the OGTT criteria for diagnosing pre-diabetes [10]. 406 healthy volunteers were taken as a comparison arm. The study subjects were matched for age, gender, and socio-economic conditions. Based on the assumption of 80% sensitivity for detecting prediabetes by using HbA1c and GA and 10% relative precision, the sample size estimated was 812. However, because of limited resources and feasibility, a sample size of 406 prediabetics and 406 controls were taken.

OGTT

A standard 75-g OGTT was performed after fasting for at least 8 h. Patients having FPG of 100 mg/dL to 125 mg/dL or 2-h plasma glucose of 140 mg/dL to 199 mg/dL after ingestion of 75 g of oral glucose load were included. The patients satisfied the screening criteria of ADA for diagnosing prediabetes [10]. Glucose concentrations during the OGTT were used as the diagnostic standard for identifying prediabetes.

Analysis of plasma glucose (FPG, 2-h plasma glucose)

3 mL of blood was withdrawn from study subjects under the sterile condition for glucose analysis. Blood was collected in a vacuum tube containing the glycolytic inhibitors potassium oxalate and sodium fluoride. Plasma glucose analysis was done by the hexokinase method using Siemens Healthineers, auto-analyzer Germany [11].

Estimation of HbA1c

For estimation of HbA1c, 2 mL of whole blood was collected in a vacutainer containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. HbA1c was estimated by the HPLC method on Bio-Rad D-10 analyzer, Hercules, California, USA, and correlated to the reference assay of the Diabetes Control and Complications Trial (DCCT). For detecting prediabetes by HbA1c, the recommended threshold of 5.7–6.4% was used [7].

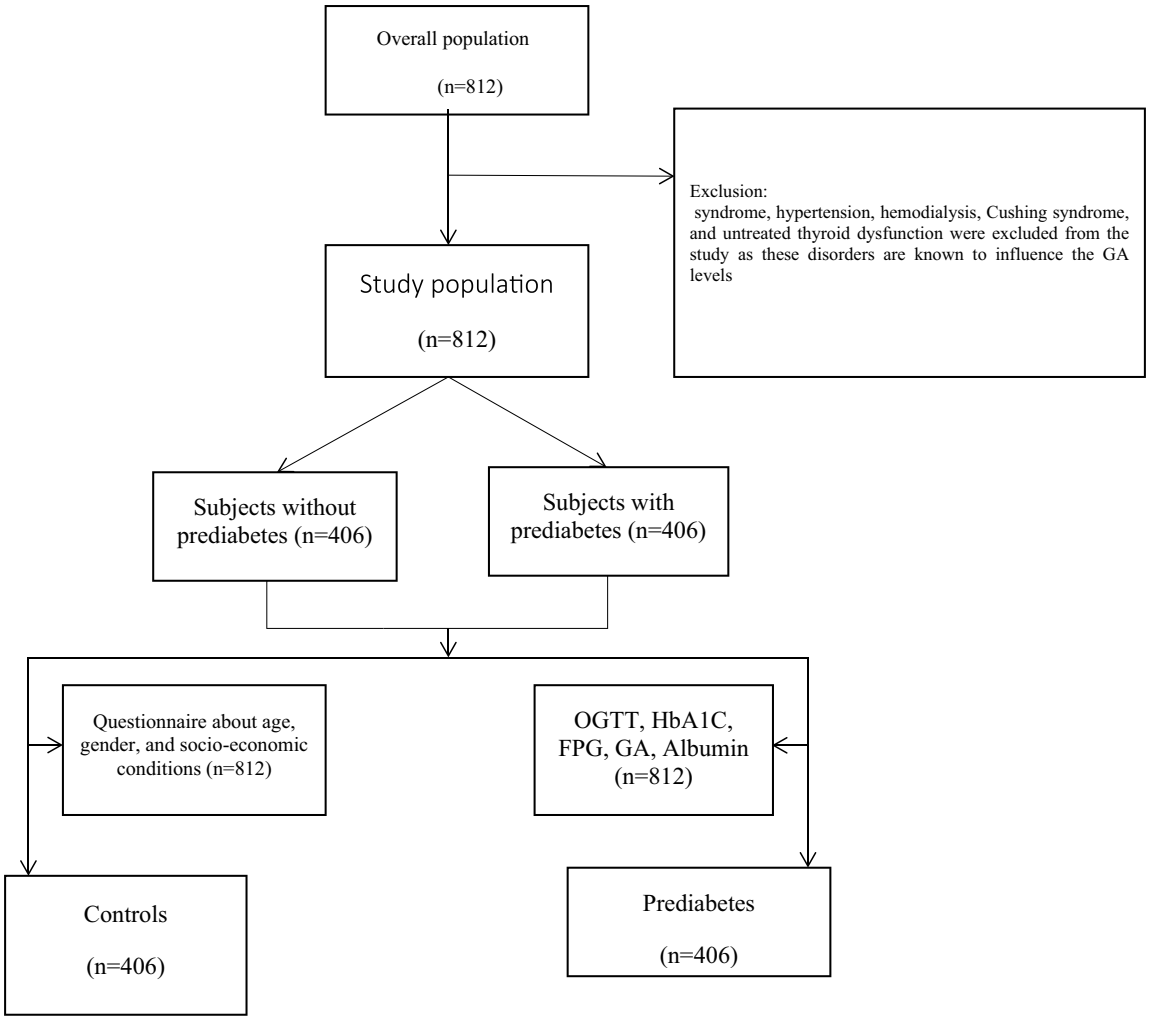

Fig. 1 Flow chart for the Study design

Estimation of albumin

2 mL venous blood was collected in a plain vacutainer under sterile conditions from the study subjects. Sera were separated for estimating albumin. Total albumin was measured by the method which is an adaptation of the bromocresol purple (BCP) dye-binding method in Siemen's autoanalyzer, Germany by using standard reagents and calibrators [12].

Estimation of GA

GA was measured by using an Enzyme-linked immunosorbent assay (ELISA) kit from Krishgen Biosystem, Mumbai, India. The ELISA procedure was performed as per standard protocol provided with the ELISA Kit from Krishgen Biosystem. Briefly, 100µL of each sample, blank (water), and controls (provided within the kit) were added to the appropriate wells followed by incubation for 1 h at 37^0C after covering wells with a plate sealer. Post incubation, the liquid from each well was removed followed by the addition of detection reagent A (100µL) in each well. Post addition, the plate was incubated again for 1 h at 37^0C. Post incubation, the liquid was aspirated followed by washing (3 times/2 min each) with 1 × wash solution (350µL) provided within the kit. Post washing the plate was placed inverted on absorbent pads for removing extra traces of wash solution. 100µL of the detection reagent B was added to each well followed by incubation for 30 min at 37^0C after covering the wells with a plastic sealer. The washing procedure was repeated as performed previously followed by the addition of 90µL substrate to each well and incubation for 15 min at 37^0C in dark. 50µL of stop solution (provided within the kit) was added to each well followed by measuring absorbance at 450 nm immediately. It was expressed as a percent of total albumin which was calculated by the formula [13]:

$$\text{GA (\%)} = 2.9\left\{\left[\text{GA(g/dL)}/\text{Total Albumin (g/dL)}\right] \div 1.4\right\} \times 100$$

Quality checks of all the routine blood assays were routinely performed by participation in internal quality-control programs provided by Bio-Rad (Hercules, CA, USA). The lab was also participating in the external quality control program namely the "CMC External Quality Assurance Scheme" from the Department of Clinical Biochemistry, Vellore, Tamil Nadu, India.

Statistical analysis

It was performed using Statistical Package for the Social Sciences (SPSS), 21st version, International Business Machines (IBM), New York (NY). The Sample size was calculated by Open Epi software, Atlanta, USA. The data were presented as mean ± standard deviation (SD). The statistical significance of the data was determined by Student's t-test. A value of $p < 0.05$ was considered to be statistically significant. Correlation analysis was done to determine the strength and degree of association among study variables. The Receiver operating characteristic (ROC) curve was used to compare the diagnostic value of study parameters and it was used to find the cut-off of parameters in diagnosing prediabetes.

Results

Comparison of OGTT with HbA1c and GA

Based on OGTT results, patients were selected. FPG values were 102.5 ± 11.49 mg/dL in prediabetic subjects as compared to healthy individuals 84.78 ± 9.89 mg/dL. Also, 2-h plasma glucose values were 163.92 ± 21.32 mg/dL in prediabetics as compared to 117.64 ± 14.88 mg/dL in healthy subjects. It was observed that mean HbA1c values were 5.83 ± 0.57% in prediabetes subjects as compared to 4.88 ± 0.60%in healthy subjects. Similarly, it was seen that GA was 14.43 ± 1.92% in prediabetics as compared to 11.15 ± 1.96% in healthy subjects. For all these parameters, the difference was statistically significant ($p < 0.05$) (Table 1).

Correlation among GA and HbA1c with blood plasma values

Pearson's correlation coefficients were used for studying correlation. In prediabetic subjects, a positive and statistically significant correlation was observed between FPG and HbA1c levels (r = 0.656) (p-value = 0.005) as well as between HbA1c and 2-h plasma glucose (r = 0.727) (p-value = 0.005) Correlation was stronger between HbA1c and 2-h plasma glucose as compared to FPG. The results of the present study also showed that the correlation of GA was positive and statistically significant (p-value = 0.01) with both FPG (r = 0.548) and 2-h plasma glucose (r = 0.647) (Table 2). Similar to HbA1c, the correlation of GA with 2-h plasma glucose was stronger as compared to FPG, although HbA1c showed a stronger positive correlation than GA. When GA was compared with HbA1c, a positive and statistically significant (p-value = 0.01) correlation (r = 0.787) was also seen. A scatter plot was made depicting the correlations between HbA1c and FPG**,** HbA1c and 2-h plasma glucose, GA and FPG**,** GA and 2-h plasma, and between HbA1c and GA and found significant**.**

Sensitivity and specificity of GA and HbA1c

ROC curve analysis showed that HbA1c, at a 5.7% cut-off, predicted prediabetes with 74% sensitivity and 90% specificity. Also, when GA was used, at 13.0% cut-off, sensitivity was 72% specificity was 80%, at 14% cut-off, sensitivity was 60%, specificity was 90% and at 13.5%

Table 2 Correlation of HbA1c and GA with FPG and 2-h plasma glucose levels

Parameters	Fasting plasma glucose		2-h plasma glucose	
	r value	R^2 value	r value	R^2 value
HbA1c	0.656	0.43	0.727	0.53
GA	0.548	0.30	0.647	0.42

Table 1 Comparison of FPG, 2-h plasma glucose, HbA1c, and GA between controls and prediabetes. Analysis was done using an independent t-test. *$p < 0.05$

Parameters	Control ($n = 406$) Mean ± SD	Pre-diabetes ($n = 406$) Mean ± SD	p value
FPG (mg/dL)	84.78 ± 9.89	102.5 ± 11.49 *	0.001
OGTT 2-h plasma glucose (mg/dL)	117.64 ± 14.88	163.92 ± 21.32 *	0.001
HbA1c (%)	4.88 ± 0.60	5.83 ± 0.57 *	0.01
Glycated Albumin (%)	11.15 ± 1.96	14.43 ± 1.92 *	0.02

cut-off, sensitivity was 66%, specificity was 85%, The sensitivity and specificity of both tests combined (HbA1c at 5.7% cut-off and GA at 13.5% cut-off) i.e. patients fulfilling both the criteria were: 84% and 72%, respectively (Table 3). To identify prediabetes, at the cut-off point of GA ≥ 13.5%, good sensitivity, and specificity (66%, 85% respectively) were seen using FPG and/or 2-h plasma glucose as reference values. The sensitivity of HbA1c and GA did not differ much (74% versus 66%, p-value < 0.05). However, the sensitivity of the combined tests was greater than that for HbA1c alone (84% versus 74%, p-value < 0.05). Specifically, 74% people were detected by HbA1c only, 66% people were detected by GA only, and 84% people were detected by both HbA1c and GA. Therefore, there was a substantial increase in the number of subjects because of the use of GA. However, the increase in sensitivity for the combined tests was associated with a decrease in specificity. The specificities for HbA1c (at cut-off 5.7%) and GA (at cut-off 13.5%) independently and in combination were: 90%, 85%, and 72% respectively. The areas under the ROC curves (AUC) for the identification of prediabetes for HbA1c and GA were (AUC: 0.864 for HbA1c, AUC: 0.831 for GA) respectively. General clinical characteristics of recruited study subjects are mentioned in Table 4. Significant changes were observed between HbA1c (%), FPG (mg/dL), PPG (mg/dL) and GA (%) in control and prediabetes study participants ($p < 0.001$).

Table 3 Sensitivity and specificity of HbA1c, GA, and both tests combined in predicting prediabetes

Diagnostic Parameters	Sensitivity	Specificity
HbA1c (cut-off 5.7%)	74%	90%
GA (cut-off 13.0%)	72%	80%
GA (cut-off 13.5%)	66%	85%
GA (cut-off 14.0%)	60%	90%
HbA1c (5.7%) + GA (13.5%) combined	84%	72%

Table 4 General characteristics of study subjects

	Control	Pre-diabetes
Total, *n* (%)	406 (50)	406 (50)
Men, *n* (%)	286 (49)	302 (51)
Women, *n* (%)	120 (54)	104 (46)
Age (years)	43.1 ± 6.6	42.9 ± 6.3
BMI (kg/m^2)	24.1 ± 3.6	25.9 ± 2.9
HbA1c (%)	4.88 ± 0.60	5.83 ± 0.57
FPG (mg/dL)	84.78 ± 9.89	102.5 ± 11.49
PPG (mg/dL)	117.64 ± 14.88	163.92 ± 21.32
GA (%)	11.15 ± 1.96	14.43 ± 1.92

BMI, body mass index; *FBG*, fasting blood glucose; *PPG*, Postprandial blood glucose; *GA*, Glycated Albumin. Continuous variables are presented as mean ± SD. Categorical variables are presented as number and percentage. Continuous variables were compared using student's t test

Discussion

OGTT has been the diagnostic standard for diagnosing diabetes for ages with associated limitations like more consumption of time, lack of reproducibility, and difficulty in taking samples. HbA1c, GA, and fructosamine can overcome these challenges. [5–7]. Albumin, being an abundant plasma protein readily participates in the non-enzymatic glycation process with a rate of 9-to 10 times higher than that of haemoglobin [14–17]. Recently, published studies demonstrated the utility of GA in the diagnosis of diabetes, renal, cerebral, and cardio-metabolic disorders [18, 19]. GA exhibits a broader fluctuation, thus rapid changes in blood glucose can be detected earlier [20]. Moreover, studies have suggested that GA is an intermediate-term glycation index for determining short-term glycemic changes over 2 weeks due to its lower half-life of 14–21 days [21]. HbA1c levels are also affected in conditions like reticulocytosis, transfusion, hyperbilirubinemia, hypertriglyceridemia, administration of drugs like dapsone, ribavirin, and uremia [20–28]. GA is a good biomarker in conditions potentially associated with an alteration of HbA1C, such as pregnancy and anaemia [29–31]. Therefore, because of these advantages of GA over HbA1c, GA could emerge as a possible marker and studies have suggested that it would represent an excellent index for monitoring short-term variations of glycemic control, pregnancy, liver diseases, chronic kidney disease undergoing dialysis, anaemia, haemoglobinopathies, and those receiving blood transfusions and microvascular complications of diabetes [32–35]. Our findings were supported by earlier studies which suggest that GA levels were raised in prediabetes [9, 36].

In the present study, prediabetic subjects showed a significant positive and stronger correlation between FPG and HbA1c levels and between HbA1c and 2-h plasma glucose. HbA1c gives an idea of overall glucose exposure which incorporates both fasting and postprandial hyperglycemia [37]. The present study also revealed an interesting finding that GA significantly and positively correlated strongly with 2-h plasma glucose as compared to FPG. Studies have shown that an independent variable for predicting cardiovascular complications and mortality in diabetes is 2-h plasma glucose, this is not the case with FPG, and therefore detection of these glucose variations is very important. Therefore, GA may reflect postprandial glucose levels and glycemic

variability more adequately than HbA1c [21, 38, 39]. Furthermore, it has been detected in our study that the correlation between GA and HbA1c was statistically significant and positive. This finding was supported by other studies which also showed a positive correlation between GA and HbA1c [40, 41]. According to a study conducted in Japan by Furusyo et al. in 2011, GA at a cut-off point of ≥ 15.5% identifies DM with both sensitivity and specificity of 83.3% [42]. Hwang et al. described a cut-off point of GA ≥ 14.3% for identifying prediabetes (sensitivity: 77.5%; specificity: 89.9%) in Korea [43]. Another study, conducted in Taiwan by Hsu et al., reported a cut-off point of 14.9% for diagnosing DM with a sensitivity of 78.5% and specificity of 80% [41]. A study conducted by Sumner et al. on 236 African Americans reported a cut-off point of GA ≥ 13.77% for diagnosing prediabetes. In the present study, we found that when HbA1c was used as a single diagnostic test to identify prediabetes, at a cut-off of 5.7%, sensitivity was 74%**,** and specificity was 90%. The cut-off point of GA ≥ 13.5% presented a good sensitivity and specificity (66%, 85% respectively) to identify prediabetes using FPG and/or 2-h plasma glucose as reference tests [6]. However, in the present study, it has been observed that diagnostic sensitivity might improve if HbA1c is combined with GA. After combining HbA1c with GA (at a cut-off of 13.5%), it was seen that sensitivity increased to 84% (combined test) from 74% (HbA1c) but the specificity decreased (HbA1c: 90%, versus combined test:72%) as seen in other studies also [9, 27]. In one of the previously published study it was also reported that GA had low sensitivity and higher specificity as it with complete agreement with our observations. The reasons behind the lower sensitivity are contributed by several factors including ethnicity that is independent of glycemia.

GA can be a better diagnostic and prognostic biomarker for the diagnosis of diabetes and its associated complications because it is associated with several advantages over glycosylated haemoglobin. In an anaemic condition, where there is a disorder of red blood cells, the rate of glycated in haemoglobin protein gets severely affected, thereby giving a false reading of glycemic index. Because GA is not affected by the red cell turnover and therefore reflects more accurate information. Similarly, in cases of iron deficiency with anaemia and without anaemia, GA can be an accurate marker compared to HbA1c [21]. Studies also support that GA is a good predictor marker of glycemic control in monitoring diabetes during pregnancy. A published study showed that the estimated glomerular filtration rate (GFR) in patients with CKD showed an inverse association with HbA1c rather than GA [44]. GA measurement can also be advantageous in conditions like nephrotic syndrome, liver, and thyroid disease [45]. Moreover, GA can predict accurately in conditions like HIV, tuberculosis, and other conditions where medication of nucleoside reverses transcriptase inhibitors were given [46]. GA assessment is also not affected by ethnicity and BMI as suggested by the previous literature [47, 48].

The finding that GA when combined with HbA1c could improve diagnostic sensitivity by detecting more cases of prediabetes would be very beneficial. Therefore, at this stage, preventive measures could be implemented and progression to diabetes could be curbed. Limitations of the present study include small sample size, cross-sectional study design, single centric study, and many unknown confounding factors that may affect the result. The strengths of this study were, age and sex-matched, standard techniques were used to measure study variables, recruited characterized study population. Further follow-up studies may be conducted on large scale to validate the results of the present study which might help in early patient management.

Conclusion

The need of the hour is to introduce a surrogate maker for detecting prediabetes so that early intervention could be done which could slow down the burden of disease and maximize health care resources. GA could be more than the missing link in controlling the diabetic epidemic. Also, additional comparison studies are to be carried out to ascertain its clinical utility. It is of utmost importance to curb progression to dysglycemic states when βcell function is still relatively more optimal and responsive to lifestyle modifications. Outstanding research would be to gain unequivocal evidence that GA could be a reasonable alternative and/or adjuvant to HbA1c in the diagnosis of prediabetes and hence diabetes.

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Data availability The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval Informed and written consent from prediabetics as well as healthy subjects was obtained before taking blood samples. An Ethical clearance certificate was obtained from the institutional ethical committee before the conduction of the study (No. JHIEC 08/2018). The present study followed the principles of the declaration of Helsinki.

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ORIGINAL ARTICLE

Targeted next-generation sequencing for maturity onset diabetes of the young (MODY) in a South Indian cohort of type 1 diabetes mellitus patients with preserved C-peptide

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Abstract

Objective MODY (maturity onset diabetes of the young) is a rare, monogenic, autosomal dominant form of diabetes occurring in young (< 25 years) individuals. It has a wide phenotypic variability and can be misdiagnosed as type 1 diabetes mellitus (T1DM). Appropriate detection of MODY has therapeutic and genetic implications. This study aimed to detect MODY among long standing, clinically diagnosed T1DM patients with preserved C-peptide levels.

Methods This was a hospital-based cross-sectional study from central South India which included 100 clinically diagnosed T1DM patients (according to ADA criteria), with a duration of > 3 years. MODY probability score, urinary C-peptide to creatinine ratio (UCPCR), and relevant biochemical investigations were performed. Targeted next-generation sequencing (NGS) for MODY-related genes (13 genes) was done for individuals with UCPCR > 0.2.

Results A UCPCR value of > 0.2 (suggestive of preserved endogenous insulin secretion) was observed in eight individuals. The mean HbA1c values were lower, and the MODY probability score was higher in individuals with preserved endogenous insulin (8.07% vs 9.53% and 2.8% vs 1.5%; *p-value*: 0.005 and 0.004 respectively). One of the eight individuals (12.5%) had a non-pathogenic gene variant in *KLF11*.

Conclusion In a South Indian cohort of T1DM with preserved C-peptide, we could not find any case of MODY through targeted NGS. UCPCR can be used as a screening tool to identify cases needing genetic testing for MODY. Larger studies utilizing whole exome sequencing should be conducted to know the actual prevalence of MODY among T1DM.

Keywords Targeted next-generation sequencing · Type 1 diabetes mellitus · Preserved C-peptide

Introduction

MODY (maturity onset diabetes of the young) is the commonest subtype of monogenic diabetes with an autosomal dominant inheritance accounting for 2.5 to 6.5% of all pediatric diabetes cases [1, 2]. The number of genetic mutations and different forms of MODY have increased from the initial description of 6 subtypes to 16 subtypes described currently [3]. The different subtypes of MODY differ in age of onset, pattern of hyperglycemia, and response to treatment. Majority of MODY subtypes present with isolated diabetes phenotype (with few exceptions like *HNF1B*-MODY, which presents with renal cysts and genito-urinary abnormalities) and can be misdiagnosed as familial type 1 DM (T1DM) or type 2 DM [4]. ISPAD (International Society Of Pediatric and Adolescent Diabetes) recommends testing for MODY in children with family history of diabetes in one parent and

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other first-degree relatives of the affected parent, absence of islet auto-antibodies at diagnosis, preserved beta cell dysfunction with low insulin requirements, and a detectable C-peptide over an extended period (> 5 years after diagnosis) [5]. However, none of the above criteria is pathognomonic in isolation and has to be considered together. Though family history of diabetes is an important criterion, sporadic de novo mutations in several causative MODY genes have been reported [6]. The prevalence of MODY in antibody negative T1DM may be as high as 6.5% [2]. Given these drawbacks and widespread lack of awareness, majority of children with MODY are misdiagnosed as T1DM. This has therapeutic implications as some subtypes of MODY can be treated with sulphonylureas with discontinuation of insulin leading to improved cost effectiveness and ultimately the quality of life [7]. Next-generation sequencing (NGS) has become a cost effective method for analyzing MODY genes. In a large Australian pediatric diabetes cohort, the prevalence of one or more antibodies among MODY cases was 18% [8]. With these considerations, we aimed to detect MODY in a South Indian cohort of clinically diagnosed T1DM individuals (both antibody positive and negative) with a preserved C-peptide through NGS.

Materials and methods

A cross-sectional single-center study was conducted on 100 individuals at a tertiary care hospital in central South India, between September 2019 and February 2021. All the patients with a clinical diagnosis of T1DM as per ADA criteria, for a duration of more than 3 years attending Endocrinology outpatient department, were enrolled in the study. Patients diagnosed withT1DM in the honeymoon phase (< 3-year duration) and those with other causes of diabetes were excluded from the study. Approval for this study was obtained from the Institutional Ethical Committee (approval number: IEC/GMC/2019/04/8, dated 05/09/2019). Written informed consent was obtained from each patient (or guardian for those under 18 years). The study was conducted as per the Declaration of Helsinki (as revised in 2013) and the International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use Guideline for Good Clinical Practice. The data was collected using a pretested questionnaire meeting the study's objectives. Detailed history, physical examination, and necessary investigations were made. Retrospective data collected from a history and medical records, age at diagnosis, disease duration, presence of diabetic ketoacidosis, episodes of hypoglycemia, insulin requirement, and family history were investigated. The examination included BMI, pubertal status, neuropathy disability score (includes vibration and touch sensation, ankle jerk, 10-g monofilament test), fundus examination for retinopathy, and other systemic examination. MODY probability scores were calculated using MODY Probability Calculator (MPC) in the Exeter Diabetes App which is based on a validated clinical prediction model by Shields et al. [9]. This program considers age at diagnosis, sex, whether or not on insulin treatment, BMI, HbA1c level, current age, and family history of diabetes, which are used to calculate a score for detecting patients who may have MODY. An MPC score ≥ 40% indicates a high likelihood of the patient having MODY. In addition, targeted NGS for MODY was planned for individuals with UCPCR > 0.2.

Procedure All patients attended following an overnight fast. Appropriate serum samples were sent immediately to the laboratory for analysis. At the same time, 2-mL EDTA blood sample was collected, and DNA was extracted and stored at –80 °C for NGS. Patients were asked to void urine before eating the breakfast. Patients self-administered their usual insulin as appropriate for the breakfast. Two hours following breakfast, patients were asked to provide a urine sample. Urine samples were stored at –80 °C until analysis.

Laboratory assays

Urinary C-peptide to creatinine ratio (UCPCR)

Postprandial UCPCR was calculated for each patient. Urinary C-peptide was analyzed using enzyme linked immunosorbent assay (ELISA) (DRG International, Inc., USA). Dynamic range of the assay was between 0.02 and 5.3 nmol/L with an intra-assay CV (%) (coefficient of variation percentage) of 5.13 to 6.54% and inter-assay CV (%) of 8.38 to 9.33%. Urine creatinine was measured by alkaline picrate method (Abbott Laboratories, USA). Results were converted from mg/dL to mmol/L using the conversion factor 0.08842. The limit of detection was 0.12 mmol/L with an intra-assay CV (%) of 0.6 to 1.4% and inter-assay CV (%) of 0.7 to 1.9%. A UCPCR ratio of ≥ 0.2 nmol/mmol was regarded as evidence of persistent insulin reserve. Molecular genetic analysis for MODY mutations was performed in subjects with a UCPCR value ≥ 0.2 nmol/mmol.

HbA1c measurements were performed using high-performance liquid chromatography (Biorad D10, USA). Anti-glutamic acid decarboxylase antibody 65(GAD) was analyzed using Isletest™ GAD ELISA diagnostic kit (BIOMERICA, USA). This method had a lower measurement level limit of 0.2 IU/mL. GAD ≥ 1.05 IU/mL was regarded as positive and value < 1 IU/mL was regarded as negative. IA-2 antibody was measured by ELISA (BIOMERICA, USA). IA-2 ≥ 1.05 IU/mL was regarded as positive and value < 0.95 IU/mL was regarded as negative. All antibody and C-peptide estimations were done using the

ThermofisherVarioskan LUXMultimode microplate reader at our department.

Molecular genetic analysis

All EDTA samples were sent to a multi-disciplinary research unit where DNA was isolated using a Fluoro AMP blood DNA kit and stored at –80 °C. MODY mutation analysis was done for subjects with UCPCR > 0.2. DNA samples were sent to Neuberg diagnostics for targeted NGS in 13 MODY-related genes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, and *KCNJ11*). Genomic DNA from the submitted specimen was enriched for the complete coding regions and splice site junction of genes listed above using a custom bait-capture system. Paired end sequencing was performed on an Illumina platform. Reads were assembled and were aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Data was filtered and analyzed to identify variants of interest.

Statistical analysis

Data were analyzed using excel and Epi Info 7.2.6.6. Mean and standard deviations were done for quantitative variables. An independent *t*-test for the mean was used wherever necessary to test for significance, and the *p*-value < 0.05 was considered significant.

Results

A total of 100 study subjects diagnosed with T1DM for more than 3 years were enrolled. The mean age at diagnosis of was 8.6 years and the mean duration was 5.3 years. Sixty percent of the study population had a history of DKA episodes. More than two-thirds of the population tested positive for GAD autoantibodies. The mean MODY probability score was 1.64%. More than 90% of the individuals had UCPCR < 0.2 nmol/mmol (Table 1).

Eight individuals out of the study population (T1DM duration > 3 years) had preserved endogenous insulin secretion (i.e., UCPCR > 0.2) (Table 3). Among them, four had a history of DKA episodes, and three had a family history of T1DM. The MODY probability score varied from 1 to 12.6, with a mean value of 2.8. Targeted NGS for the MODY gene panel was done in individuals with preserved endogenous insulin secretion. One study individual had a gene variant for *KLF11*. She was diagnosed with T1DM at the age of 21 years. There was an episode of DKA during the initial presentation. Both father and paternal grandmother were diagnosed with diabetes at about 35 years of age and were well-controlled with oral anti-diabetic drugs. The UCPCR value was 0.9, and MODY probability score was 12.6%. NGS revealed a heterozygous variant at Exon 3, c.1120G > A (p.Val374Met) position of the *KLF11* gene (Fig. 1). This variant was described as VUS (variant of uncertain significance) in the ACMG classification system and is not considered pathogenic in GnomAD database. Sanger sequencing for this gene variant, in the father and paternal grandmother, revealed the same variant in KLF11. Poor response to sulfonylurea was observed, and she was continued on insulin. Appropriate genetic counseling was done.

Table 1 Depicting the baseline clinical and biochemical characteristics

Variables	Mean ± SD (95%CI)
Age at diagnosis (years)	8.6 ± 3.6 (7.8–9.3)
Duration of diabetes (years)	5.3 ± 2.2 (4.8–5.7)
Sex (%)	
Male	41%
Female	59%
Family history of diabetes (%)	18%
History of DKA episodes (%)	60%
Insulin requirement (U/kg)	0.9 ± 0.3 (0.8–0.9)
Hypothyroidism (%)	9%
Mean BMI (kg/m^2)	20.3 ± 3.5 (19.6–21)
Neuropathy (%)	9%
HbA1c (%)	9.4 ± 1.1 (9.2–9.6)
GAD autoantibodies (%)	68%
IA2 autoantibodies (%)	56%
GAD65-positive patients according to duration (%)	
> 5 years	28%
< 5 years	40%
IA-2-positive patients according to duration (%)	
> 5 years	14%
< 5 years	42%
MODY probability score (%)	1.64 ± 2.2 (1.1–2)
UCPCR (nmol/mmol)	0.17 ± 0.25 (0.1–0.2)
> 0.2 nmol/mmol (%)	8%
< 0.2 nmol/mmol (%)	92%

The antibody IA-2 was more prevalent in individuals with T1DM for less than 5 years, and anti-GAD was more prevalent in those with more than 5 years of disease duration

Discussion

In this study, we found that, out of hundred clinically diagnosed T1DM patients for more than 3 years, eight individuals had a preserved endogenous insulin reserve, and one patient had *KLF11* gene variant with uncertain significance. UCPCR was utilized for assessing the beta cell function in the present study considering the limitations with serum C-peptide estimations. Serum C-peptide can be degraded

Table 2 Comparison between individuals with preserved and absent insulin reserve

Parameter	UCPCR > 0.2	UCPCR < 0.2	*p-value*
Mean UCPCR (nmol/mmol)	0.95	0.1	*0.001*
Mean duration of T1DM (years)	4.1	5.3	0.06
Mean HbA1c (%)	8.07	9.53	*0.005*
Mean MODY probability score (%)	2.8	1.5	*0.004*
Mean insulin requirement (IU/kg)	0.7	0.9	0.861

A significant difference was found between the HbA1c and mean MODY probability score in individuals with a UCPCR below and above 0.2 (Table 2)

Table 3 Clinical, biochemical, and targeted next-generation sequencing characteristics of individuals with preserved C-peptide

S.No	Age at diagnosis (yrs)	Duration (yrs)	HbA1c (%)	GAD	IA-2	DKA episodes	Family history	UCPCR	MODY probability score	Gene variant
1	12	12	8.4	Present	Present	Yes	No	0.4	1	None
2	12.5	3.5	6.5	Negative	Negative	No	Yes	1.2	4	None
3	15	4	6.9	Negative	Present	No	Yes	1	2.8	None
4	9	4.8	7.3	Present	Present	No	No	0.8	1.2	None
5	13.5	3.6	9.2	Present	Present	Yes	No	1.2	1.1	None
6	21	6	8.5	Negative	Negative	Yes	Yes	0.9	12.6	***KLF11***
7	14.5	3.5	9.2	Negative	Present	No	No	0.6	0.4	None
8	13	2.8	7	Negative	Negative	Yes	No	0.8	1	None

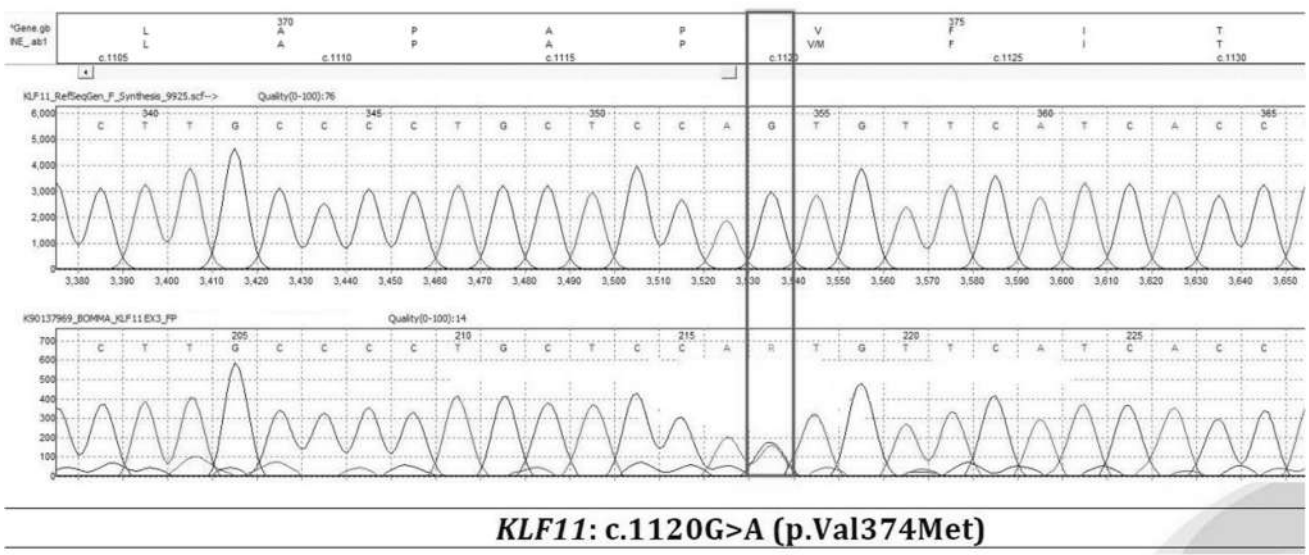

Fig. 1 Sangers phrenogram of subject 6 depicting the location of KLF11 gene variant

by proteases and requires cold chain maintenance for accurate estimation [10]. Urinary C-peptide is more stable and provides for non-invasive estimation, which is useful in pediatric populations [11]. Prior studies have shown a good correlation between UCPCR and stimulated serum C-peptide measurements. A UCPCR cut-off of 0.2 nmol/mmol had a sensitivity of 96.3% and specificity of 85.7% for identifying MODY cases among pediatric T1DM patients [12]. The same cut-off (0.2 nmol/mmol) was used in our study for selecting cases for MODY. In a Turkish study, 15% of T1DM patients (> 3-year duration) had preserved endogenous insulin secretion [13]. In comparison, only 8% had residual insulin secretion in our study which is significantly lower compared to the other studies. This can be attributed

to the delay in diagnosing T1DM and also to ethnic and geographical differences. Seventy-six percent of subjects had one or more anti-islet auto antibodies which is higher in comparison to a recent Indian study which revealed a lower percentage (70%) in newly detected T1DM [14]. Sero-conversion occurring later in the course of the disease may be the reason for this discrepancy. This concept was supported by a study conducted by Landin-Olsson Met al., where they concluded that newly diagnosed patients might be negative for auto-antibodies at diagnosis [15] and develop antibodies during the later stages of the disease. When the antibodies were studied in relation to the duration of disease, among individuals with a duration of T1DM < 5 years, IA-2 antibodies were more prevalent. In individuals with a duration of T1DM > 5 years, anti-GAD antibodies were more prevalent. The HbA1c values were lower, and the MODY probability score was higher in individuals with preserved endogenous insulin (8.07% and 2.8%; *p-value*: 0.005 and 0.004 respectively). Low MODY probability scores ranging from 1 to 12.6 among individuals with UCPCR > 0.2 are likely due to an early administration of insulin and its continued usage because of T1DM. One individual among eight individuals (12.5%) with preserved endogenous insulin had *KLF11* gene variant of uncertain significance. V Mohan et al. found that *HNF1 alpha* was the most common MODY variant (7.6%), followed by ABCC8 (3.3%) and KLF11 mutation is considered an infrequent cause of MODY in South India [16]. The patient with *KLF11* gene variant was overweight (BMI 23.5 kg/m^2) with a low insulin requirement. Anti-islet antibodies were absent, and the MODY probability score was lower (12.6%). Neve B et al. studied the role of transcription factor, *KLF11* on pancreatic beta cell function and prior to 2022 (during the conduct of the study), it was considered as a cause of MODY 7 [17]. However, Laver et al. demonstrated that the published variants in KLF11 (MODY 7), PAX4 (MODY 9), and BLK (MODY 11) showed poor co-segregation with diabetes (combined logarithm of the odds scores ≤ 1.2) and are all too common to cause MODY and recommended that these genes should not be included for MODY genetic testing [18]. Still many commercial labs until today include these genes for MODY genetic testing despite being refuted as cause for MODY. Reporting of these refuted variants may lead to inappropriate stopping of insulin and can increase the risk for DKA. Earlier, the occurrence of DKA at initial diagnosis is considered a hallmark of T1DM or an exclusion criterion for MODY. However, it is considered a non-specific feature which can occur in MODY as well [19]. There is a significant overlap in clinical characteristics like DKA, presence or absence of auto-antibodies, and MODY probability scores inT1DM and MODY. Employing a single criterion like MODY probability score or auto-antibodies may potentially lead to a misdiagnosis.

Despite the rigorous research in T1DM, there is a potential lacuna in the criteria for the selection of patients for genetic testing of MODY. Testing all the individuals is not feasible and may detect clinically non-significant variants leading to a predicament. Despite appropriate selection (T1DM with preserved C-peptide), we did not find any pathogenic variants for MODY suggesting that it may not be common in T1DM. However, this may not be true as the current MODY gene panel test for a limited number of genes and whole exome sequencing may be more appropriate to know the true prevalence.

Limitations The sample size of our study was small. We estimated only two auto-antibodies, i.e., GAD-65 and IA-2. Whole exome sequencing should have been done given the expanding number of genes responsible for MODY.

Conclusion

In a South Indian cohort of T1DM with preserved C-peptide, targeted NGS did not reveal any pathogenic/likely pathogenic variants. UCPCR can be used as a screening tool to identify cases needing genetic testing for MODY. The existing clinical criteria and MODY probability calculator are inadequate and can lead to misdiagnosis of MODY. Larger studies utilizing whole exome sequencing should be conducted to know the actual prevalence of MODY among T1DM.

Author contribution The present manuscript has been read and approved by all the authors. The requirements for authorship have been met, and each author believes that the manuscript represents honest work.

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Design: Contributor 1, Contributor 2, Contributor 3, Contributor 4, Contributor 5

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Clinical studies: Contributor 1, Contributor 2, Contributor 3, Contributor 4, Contributor 5

Experimental studies, Data acquisition: Contributor 1, Contributor 2, Contributor 3, Contributor 4, Contributor 5

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Data Availability The data from the present study are not publicly available but can be made available from corresponding author upon reasonable request.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for this study was obtained from the Institutional Ethical Committee (approval number: IEC/GMC/2019/04/8, dated 05/09/2019).

Consent to participate Informed consent was obtained from all individual participants (or parents if minor) included in the study.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Family-based association of 4q27chromosomal region covering IL2-IL21 genes with type 1 diabetes (T1D)—a study of genetic risk factors

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Abstract

Objective Pancreatic beta cell destruction is a hallmark of type 1 diabetes (T1D), a heterogeneous disorder with a wide range of potential causes. T cell activation molecules have been shown to play an important role in the development of T1D, according to the majority of studies. Some autoimmune diseases have been linked to SNPs in the 4q27 region, specifically in the KIAA1109-interleukin 2 (IL2)-IL21 block. The purpose of this research was to look into how certain polymorphic variants in the 4q27 region are linked to T1D.

Methods We investigated whether variants in the 4q27 region could be a causal factor in T1D susceptibility. Polymorphisms of ten single-nucleotide polymorphisms (SNPs) belonging to the KIAA1109/IL21/IL2 block were studied in 255 individuals from 59 families using the Sequenom MassARRAY platform.

Results The IL21/IL2 region was found to have a significant association with T1D in Tunisian cohorts. We found that the T allele of the rs2221903 marker is disproportionately passed down from parents to their children. In addition, haplotype analyses encompassing all of the SNPs under consideration show that the GACAGGA and the shortly TT haplotypes were significantly over-transmitted from parents to their children, suggesting they may be a T1D genetic susceptibility factor in our population.

Conclusion Several autoimmune disorders (ADs) have been linked to the IL2/IL21 genes, suggesting that there is a shared genetic background that confers a common genetic predisposition across ADs. More research into the genetic and functional aspects of the 4q27 region is needed to better explain the role it plays in the risk of ADs.

Keywords 4q27 · T1D · Polymorphism · Genetics · Autoimmunity · IL2/IL21 · Fbat

Introduction

Absolute insufficiency of insulin secretion is the hallmark of T1D, a heterogeneous disorder characterized by the destruction of pancreatic beta cells [1]. Research into the genetics of T1D and how it interacts with specific demographic, clinical, and biologic markers has the potential to enhance current methods of prediction, prevention, and intervention in both individual patients and the population at large.

Research into the genetics of T1D has yielded several candidate gene studies, and efforts are currently underway to identify the risk genes. Several genes, including CTLA-4, IL2RA, PTPN22, and INS-VNTR, have been linked to an increased risk of T1D, in addition to the human leukocyte antigen (HLA) on chromosome 6p21. It is now generally agreed that many different genes play a role in T1D, and that studying these genes may shed light on the disease's complex, multifactorial pathogenesis [2, 3]. T1D has been linked to a number of genes, including PTPN22, IL2RA (CD25), and CD28 [4, 5], and our research confirms the results of many previous studies [6–8]. Recent studies in the UAE, Sudan, and southern India have linked multiple variants in the CTLA-4, PTPN22, INS, and IL2-RA genes to T1D [9–11]. Although variations in these genes are a major

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contributor to the development of T1D, it is possible that other genes play a role in the pathogenesis of the disease as well.

Numerous autoimmune disorders (ADs), including T1D [12–14], ulcerative colitis [15], celiac disease (CD) [16], systemic lupus erythematosus (SLE) [17], and Grave's disease (GD) [18] have been linked to the KIAA1109-IL2-IL21 region at 4q27. Recent research has pinpointed the IL2/IL21 locus on 4q27, which contains genes involved in controlling immune responses, as a potential risk factor for developing coeliac disease [19]. This data supports the hypothesis that the KIAA1109-IL2-IL21 pathway affects the heritability of Alzheimer's disease in humans. The 4q27 locus, which contains the IL2 and IL21 genes, spans about 200 kb. Both of these genes are promising biological candidates for ADs because their encoded cytokines play important roles in T- and B-cell proliferation and distinct immunological activation pathways [20].

First of all, IL2 is a growth factor, a differentiation factor, and a regulator of cell death, so it is no surprise that it is on the list of promising candidate genes for autoimmunity disease [21]. IL2 is a pleiotropic cytokine, meaning that it not only plays a role in the termination of Tcell responses but also in the promotion and induction of T cell proliferation. Therefore, IL2 can stimulate the development and expansion of natural killer T cells (NKT) and induce immunoglobulin production in B cells [20, 22]. T helper 17 (Th-17) cells have been shown to be the primary producers of the effector cytokine IL-21, which has been shown to have pleiotropic effects on both innate and adaptive immune responses by, for example, stimulating CD8 T cells and NKT to acquire a more potent cytotoxicity and promoting T cell proliferation and differentiation [23, 24]. Because of this, alterations in the expression of these genes may lead to immune regulation disorders that manifest as autoimmunity.

Many autoimmune diseases (ADs) have been linked to genetic variation within the chromosome 4q27 locus, which contains the IL2/IL21 genes [13, 25]. These include T1D, coeliac disease, Graves' disease, and systemic rheumatoid arthritis. Multiple subsequent studies in various populations have confirmed these findings, and the application of this strategy to additional autoimmune diseases (ADs) like inflammatory bowel disease, lupus, and psoriasis has also been demonstrated [26, 27].

T1D susceptibility has been linked to a number of candidate genes over the past decade, but many of these genes still need to be studied in independent populations. Given the significance of the interleukin (IL) 2/interleukin (IL) 21 pathway in immune response, autoimmunity, and the increased efficacy of haplotype approaches, we set out to assess the role of the 4q27 region, which contains the IL2 and IL21 genes, in T1D genetic susceptibility.

Methods and materials

Study design

Blood samples were collected from members of 59 families that including 86 childrens with T1D (mean age, 12 ± 6.36 years with a range of 2–45 years) and 169 of their parents (mean age, 30 ± 10.60 years with a range of 3–57 years) (Table 1). Clinically affected patients and their first-degree relatives were recruited at the pediatric departments of Hedi Chaker Hospital (Sfax, Tunisia). The inclusion criteria for the recruitment of T1D patients were the presence of diabetic ketosis at onset, a dependence on insulin therapy for controlling hyperglycemia, their serum blood contain at least one of the anti-islet auto-antibodies (glutamate decarboxylase (GADA), insulin (IAA), zinc transporter 8 autoantibodies (ZnT8A), islet cell antigen (ICA), and IA2 protein (IA2A)). We excluded studies of patients who have other types of diabetes. The prospective study included patients who were not obese, free of concomitant complications, did not receive any further treatment. All subjects were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

DNA extraction and genotyping

Genomic DNA was purified from whole blood by overnight proteinase K digestion of lysed peripheral lymphocytes followed by phenol/chloroform extraction, according to a previously described protocol [28]. Ten

Table 1 Demographic and clinical characteristics of the sample

Features	T1D patients	Relatives (controls)
Number	86	169
Mean age	12 ± 6.36	30 ± 10.60
Male	49	86
Female	37	83
Origin	South/center	South/center
Serology (anti-GAD, anti-IAA, anti-ZnT8A, anti-ICA, anti-IA2)	Positive for at least one of the auto-antibodies	Negative

single-nucleotide polymorphisms (SNPs) were chosen from the KIAA1109-IL21-IL2 region (Table 2) and tested for their association with T1D genetic risk. SNPs for this study were chosen from HapMap, and mapping data was obtained from the db SNP built 126 database, both of which can be found at http://www.ensembl.org (Table 2). Genomic sequences containing target SNPs were amplified by multiplex polymerase chain reactions (PCR). The amplified product was then cleaned using shrimp alkaline phosphatase and used for allele-specific primer extension reaction. The reaction mixture was then spotted into a SpectroCHIP microarray. The extended products were analyzed by MALDI-TOF (matrix-assisted laser desorption ionization–time-of-flight) mass spectrometry; the time-of-flight is proportional to mass, which allows to determine the size of products generated. Sequenom supplies SpectroTYPER software that automatically translates the mass of observed primers into a genotype for each reaction. SNPs that have a call rate of 80% or higher in Hap Map's control samples and that have been verified to be in Hardy–Weinberg equilibrium in healthy parent samples ($p > 0.05$) have been chosen.

Statistics

Using the family based association test "FBAT" software (https://sites.google.com/view/fbat-web-page), we analyzed the genetic association of T1D with a transmission disequilibrium test (TDT). A variety of TDT, including allelic/genotypic and haplotype analyses, were performed by using generalized mixed genetic models, and the best fitting model results were showed. Furthermore, in order to obtain more information and maximize the power analysis of the FBAT test, we include in the study all family members in the pedigree, notably the families with missing parents. For all statistical tests, p value was considered statistically significant if it is less than or equal to 0.05 ($p \leq 0.05$). To estimate the LD between different markers, the haploview programme (http://www.broad.mit.edu/mpg/haploview/) was used to conduct a pairwise LD analysis.

Table 2 Polymorphisms and investigated genes

Genes	Variants	Chromosomes	Location
KIAA1109	rs6534347	4	123,198,435
IL2	rs11575812	4	123,371,049
IL2	rs2069778	4	123,376,135
IL2	rs2069763	4	123,377,482
Upstream IL2	rs2069762	4	123,377,980
Upstream IL2	rs1479924	4	123,387,600
Intergenic	rs6852535	4	123,478,716
Intergenic	rs12642902	4	123,508,501
Intergenic	rs6822844	4	123,509,421
IL21	rs2221903	4	123,538,912
IL21	rs17005931	4	123,545,648

Results

From 10 variants spanning IL21/IL2 region, the risk T allele of rs2221903 was significantly more transmitted from parents to T1D's children ($p = 0.035$, $z = 2.100$). However, the C allele was less transmitted from parents to their affected children ($p = 0.035$, $z = -2.100$). In the genotypic analysis, the homozygous CC genotype was less transmitted than what would be expected by chance from informative parents and seems to be less risk factor for T1D ($p = 0.030$; $z = -2.160$). The remaining SNPs of IL2/IL21 region were not confirmed any association between T1D and genotyped families (Table 3).

FBAT analysis was indicated that T allele (rs6822844) that is located within a noncoding region upstream of IL21 and downstream of IL2 was over-transmitted than what would be expected by chance ($p = 0.057$, $z = 1.897$), but it is still statistically insignificant to conferred a T1D genetic risk (Table 3).

LD analysis revealed two blocks across the IL21-IL2 SNPs. The first little evidence for LD is between rs2221903 and rs6822844, covering 29 kb (Fig. 1). The second block is covering 137 kb that encompassed seven analyzed SNPs (rs6534347, rs11575812, rs2069778, rs2069763, rs2069762, rs1479924, and rs6852535). In contrast to rs6822844 that has been found in complete LD with three SNPs (rs11575812, rs2069778, and rs1479924),whereas SNP (rs2221903) showed a little LD with other variants, indicating a statistical separate association of rs6822844 and rs2221903 with disease risk (Fig. 1). The haplotype association analyses including all studied SNPs for IL21-IL2 genes (block 1 and 2), indicate that GACAGGA haplotype derived from block 1 and TT haplotype derived from block 2, were significantly over-transmitted from parents to affected offspring (Table 4), proving their possible role in T1D genetic risk.

Discussion

In this work, we found a significant and marginal genetic linkage, repectively between rs2221903 and rs6822844 variants located in the IL2/IL21 locus and T1D among some Tunisian families. We found a similar positive association between the IL2/IL21 region and T1D pathology in our previous case–control study [5]. Over the past decade, researchers have found more and more evidence linking the IL2-IL21 region at 4q27 to multiple ADs. Wellcome Trust Case Control Consortium GWAS data have also reported this region in their search for T1D genetic risk [25], which

Table 3 FBAT analysis of SNPs markers in IL21

Marker	Allele	freq	fam#	*S*	*E*(*S*)	*Z*	*p*
rs2221903	C	0.193	25	18.000	25.500	−2.100	0.035
	T	**0.807**	**25**	**56.000**	**48.500**	**2.100**	**0.035**
	CC	**0.000**	**8**	**0.000**	**3.500**	**−2.160**	**0.030**
	CT	0.385	25	18.000	18.500	−0.164	0.869
	TT	0.615	25	19.000	15.000	1.382	0.166
rs6822844	G	0.952	7	12.000	15.000	−1.897	0.057
	T	0.048	7	8.000	5.000	1.897	0.057
	GG	0.905	7	2.000	5.000	−1.897	0.057
	GT	0.095	7	8.000	5.000	1.897	0.057
	TT	0.000	0	*****			

This table contains only the associated markers

FBAT family-based association test, *freq* allelic and genotypic frequencies, *Fam#* number of informative families, *S* test statistics for the observed number of transmitted alleles, *E(S)* expected value of S under the null hypothesis (i.e., no linkage or association)

Significant *p* values ($p < 0.05$) are in boldface

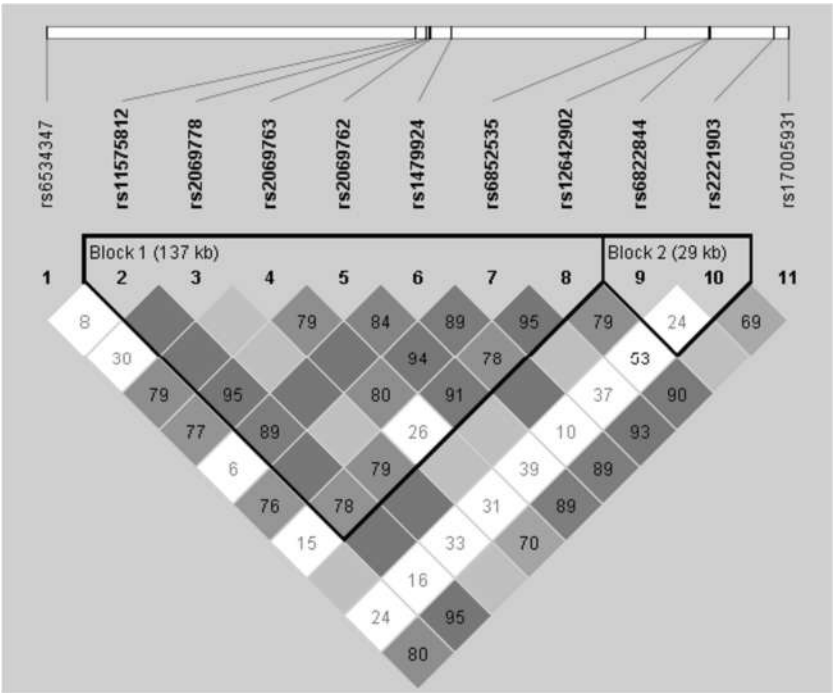

Fig. 1 Haploview analysis for LD (D') measures between SNPs genotyped in *IL2/IL2*. The blocks generated (blocks 1 and 2) under confidence interval algorithm of HAPLOVIEW are marked. Block 1 is constituted by seven SNPs: 5 variants from IL2 (rs11575812, rs2069778, rs2069763, and rs2069762) and two variants from IL21 (rs6852535 and rs12642902). Block 2 is generated between two SNPs from IL21 gene

is consistent with our findings [25]. Studies that followed GWAS included a meta-analysis of 305,090 SNPs from three GWAS [14]. This meta-analysis, along with others found that the 4q27 region was strongly associated with an increased risk of T1D [13, 26, 29]. KIAA1109/Tenr/IL2/IL21 of 4q27 has been previously linked to type T1D risk, and our study confirms this association in the Tunisian population, suggesting that some genetic risk factors are shared by people of different racial and ethnic backgrounds. A better understanding of T1D and its treatment will result from research into the similarities and differences in genetic susceptibility across populations.

4q27 is a long region with a large block (480 kb) of linkage disequilibrium known KIAA1109/Tenr/IL2/IL21 [16] that contain several gene including the KIAA1109 gene that encoding a protein of unknown function, the Testis Nuclear RNA-binding protein (TENR), and the IL2 and IL21 genes, which are both plausible functional candidate genes risk for T1D. As far as we are aware, no single variant can trigger the autoimmune response on its own. The causal variant(s)

Table 4 Results of haplotype association analysis for the SNPs with linkage disequilibrium

Block	Haplotype	Freq	*T*: *U*	Chi square	*p* value
Block 1	AGCCAAG	0.369	23.0: 28.0	0.49	0.4841
	GGCAGGA	0.217	22.9: 20.7	0.112	0.7383
	AGCAAGG	0.123	13.1: 13.6	0.009	0.9262
	AGAAAGG	0.102	11.5: 10.4	0.051	0.8207
	GGCAGGG	0.028	3.8: 2.6	0.207	0.6494
	GACAGGA	**0.024**	**7.0: 1.0**	**4.5**	**0.0339**
	AGAAAGA	0.020	3.0: 2.0	0.2	0.6544
	AGCAAAG	0.019	3.0: 5.0	0.497	0.4809
	GGCAAGG	0.017	1.6: 0.5	0.556	0.4557
	AGCCGAG	0.011	1.2: 2.3	0.304	0.5813
	AGACAAG	0.010	1.0: 2.0	0.309	0.5783
	AGCAGGA	0.010	1.0: 1.1	0.001	0.9783
Block 2	GT	0.784	34.2: 26.2	1.059	0.3035
	GC	**0.184**	**19.2: 33.2**	**3.736**	**0.0532**
	TT	**0.028**	**8.0: 1.0**	**5.444**	**0.0196**

that may correlate with mRNA translation or regulate the expression or function of the gene may be in LD with the associated SNP. In our study, we found that the GACAGGA and TT haplotypes for the KIAA1109/Tenr/IL2/IL21 SNPs were significantly associated with T1D. The IL2/IL21 genes are both plausible functional candidates as genetic modifiers of autoimmunity [13], and extensive LD within this block means that none of these genes can be ruled out as the causal one.

IL21 is a cytokine that has potent immunomodulatory activity and predominantly produced by Th17 and NKT cells [30]. This cyokine stimulates T cell proliferation, increases cytotoxicity of two cytotoxic lymphocyte subsets; CD8 + T cells and NKT cells, and enhances the naïve B cell differentiation [31]. A previous study showed that increased IL-21 production, elevated IL21R expression, and polymorphisms in either gene have been documented [32, 33]. While increased IL-21/IL21R signaling has been described before as a driver of autoantibody generation [34]. The combination of anti-IL-21 and the glucagon-like peptide-1 receptor antagonist Liraglutide was able to reverse T1D in NOD mice, and histological sections of the pancreata have a visually smaller CD8 T cell infiltrate into the islets [35]. The fact that IL21 exhibits these features raises the possibility that this cytokine has a wide range of effects on both the innate and adaptive immune systems [36]. Any change in IL21 production leads to defective IL-21 signalling in T cells, suggesting that IL21 has a different biological effect on different lymphoid cells. IL21 activates the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, in particular Jak1 and Jak3 and STAT1 and STAT3 [23, 37, 38].

By downregulating FOXP3 expression in CD4 + T cells [39] and making CD4 + CD25-T cells resistant to regulatory T-cell-mediated suppression [40], IL-21 plays a crucial role in Th17 differentiation [41].

High levels of IL-21 expression have been found in Th-17-related autoimmune diseases (ADs), such as multiple sclerosis [42], inflammatory bowel disease [43], and rheumatoid arthritis [44]. Rosanne et al. conducted an analysis of IL-21's role in the onset of diabetes in NOD mice and found that blocking IL-21 signalling completely prevented the disease from manifesting itself [45]. Animal models in which IL2 and IL21 cytokine genes were deleted showed an increased risk of developing ADs, demonstrating the early importance of these cytokines in the immune response [46]. By the same, it has been found that mice deficient in IL2, show hyperativation and uncontrolled proliferation of T cells leading to rapid lethal autoimmunity. A defect in Treg cells due to reduced IL-2 levels coincided with the IL-21-driven expansion of diabetogenic T cells, which provides a scenario by which researchers can strengthen connections between the roles of both IL-2 and IL-21 in T1D [47]. Indeed, another study showed that depletion of IL-2-dependent Treg cells improved the efficacy of IL-21 to mediate supression of FoxP3 + Treg cells [39] argues that these two cytokines work toguether and they need to communicate with each other.

Conclusion

In this study, we confirm that IL2/IL21 region is associated with T1D genetic risk. This association has been found with several ADs, suggests that there is a common genetic background bconfering a genetic predisposition across the different ADs. Much more genetic and functional works is required before researchers can obtain more precise details to explain the role of 4q27 region in the risk to ADs.

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Declarations

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

Ethical approval All subjects were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

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ORIGINAL ARTICLE

The effect of atorvastatin on the concentrations of methylglyoxal, glyoxalase 1, and aldo–keto reductase family 1 member B10 in patients with type 2 diabetes mellitus and prediabetes

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Abstract

Background Type 2 diabetes mellitus (T2DM) is often associated with metabolic disorders. Statin drugs are potent inhibitors of cholesterol biosynthesis.

Objective The aim of this study was to evaluate the effect of atorvastatin on the concentrations of methylglyoxal (MGO), glyoxalase 1 (GLO-1), and aldo–keto reductase family 1 member B10 (AKR1B10) in patients with T2DM and prediabetes.

Methods This study was conducted on 80 subjects with and without T2DM and prediabetics divided into 5 groups: patients with T2DM receiving statins (group A, $n=17$), patients with T2DM not receiving statins (group B, $n=17$), patients with prediabetes receiving statins (group C, $n=12$), patients with prediabetes not receiving statins (group D, $n=17$), and healthy controls without T2DM (control group, $n=17$). Patients with T2DM and prediabetes received atorvastatin 20 mg/day for 3 months. The measurement of MGO and AKR1B10 was performed with a non-competitive sandwich-type enzyme-linked immunosorbent assay (ELISA) at 450 nm. The measurement of GLO-1 was performed by an enzymatic method at 240 nm.

Results The serum level of MGO was significantly higher in patients with T2DM and prediabetes than that of healthy controls ($p=0.001$). In patients with T2DM, statins decreased the serum level of MGO, but in patients with prediabetes, statins increased the serum level of MGO ($p=0.001$). The level of GLO-1 activity was significantly higher in healthy controls than that of patients with T2DM and prediabetes ($p=0.001$). Furthermore, the level of GLO-1 activity was significantly higher in patients with T2DM and prediabetes receiving statins than that of patients with T2DM and prediabetes not receiving statins ($p=0.002$). The serum level of AKR1B10 was significantly higher in groups C and D than that of the other groups ($p=0.001$).

Conclusion Atorvastatin can improve the level of GLO-1 activity and thereby prevent diabetic complications.

Keywords Type 2 diabetes mellitus · Prediabetes · Atorvastatin · Aldo–keto reductase family 1 member B10 · Glyoxalase 1 · Methylglyoxal

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Introduction

Prediabetes is a condition in which blood glucose or hemoglobin A1c (A1c) levels are higher than normal but not high enough to be classified as diabetes. People with prediabetes are at high risk for developing type 2 diabetes [1]. Patients with prediabetes are at high danger of developing type 2 diabetes mellitus (T2DM) [1, 2]. T2DM is often associated with metabolic disorders, including hyperinsulinemia, insulin resistance, beta cell dysfunction, dyslipidemia, and obesity. Also, in patients with T2DM, triglyceride, cholesterol, very low-density lipoprotein cholesterol (VLDL-C), and LDL-C levels increase, and high-density lipoprotein cholesterol (HDL-C) levels decrease [3, 4]. Statins are potent inhibitors of cholesterol biosynthesis. Thus, these drugs reduce cholesterol, VLDL-C, LDL-C levels, and thereby the prevalence of cardiovascular diseases (CVDs). Statins inhibit cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA-R) activity [5–7]. Isoprenoids, such as farnesyl pyrophosphate (FPP) and geranyl–geranyl pyrophosphate (GGPP), are also produced in the cholesterol biosynthesis pathway; statins also inhibit the synthesis of these isoprenoids. Effects beyond inhibition of cholesterol biosynthesis of statins are branded as pleiotropic benefits [8]. Membrane permeability varies between different statins; thus, lipophilic statins (such as atorvastatin, simvastatin, lovastatin, fluvastatin, and pitavastatin) enter the liver and non-liver cells through passive transmission. However, hydrophilic statins (such as rosuvastatin and pravastatin) enter the liver cells through active transmission and specific carriers. These statins cannot enter the non-liver cells; this justifies the diverse impacts of statins [9–12]. Rho guanosine triphosphatases (GTPases) are members of small G-proteins or GTPase superfamily requiring the GGPP for activations [13, 14]. Lipophilic statins inhibit the function of Rho family members in non-liver cells, such as vascular endothelial cells, by inhibiting GGPP synthesis [15]. Rac-1 is a member of the Rho family and plays a role in vascular inflammation [15–17]. Accordingly, Rac-1 produces superoxide anion (O_2^-), thereby creating oxidative stress by activating NADPH oxidase [18–22]. Moreover, it has been shown, statins increase the expression of catalase [23]. Therefore, statins can act as an antioxidant.

Increased blood glucose in diabetes results in increased α-dicarbonyls [24]. α-Dicarbonyls are primarily formed as glycolytic intermediates during glucose metabolism. These compounds include methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG). Excessive accumulation of these compounds results in dicarbonyl stress [25, 26]. α-Dicarbonyls are highly unstable and reactive and react quickly with other compounds (including proteins, lipids, and nucleic acids), leading to glycation of these compounds and alteration of their functions. Accordingly, these compounds are known as important intermediates for diabetic complications [27–29]. The creation of α-dicarbonyls occurs during glycolysis, polyol pathways, Maillard reactions and autoxidation of carbohydrates [30–35]. Maillard reactions and autooxidation of carbohydrates require oxidative factors. In addition, reactive oxygen species (ROS) are produced in these reactions; therefore, dicarbonyl stress and oxidative stress are interrelated [34–38]. The degradation of α-dicarbonyls prevents the toxic impacts of these compounds. MGO and GO are primarily degraded by the glyoxalase 1 (GLO-1) enzyme. This enzyme needs reduced glutathione (GSH) for its activity [39–43]. Moreover, MGO is partially degraded by the *aldo–keto reductase* (AKR) enzyme. AKR can play a role in the decomposition of 3-DG [43, 44]. The AKR superfamily contains several enzymes that are similar in structure and enzymatic activity [45]. AKR family 1 member B10 (AKR1B10) is a member of this superfamily, which can result in the reduction of various kinds of aldehydes and ketones. However, the physiological performance of AKR1B10 has not been completely elucidated [46]. It has been shown, AKR1B10 can be associated with diabetes progression. Besides, it can be contributed to the decrease in diabetic complications through MGO and 3-DG degradation [47, 48].

Materials and methods

Study design

This case–control study was conducted on patients with T2DM and prediabetes referred to a department of endocrinology, as well as on healthy controls referred to a medical diagnostic laboratory (Babol, Iran) for checkup. Serum samples were obtained from the patients and healthy controls from May 2016 to September 2018. The healthy controls were selected under the supervision of an endocrinologist. These people had normal levels of (FBS), cholesterol, LDL-C, HDL-C, HbA1c triglycerides and blood pressure. They did not have any underlying diseases and were not included in the exclusion criteria. The subjects ($N=80$) were divided into 5 groups: patients with T2DM receiving atorvastatin 20 mg/day for 3 months (group A, $n=17$), patients with T2DM not receiving statins (group B, $n=17$), patients with prediabetes receiving atorvastatin 20 mg/day for 3 months (group C, $n=12$), patients with prediabetes not receiving statins (group D, $n=17$), and healthy controls without T2DM (control group, $n=17$). The healthy controls did not have a specific disease or history of diabetes.

The characteristics of the subjects are as follows: In group A, the age range was 50–69 years, the weight range was 56–98 kg, the height range was 144–172 cm, the blood systolic pressure range was 110–140 mm Hg and the blood diastolic pressure range was 70–90 mm Hg. In group B, the age range was 30–67 years, the weight range was 66–97 kg, the height range was 148–177 cm, the blood systolic pressure range was 120–140 mm Hg and the blood diastolic pressure range was 70–80 mm Hg. In group C, the age range was 37–68 years, the weight range was 47–90 kg, the height range was 148–169 cm, the blood systolic pressure range was 110–140 mm Hg and the blood diastolic pressure range was 70–90 mm Hg. In group D, the age range was 25–58 years, the weight range was 47–85 kg, the height range was 153–177 cm, the blood systolic pressure range was 110–130 mm Hg, and the blood diastolic pressure range was 70–80 mm Hg. In the control group, the age range was 19–40 years, the weight range was 48–78 kg, the height range was 140–180 cm, the blood systolic pressure range was 110–120 mm Hg and the blood diastolic pressure range was 70–80 mm Hg.

Sample collection

After 3 months, blood samples (5 mL) were collected from all subjects in serum tubes and then isolated.

Inclusion and exclusion criteria

The inclusion criteria were patients with T2DM (FBS ≤ 126 mg/dL, 2hpp ≤ 200 mg/dL, HbA1c ≤ 6.5%), patients with prediabetes (FBS 100–125 mg/dL, 2hpp 140–199 mg/dL, HbA1c 5.7–6.4%), Patients had diabetes for at least three years and did not receive insulin. Some of patients with T2DM and prediabetes used atorvastatin 20 mg/day, healthy controls referred to a medical diagnostic laboratory (Babol, Iran) for checkup. The exclusion criteria were pregnant or breastfeeding women, women using contraceptives, being under the treatment of chronic disease requiring steroids (such as prednisone), consuming cigarettes/alcohol, failure of important organs (such as the liver, lung, kidney, heart, and brain) and subjects with active infections (such as hepatitis B, hepatitis C, HIV, and tuberculosis), history of cancer, heart attack in the last 6 months/peripheral coronary artery disease, indigestion/chronic diarrhea, anemia, and diabetic nephropathy.

Measurement methods

The measurement of MGO and AKR1B10 was performed with a non-competitive sandwich-type enzyme-linked immunosorbent assay (ELISA) at 450 nm. The ELISA kits were purchased from Shanghai Crystal Day Biotech Company (China). The measurement of GLO-1 was performed by an enzymatic method at 240 nm. the GLO-1 kit was purchased from Sigma Aldrich (USA).

Since the measurement of MGO and AKR1B10 was performed with a non-competitive sandwich-type ELISA at 450 nm, the desired factor or antigen was placed between 2 specific antibodies (one antibody was coated at the bottom of the well and the other was a conjugated antibody). This type of sandwich ELISA is called Ag capture.

The procedure was initiated with the preparation of 5 concentrations of standard solutions. The standard solutions were added to the wells of the microplate, then the samples were added. Next, a biotin-conjugated antibody and streptavidin-HRP complex solution were added. Then, the microplate was placed in an incubator shaker at 37 °C for 1 h. In the next step, washing solution 1 × was added to each well 5 times, and chromogen solutions A and B were added to each well. Furthermore, the plate was incubated at 37 °C for 10 min; at this time, the color of the wells turned blue. The blue color was due to the reaction between HRP and its substrate (H_2O_2) and the effect of free oxygen produced on the dye. Moreover, a stop solution was added to each well, and the enzymatic reaction was stopped. In this case, the blue color of the wells was quickly changed to yellow. In general, the intensity of the color was directly related to the concentration of the desired factor in the serum sample. The absorbance of the wells was read at a wavelength of 450 nm using an ELISA reader.

The measurement of GLO-1 was carried out by an enzymatic method at 240 nm. First, buffer and master mix were added to all samples; after mixing, the samples were incubated at room temperature. Then, perchloric acid (4M) was added to the samples and the samples were centrifuged at 14 000 rpm. Furthermore, 200 µL of the supernatant solution was separated and diluted 3 times with distilled water and the absorbance of the samples was determined by spectrophotometer at 240 nm.

Then, we calculated the level of GLO-1 enzyme activity using the following formula:

$$\text{Activity} = 175\text{x}\{(A_{240})_{\text{Sample}} - (A_{240})_{\text{Blank}}\}\text{x}1.35\text{xnunits/L}$$

$$\text{n} = \text{DilutionFactor}$$

Blank did not contain the sample.

Statistical analysis

The data were calculated by descriptive statistics (middle, mean and SEM). The correlation between different

variables was calculated based on the Pearson correlation coefficient. The results were analyzed using SPSS version 24 (SPSS Inc, Chicago, Ill, USA). An analysis of variance (ANOVA) was used to compare the mean of data. Furthermore, a box plot was used to compare the middle of obtained data. Then, a receiver operating characteristic (ROC) curve was used to assess the sensitivity and specificity of the variables in each group. P values less than 0.05 were considered statistically significant.

Results

Results of mean comparison of the variables measured in 5 groups

The comparison of the mean and SEM of the variables measured in all groups is presented in Table 1 based on ANOVA. The results showed, the serum level of MGO was significantly higher in patients with T2DM and prediabetes than that of healthy controls ($p = 0.001$; Table 1). In addition, the serum level of MGO was significantly higher in prediabetes on statin than that of the other groups ($p = 0.001$). In patients with T2DM, statin decreased the serum level of MGO; however, in patients with prediabetes, statins increased the serum level of MGO ($p = 0.001$). The level of GLO-1 activity was significantly higher in healthy controls than that of patients with T2DM and prediabetes ($p = 0.001$). Moreover, the level of GLO-1 activity was significantly higher in patients with T2DM and prediabetes receiving statins than that of patients with T2DM and prediabetes not receiving statins ($p = 0.002$). The serum level of AKR1B10 was significantly higher in prediabetes on statin and prediabetes not on statin than that of the other groups ($p = 0.001$).

Correlation between the variables

In the healthy control, based on the Pearson correlation coefficient, the serum level of MGO had a positive and significant correlation with the serum level of AKR1B10 ($R = 0.7$; $p = 0.03$). In T2DM on statin, the serum level of MGO had a negative and significant correlation with the level of GLO-1 activity and insulin level, respectively ($R = -0.5$; $p = 0.04$ and $R = -0.5$; $p = 0.02$). Furthermore, the serum level of MGO had a positive and significant correlation with the serum level of AKR1B10 ($R = 0.5$; $p = 0.04$).

Results of box plots

In the current study, a box plot was used to compare the middle of the obtained data which was shown as the healthy control, T2DM on statin, T2DM not on statin, prediabetes on statin and prediabetes not on statin in box plots. Based on the obtained data, the serum level of MGO was higher in prediabetes on statin than that of the other groups (Fig. 1). The level of GLO-1 activity was higher in prediabetes not on statin than that of the other groups (Fig. 2). The serum level of AKR1B10 was higher in prediabetes on statin than that of the other groups (Fig. 3).

Results of the ROC curve

In Fig. 4, the ROC curve shows the sensitivity and specificity of the measured variables between T2DM not on statin and healthy control, predicting the probability of developing diabetes in a healthy person. GLO-1 was significantly able to predict passing from the healthy phase to the diabetic phase.

In Table 2, a ROC test assessed the sensitivity and specificity of the measured variables between T2DM not

Table 1 The comparison of the mean and SEM of the variables measured in 5 groups, based on ANOVA

Variables	Control group ($n = 17$)	Diabetic group receiving statins ($n = 17$)	Diabetic group not receiving statins ($n = 12$)	Prediabetic group receiving statins ($n = 17$)	Prediabetic group not receiving statins ($n = 17$)	*p value*
MGO (mean ± SEM) ng/mL	38 ± 7.43	56 ± 4.13*	58 ± 2	72 ± 6.3*	56 ± 3.9	0.002
GLO-1 activity (mean ± SEM) IU/L	253 ± 32.8*	211 ± 31.8*	134 ± 24	181 ± 31.5*	114 ± 10.9	0.001
AKR1B10 (mean ± SEM) ng/mL	3 ± 0.48	3 ± 0.5	3 ± 0.57	4 ± 0.57*	4 ± 0.73*	0.001

Abbreviations: *MGO* methylglyoxal; *GLO-1* glyoxalase 1; *AKR1B10* aldo-keto reductase family 1 member B10

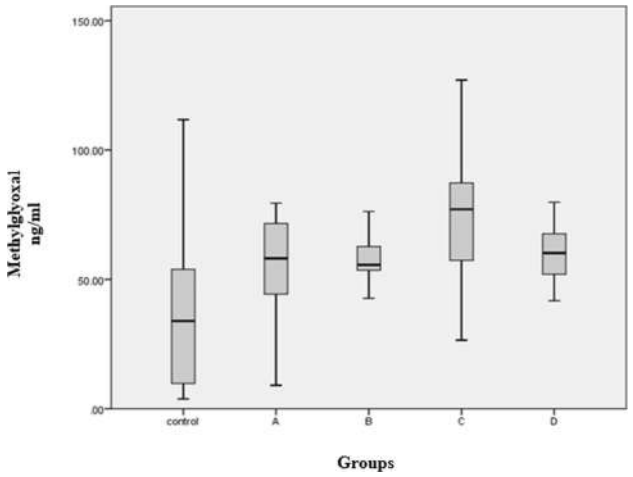

Fig. 1 The s**erum** level of MGO in 5 groups based on the box plot

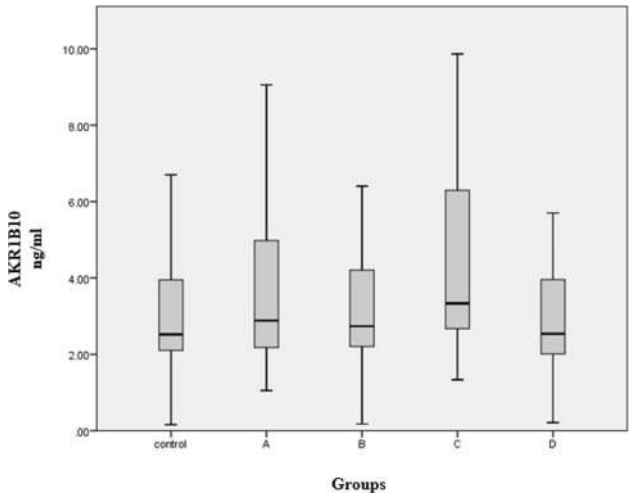

Fig. 3 The s**erum** level of AKR1B10 in 5 groups based on the box plot

on statin and healthy control, predicting the probability of developing diabetes in a healthy person. GLO-1 was significantly able to predict passing from the healthy phase to the diabetic phase with a sensitivity of 70%, specificity of 71%, and cutoff of 190 IU/L.

In Fig. 5, the ROC curve shows the sensitivity and specificity of the measured variables between prediabetes not on statin and healthy control, predicting the probability of developing prediabetes in a healthy person. MGO was significantly able to predict passing from the healthy phase to the prediabetes phase.

In Table 3, a ROC test assessed the sensitivity and specificity of the variables between prediabetes not on statin and healthy control, predicting the probability of developing prediabetes in a healthy person. MGO was significantly able to predict passing from the healthy phase to the prediabetes phase with a sensitivity of 94%, specificity of 60%, and cutoff of 41 ng/dl.

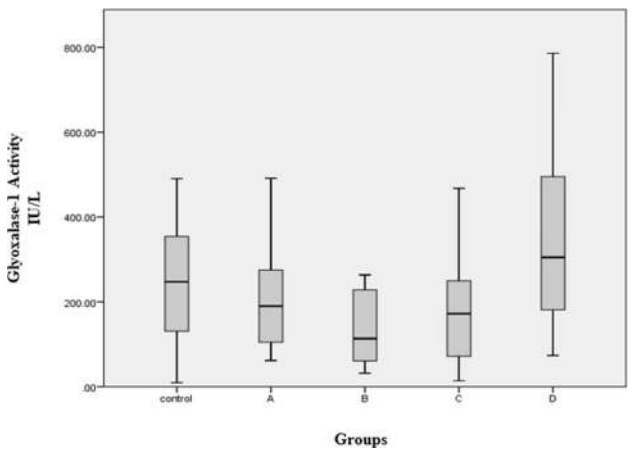

Fig. 2 The level of GLO-1 activity in 5 groups based on the box plot

Discussion

The current study indicated, the serum level of MGO was significantly higher in patients with T2DM and prediabetes than that of healthy controls ($p=0.002$). Eppinger showed in the mice, in patients with lately diagnosed diabetes, changes in the plasma level of MGO occurred 3 years before the diagnosis of diabetes or prediabetes. Moreover, an increased plasma level of MGO was observed in diabetic mice. These results indicate that changes in the level of MGO are associated with the occurrence of diabetes [49].

In patients with diabetes, atorvastatin decreased the serum level of MGO, but in patients with prediabetes, atorvastatin increased the serum level of MGO. Therefore, the effect of atorvastatin on the serum level of MGO is complex. These effects may be due to the oxidation reactions involved in the formation of MGO [34–38]. It is probable that atorvastatin is an antioxidant; this drug reduced the serum level of MGO. To our knowledge, no study has been conducted on the influence of statins on the serum level of MGO. As described in the introduction section, The creation of α-dicarbonyls occurs during Maillard reactions and autoxidation of carbohydrates [30–35]. Maillard reactions and autooxidation of carbohydrates require oxidative factors [34–38]. Also, it has been shown, Statins increase the level of free fatty acids (FFAs) through the accumulation of acetyl coA and increasing the expression of genes related to fatty acid synthesis, such as Fatty acid synthase (FAS) and Acetyl CoA carboxylase 1 (ACC1) (via SREBP2) [50]. FFAs and intermediates of its metabolism such as diacylglycerol (DAG) activate protein kinase C [51]. PK-C can increase superoxide anion production by activating NADPH oxidase [52]. Perhaps, the increase in the level of atorvastatin mediated methylglyoxal

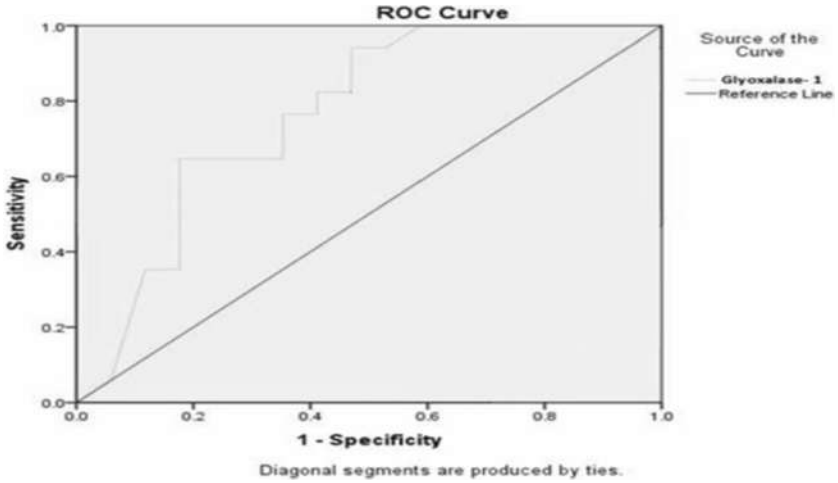

Fig. 4 The ROC curve between T2DM not on statin and the healthy control

in prediabetes is due to the production of superoxide anion by PK-C.

In the healthy control, the serum level of MGO had a positive and significant correlation with the serum level of AKR1B10 ($R = 0.7$; $p = 0.03$). This result may be related to two mechanisms. First, AKR1B10 can contribute to the complications of diabetes via increasing MGO formation. Second, owing to the similar function between AKR1B10 and AKR1B1, AKR1B10 perhaps increased MGO formation by reducing glucose in the polyol pathway. For the first time, Shaw et al. indicated, AKR1B10 contributed to the complications of diabetes. They showed, hyperglycemia induced the expression of the AKR1B10 gene and increased the activity of this enzyme contributed to the promotion of diabetic nephropathy [48]. The other mechanism is that the expression of AKR1B10 was enhanced by increasing the MGO level. As a result, AKR1B10 reduced the toxic effects of MGO. These two mechanisms indicate the dual effects of AKR1B10 in increasing or decreasing the α-dicarbonyls levels and adverse effects of diabetes.

In T2DM on statin, the serum level of MGO had a negative and significant correlation with the level of GLO-1 activity ($R = -0.5$; $p = 0.04$), showing that GLO-1 was involved in decreasing toxicity of MGO. On the other hand, in T2DM on statin, the serum level of MGO had a negative and significant correlation with the serum insulin level ($R = -0.5$; $p = 0.02$). Several studies have shown, statins can impair insulin secretion [53–56]. In addition, diabetes can increase the level of MGO [49]. In fact, insulin and MGO were indirectly linked through statin effects. To elucidate this issue, more comprehensive studies are required. The results of the ROC test between prediabetes not on statin and healthy control showed that a level of MGO greater than 41 ng/mL was associated with prediabetes (Table 3).

Giacco et al. showed that excessive expression of GLO-1 decreased the glucose level mediated epigenetic and gene expression changes [57]. Brouwers et al. indicated that increased expression of GLO-1 reduced the glycation process in diabetic rats [58]. As mentioned previously, one of the main physiological substrates for GLO-1 was MGO. Thus, GLO-1 decreases diabetic complications by degrading MGO [40–43]. In the current study, the level of GLO-1 activity was significantly lower in patients with T2DM and prediabetes than that of healthy controls ($p = 0.001$), showing that hyperglycemia decreased the expression of GLO-1. In addition, statins enhanced GLO-1 activity in patients with T2DM and prediabetes. Since GLO-1 needs reduced GSH for its activity, statins can increase the level of GSH as an antioxidant; therefore, statins improve the activity of GLO-1. For the first time, the current study showed this result. Further studies are needed to clarify this issue. The results of the ROC test between T2DM not on statin and healthy control were showed in Table 2. Given that, GLO-1 is involved in the breakdown of MGO and can reduce inflammatory conditions. GLO-1 activity higher than 190 IU/L indicates being healthy, and GLO-1 activity lower than 190 IU/L indicates diabetic conditions.

In the current study, the serum level of AKR1B10 did not significantly differ between T2DM on statin, T2DM not on statin, and healthy control. However, in prediabetes on statin and prediabetes not on statin, a significant increase was observed in the level of this enzyme ($p = 0.001$; Fig. 3). Shaw et al. indicated that the polymorphisms of AKR1B10 were observed in diabetic patients, leading to the development of diabetic nephropathy

Table 2 The ROC test between T2DM not on statin and the healthy control

Variable	Sensitivity	Specificity	AUC	Cutoff	*p* value
GLO-1	70%	71%	0.73	190	0.008

Abbreviations: *GLO-1* glyoxalase 1; *AUC* area under the curve

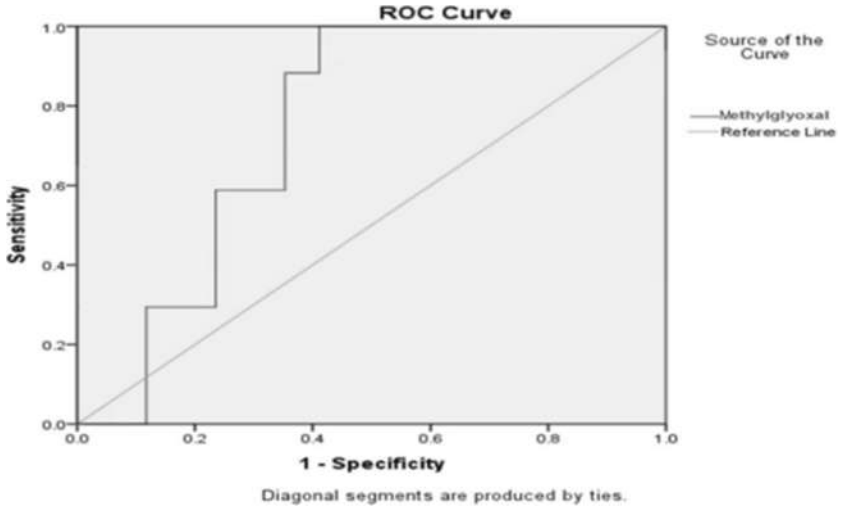

Fig. 5 The ROC curve between prediabetes not on statin and healthy control

through its impact on the activity and serum level of AKR1B10 [48]. Also, Ruf et al. showed, statins reduced the expression of AKR1B10; however, various polymorphisms may neutralize the effect of statins [59]. Lack of difference in serum levels of AKR1B10 among different groups may be due to the similar polymorphisms.

Conclusion

Atorvastatin, as an antioxidant, can improve GLO-1 and MGO activity and thereby prevent diabetic complications. The serum level of AKR1B10 did not significantly differ between T2DM on statin, T2DM not on statin, and healthy control; it may be due to the similar polymorphisms that neutralize the effect of atorvastatin on AKR1B10.

Article highlights

Research background

The effect of atorvastatin on methylglyoxal, glyoxalase 1 and aldo–keto reductase family 1 member B10 concentrations were studied in patients with type 2 diabetes mellitus and prediabetes.

Table 3 The ROC test between prediabetes not on statin and healthy control

Variable	Sensitivity	Specificity	AUC	Cutoff	*p* value
MGO	94%	60%	0.74	41	0.015

Abbreviations: *MGO* methylglyoxal; *AUC* area under the curve

Research motivation

We tried to provide new insight into the relationship between atorvastatin consumption and concentrations of methylglyoxal, glyoxalase 1, and aldo–keto reductase family 1 member B10 in patients with type 2 diabetes mellitus and prediabetes.

Research method

The study was performed on 5 groups: patients with type 2 diabetes mellitus receiving statins (group A), patients with type 2 diabetes mellitus not receiving statins (group B), patients with prediabetes receiving statins (group C), patients with prediabetes not receiving statins (group D), and healthy controls without type 2 diabetes mellitus (control group).

Research results

In patients with type 2 diabetes mellitus, atorvastatin decreased the serum level of methylglyoxal, but in patients with prediabetes, atorvastatin increased the serum level of methylglyoxal. The level of glyoxalase 1 activity was significantly lower in patients with type 2 diabetes mellitus and prediabetes than that of healthy controls. In addition, the serum level of aldo–keto reductase family 1 member B10 did not differ significantly between group A, group B, and control group. However, in groups C and D, a significant increase was observed in the level of this enzyme.

Research conclusion

Atorvastatin, as an antioxidant, can improve glyoxalase 1 activity and thereby prevent diabetic complications.

Research perspective

Glyoxalase 1 needs reduced glutathione for its activity, and statins can increase the level of glutathione as an antioxidant; further studies are needed to clarify this issue.

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Authors' contribution DQ designed the experiments. AN performed the experiments. KH analyzed the data, and SM contributed to the writing and revising of the manuscript.

Data availability No additional data are available.

Declarations

Institutional review board statement The study was approved by the Ethics Committee of Hormozgan University of Medical Sciences.

Arrive guidelines statement The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Conflict of interest The authors declare no conflict of interests.

Ethical clearance and consent of participation This study was approved by the Ethics Committee of Hormozgan University of Medical Sciences (HUMS.REC.1395.127). Written consent was obtained from all subjects.

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ORIGINAL ARTICLE

β cell function and insulin resistance have gender-specific correlations with carotid intima-media thickness in type 2 diabetes

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Abstract

Objective The relationships between carotid intima-media thickness (C-IMT) and β cell function and insulin resistance in patients with type 2 diabetes (T2D) have not been fully elucidated. This study is to investigate whether impaired glucose metabolism is etiologically associated with C-IMT in patients with T2D.

Methods The study group consisted of 490 (284 men, 206 women) participants. Venous blood specimens were obtained from all subjects for biochemical profiles after an >8-h overnight fast. C-IMT was measured as the distance between the luminal-intimal leading edge (I-line) and the medial-adventitial leading edge (M-line) on the far wall. Insulin resistance was estimated with the homeostasis model assessment of insulin resistance (HOMA-IR). The acute insulin response to arginine was calculated as the mean of the three plasma insulin levels obtained within 2, 4, and 6 min after the arginine bolus minus the pre-stimulus plasma insulin levels.

Results There was a graded increase in C-IMT values according to tertiles of HOMA-IR in men; the values of C-IMT were significantly decreased across the tertiles of acute insulin and C-peptide responses in women. Multivariate analysis revealed that HOMA-IR and age were positively associated with C-IMT among men participants, and acute insulin response and current smoking were the independent determinants of C-IMT in women.

Conclusion Early insulin response stimulated by arginine is independently associated with C-IMT in women T2D individuals, whereas insulin resistance is positively correlated with C-IMT in men T2D subjects.

Keywords β cell function · Insulin resistance · Carotid intima-media thickness · Type 2 diabetes

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Introduction

Type 2 diabetes (T2D) characterized by pathological progressive hyperglycemia is caused by insulin resistance and pancreatic β cell dysfunction. There is evidence that T2D is associated with early atherosclerosis, and is a well-established risk factor for cardiovascular diseases [1, 2]. Carotid intima-media thickness (C-IMT) is currently a widely used marker for cardiovascular conditions, in particular for atherosclerotic disease [3]. It has been addressed to be directly associated with the development of cardiovascular disease [4, 5]. On the other hand, there is growing evidence that inflammation represents as a key pathophysiological etiology in the development and progress of both atherosclerotic cardiovascular disease and T2D [6, 7]. Notably, patients with T2D were reported to have a marked increment in C-IMT compared to the age-matched non-diabetic individuals [8]. Some atherosclerosis-promoting factors may be influenced by insulin resistance alone or together with hyperinsulinemia and hyperglycemia [9–11]. However, previous studies have shown contradictory results regarding the relationship between C-IMT and T2D. In non-diabetic individuals, C-IMT was shown to be negatively associated with insulin secretion independently of other risk factors such as insulin sensitivity [12]. Interestingly, another study identified a gender difference suggesting that C-IMT is inversely correlated with insulin sensitivity in men, but is independently associated with fasting plasma glucose levels in women [13]. In this respect, both insulin resistance and insulin secretion should be accurately measured. Defects in insulin action and β cell function are known as hallmarks of T2D with resultant relative or absolute inadequacy of insulin secretion in response to hyperglycemia. However, the relationship between C-IMT and β cell function in T2D individuals has not been fully elucidated.

There is a need to establish novel methods that can appropriately assess disease progression and β cell mass in clinical and epidemiological studies. Currently, HOMA-β (homeostasis model assessment of β cell function) is commonly used to assess β cell function. However, this score is calculated by using fasting plasma glucose and insulin levels, which only indicates β cell function under fasting non-dynamic conditions. Furthermore, arginine-induced insulin secretion is also able to evaluate β cell function in T2D patients as well as in islet auto-transplantation studies [14, 15]. In a clinical setting, this stimulus is applied intravenously to elicit an acute insulin response (AIR) or an acute C-peptide response (ACR). The aim of the present study is to investigate the association between C-IMT and β-cell function in individuals with T2D. Physiological approaches, e.g., arginine-stimulated test (AST), were applied to participants to determine insulin resistance and early insulin secretion.

Materials and methods

Study design

The study was designed as a cross-sectional case-control study, and was conducted at the Fudan University, Zhongshan Hospital, Xiamen Branch, over a period of 2 years.

From August 2018 to February 2021, a total of 490 patients with T2D (284 men, 206 women) aged between 40 and 70 year-old from the department of Endocrinology, Zhongshan Hospital (Xiamen), Fudan University, China, had been recruited into this present ongoing cohort. Patients were diagnosed as diabetes based on American Diabetes Association (ADA) 2018 criteria: (1) a self-reported history of diabetes previously diagnosed by health care professionals; (2) fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L); (3) 2-h plasma glucose (2-h PG, OGTT) ≥200 mg/dL (11.1 mmol/L); or (4) glycosylated hemoglobin A1c (HbA1c) ≥6.5% (48 mmol/mol). All patients were examined Glutamic acid decarboxylase antibody (GAD-Ab) negative to exclude type 1 diabetes. Subjects with the following conditions were also excluded: specific types of diabetes due to other causes, cancer, pregnancy, lactation, abnormal liver or/and renal function, infectious diseases, evidence of hyperthyroidism, hypothyroidism, alcoholism, viral hepatitis (type B or C), or those who underwent vascular surgery such as carotid endarterectomy or stenting. The study protocol was approved by the Human Research Ethical Committee of the Zhongshan Hospital (Xiamen), Fudan University. Written informed consent was obtained from each participant before the start of the study.

Waist circumference, heart rate, and blood pressure (BP) were measured in all participants. After an >8-h overnight fast, venous blood specimens were obtained from all subjects for biochemical profiles as well as low density lipoprotein (LDL-cholesterol), high density lipoprotein (HDL-cholesterol), triglycerides, high-sensitivity C-reactive protein (hs-CRP), uric acid, and estimated glomerular filtration rate (eGFR); the information of current smoking and body mass index (BMI) were also obtained.

Carotid intima-media thickness measurement

High resolution B-mode ultrasound of the extracranial carotid arteries was performed by trained and certified technicians following a standardized protocol [13]. A segment about 1 cm proximal to the carotid bifurcation was imaged in the longitudinal plane and C-IMT was measured as the distance between the luminal–intimal leading edge (I-line) and the medial–adventitial leading edge (M-line)

on the far wall. C-IMT was assessed in three contiguous sites at 1-mm intervals and the average of the three values was used for analyses. The mean of the left and right measurements was used in this analysis.

Clinical and biochemical measurements

Clinical parameters, such as age, gender, systolic blood pressure, heart rate, body mass index (BMI), and waist circumference were collected. Blood pressure was measured using a standard sphygmomanometer in the sitting position, as the average of the last two of three consecutive measurements obtained at 3-min intervals. BMI was calculated as body weight in kilograms divided by the square of body height in meters (kg/m^2). Waist circumference was taken at umbilical level to the closest centimeter. Fasting blood samples were obtained in the early morning for biochemical studies including serum creatinine, high-density lipoproteins (HDL), low-density lipoproteins (LDL) triglyceride, uric acid, glycated hemoglobin (HbA1c), and high-sensitivity C-reactive protein (hs-CRP). The glomerular filtration rate was estimated by the modified modification of diet in renal disease equation with the new Japanese coefficient [16].

Arginine stimulation test

Dynamic testing of β cell function was evaluated by AST performed according to the method of Robertson [17]. Before the test, glucose levels were measured to ensure that they were between 4 and 12 mmol/L. After baseline samples were drawn for glucose, insulin, and C-peptide at 0 min, an intravenous injection of 5 g of arginine (given as 50% arginine HCl) was administered over 30 s, with time 0 set halfway through the arginine injection. Samples for plasma glucose, insulin, and C-peptide were collected from the contralateral arm at 2, 4, and 6 min after the arginine injection.

Insulin secretion and insulin resistance

Insulin resistance was estimated with the homeostasis model assessment of insulin resistance (HOMA-IR) according to the formula HOMA-IR = fasting insulin (FINS) (μU/ml) × fasting plasma glucose (FPG) (mmol/L)/22.5 [18]. For the assessment of pancreatic β cell function, we used the homeostasis model assessment of β cell function (HOMA-β) according to the formula HOMA-β = 20×FINS (μU/ml)/(FPG mmol/L-3.5). The pancreatic β cell function was also evaluated by area under curve of insulin (INS_{AUC}) according to the formula $INS_{AUC} = INS_0+2\times INS_2+2\times INS_0+INS_6$, where $INS_{0, 2, 6}$ is the plasma insulin levels obtained within 0, 2, and 6 min after the arginine bolus. The acute insulin response (AIR) to arginine was calculated as the mean of the three plasma insulin levels obtained within 2, 4, and 6 min after the arginine bolus minus the prestimulus plasma insulin level. In addition, the acute C-peptide response (ACR) to arginine was calculated as described for insulin.

Statistical analysis

The statistical analyses were carried out by Statistical Package for Social Sciences 18 (SPSS). Continuous variables are presented as mean ± SD (standard deviation) or median (interquartile range) and categorical variables as percentages of patients in the study. Subjects were divided into two groups according to gender and each group was further subdivided into tertiles of fasting plasma insulin, glucose concentrations and index of insulin secretion and resistance. Comparisons were drawn by chi-square test, unpaired Student *t* test, and one-way analysis of variance, as appropriate. Two-sided *p* values of less than 0.05 were considered statistically significant. To find the parameters that explain the significance of the variance of the dependent variables, stepwise multivariate linear regression analysis was performed to correlated parameters with C-IMT and *p* value <0.05 was considered as indication of statistical significance. Regression models, with standardized baseline C-IMT as the dependent variable and established risk factors as independent variables, were run separately for men and women.

Results

Demographic, clinical, and laboratory data

The demographic, clinical, and laboratory data from participants were shown in Table 1. At baseline, men and women differed in age, waist circumference, systolic BP, HDL-cholesterol, current smoking, uric acid, fasting insulin, INS_{AUC}, HOMA-IR, HOMA-β%, and C-IMT (Table 1). There were no significant differences in fasting glucose, AIR, ACR, and HbA1c.

Correlations between C-IMT and insulin secretion related factors

At baseline, C-IMT was detected to be significantly higher in men. We next examined C-IMT across the tertiles of fasting plasma insulin and glucose concentrations, and index of insulin secretion and insulin resistance in men and women. Notably, C-IMT values were positively correlated with HOMA-IR, but no significant differences in IMT values were identified across tertiles of HOMA-β, fasting glucose concentrations, INS_0, AIR, INS_{AUC}, and ACR (Table 2), suggesting that the elevated C-IMT values may be attributed by the progress of insulin resistance in men. However, completely different conclusions were drawn from the data

Table 1 Clinical and metabolic characteristics, mean ± SD, median (interquartile range), percentages of patients in the study

	Men	Women	*p* value
n	284	206	
Age, y	53±12	60±11	<0.0001
BMI, kg/m^2	25.0±3.5	24.7±4.3	0.37
Waist circumference, cm	90.6±10.8	85.5±9.9	<0.0001
Heart rate, bpm	80.2±9.2	79.4±8.5	0.32
Systolic BP, mm Hg	129.7±16.7	133.1±18.6	0.04
LDL-cholesterol, mmol/L	2.6±0.9	2.6±1.1	0.71
HDL-cholesterol, mmol/L	1.1±0.4	1.2±0.4	<0.0001
Triglycerides, mmol/L	4.6±1.2	4.7±1.2	0.21
hsCRP, mg/L	1.3 (2.3)	1.3 (2.2)	0.52
eGFR, (estimated glomerular filtration rate)	94.1±22.4	93.8±20.0	0.87
Current smoking, %	35.9	1.5	<0.0001
Uric acid	363.0±106.8	324.6±96.3	<0.0001
Fasting glucose, mmol/L	7.88±2.35	7.84±2.46	0.88
Fasting insulin, pmol/L	6.5 (6.75)	8.3 (8.3)	0.002
Acute insulin response, AIR	17.8 (25.5)	18.8 (21.3)	0.65
Area under curve of insulin, INS_{AUC}	97.1 (113.7)	121.8 (117.9)	0.01
Homeostasis model assessment of insulin resistance, HOMA-IR	2.3 (2.6)	2.9(3.1)	0.01
Homeostasis model assessment of beta cell function (HOMA-β)	33.3 (45.3)	42.4 (60.7)	0.001
Acute C-peptide response, ACR	1.4 (1.4)	1.3 (1.2)	0.18
HbA1c	9.3±2.3	9.2±2.2	0.57
Intima-media thickness (IMT)	0.81±0.28	0.75±0.28	0.01

SD Standard deviation, *BMI* body mass index, *BP* blood pressure, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *CRP* C-reactive protein, *HbA1c* glycosylated hemoglobin

Table 2 Values of C-IMT by tertiles of fasting plasma insulin, glucose concentrations and index of insulin secretion and resistance

	Men				Women			
	1	2	3	*p* value	1	2	3	*p* value
HOMA-IR	**0.04–1.57**	**1.58–3.12**	**3.13–284.4**		**0.17–1.85**	**1.86–3.73**	**3.74–213.33**	
IMT, mm	0.74±0.32	0.82±0.19	0.90±0.27	0.01*	0.79±0.19	0.73±0.29	0.74±0.32	0.77
HOMA-β	**4.29–22.22**	**22.23–51.67**	**51.68–6896.55**		**3.64–32.57**	**32.58–67.10**	**67.11–15384.62**	
IMT, mm	0.80±0.30	0.83±0.26	0.83±0.26	0.65	0.76±0.25	0.76±0.27	0.74±0.30	0.83
FG, mmol/L	**3.60–6.80**	**6.81–8.50**	**8.51–17.20**		**3.70–6.60**	**6.61–8.50**	**8.51–19.50**	
IMT, mm	0.76±0.27	0.82±0.30	0.88±0.24	0.12	0.78±0.25	0.73±0.31	0.75±0.26	0.61
INS_0, pmol/L	**0.20–4.90**	**4.91–8.70**	**8.71–1000**		**0.60–5.90**	**5.91–11.30**	**11.31–1000**	
IMT, mm	0.76±0.30	0.84–0.27	0.87±0.23	0.05	0.78±0.21	0.75±0.28	0.73±0.32	0.86
AIR	**0.90–11.77**	**11.78–27.73**	**27.74–185.47**		**0.27–12.43**	**12.44–25.97**	**25.98–278.67**	
IMT, mm	0.80±0.27	0.82±0.27	0.85±0.28	0.42	0.84±0.17	0.81±0.22	0.65±0.36	0.005*
INS_{AUC}	**5.60–64.80**	**64.81–132.40**	**132.41–6000**		**8.80–75.90**	**75.91–166.50**	**166.51–6000**	
IMT, mm	0.78±0.30	0.85±0.25	0.85±0.27	0.11	0.83±0.14	0.79±0.30	0.69±0.34	0.09
ACR	**0.10–1.02**	**1.03–2.07**	**2.08–20.07**		**0.04–1.01**	**1.02–1.74**	**1.75–46.60**	
IMT, mm	0.81±0.27	0.81±0.26	0.86±0.28	0.55	0.83±0.22	0.78±0.25	0.69±0.33	0.03*

HOMA-IR Homeostasis model assessment of insulin resistance, *HOMA-β* homeostasis model assessment of beta cell function, *FG* fasting glucose, *AIR* acute insulin response, *INS_{AUC}* area under curve of insulin, *ACR* acute C-peptide response, *IMT* intima-media thickness. *, significant changes in C-IMT values across the indicated tertiles (defined as *p* values less than 0.05).

in women. C-IMT levels were markedly decreased across the tertiles of AIR and ACR in female participants. No significant differences were found in C-IMT values across the tertiles of HOMA-IR, HOMA-β, fasting glucose concentrations, INS_0, and INS_{AUC} (Table 2). Collectively, these data indicate that changes in C-IMT are strongly associated with

insulin resistance status in men, but are more prone to be controlled by β cell function in women.

Independent determinants of C-IMT in men and women

To further explore the independent determinants of C-IMT in men and women, we then assessed the roles of insulin resistance or insulin secretion in association with C-IMT in our study participants. Multivariate analysis revealed that HOMA-IR and age were positively associated with C-IMT in male participants, after adjusting for other traditional risk factors (waist circumference, systolic BP, LDL-cholesterol, and current smoking) (Table 3). Whilst, in women, AIR and current smoking were unveiled to be the independent determinants of C-IMT by multivariate analysis (Table 3), suggesting that both the risk factors and the mechanism underlying of carotid plaques differ in gender. Taken together, C-IMT is significantly higher in the highest HOMA-IR tertile in men ($p<0.001$), and in contrast, C-IMT is higher in the lowest tertile of AIR index in women ($p<0.001$; Fig. 1).

Discussion

In the present study, we assessed C-IMT in 490 T2D participants. The arginine stimulation test (AST) method was applied as the established measurement to assess insulin secretion in patients. We for the first time demonstrated that insulin resistance and insulin secretion are independent risk factors of C-IMT in men and women with T2D, respectively.

Elevated C-IMT has been demonstrated to be associated with insulin resistance (evaluated by HOMA-IR), glucose intolerance, and higher fasting glucose levels, especially in non-diabetic individuals and even in obese children [9, 13, 17, 19], indicating that early atherosclerosis in prediabetes may be causally linked to endothelial insulin resistance. In fact, hyperinsulinemia resulted from insulin resistance

Table 3 Independent determinants of C-IMT in men and women

C-IMT	β±SE Men	*p* value		β±SE Women	*p* value
HOMA-IR	0.106±0.023	<0.001	AIR	−0.083±0.024	0.001
Age	0.004±0.002	0.033	Current smoking	−0.493±0.144	0.001
			Constant	0.954±0.051	<0.001
Total model R^2	0.361	<0.001	Total model R^2	0.367	<0.001

C-IMT Carotid intima-media thickness, *SE* standard error of the mean, *HOMA-IR* homeostasis model assessment of insulin resistance, *AIR* acute insulin response

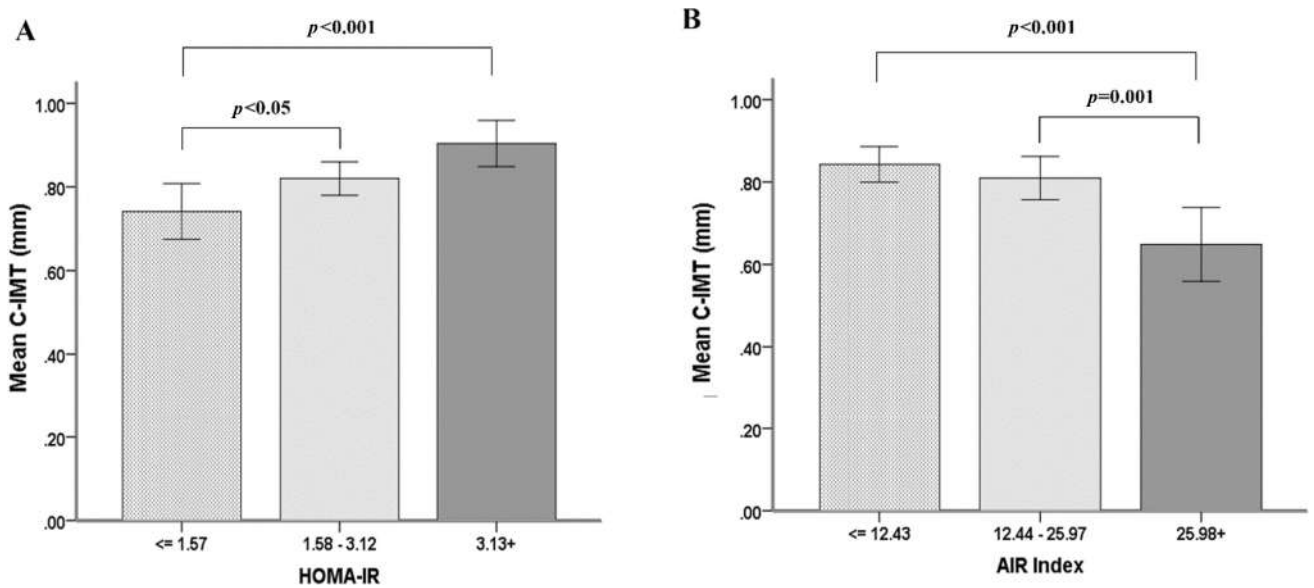

Fig. 1 Independent gender-specific determinants of C-IMT by tertiles in T2D individuals. **A** C-IMT with insulin resistance (HOMA-IR) in men. **B** C-IMT with insulin secretion (AIR index) in women. *p* values indicated are adjusted for age, BMI, waist circumference, systolic BP, low-density lipoprotein-cholesterol, eGFR, and current smoking

has been reported to cause endothelial dysfunction and atherosclerosis [20]. More importantly, insulin supplementation showed the ability to improve several vascular functions in animal models and people with insulin resistance [21]. On the other hand, Roussel et al. demonstrated that insulin secretion (evaluated by the early insulin response index which was calculated as the ratio of insulin change over the first 30 min of the OGTT, to plasma glucose at 30 min) is also associated with early carotid atherosclerosis in non-diabetic population independently of other risk factors such as insulin resistance [12]. Similarly, β cell function (estimated by HOMA-β) was still significantly related to an increased risk of poor functional outcomes in non-diabetic ischemic stroke patients after adjusting insulin resistance [18]. Furthermore, C-IMT was addressed to be associated with impaired β cell function in non-diabetic people [11]. Together these hinted that defects in insulin resistance and/or insulin secretion may pathophysiologically be connected with the development of carotid atherosclerosis. Given the above evidence in non-diabetic population, we raised the likelihood that this association may also exist in T2D population. Indeed, our data clearly revealed that C-IMT value is significantly correlated with insulin resistance (evaluated by HOMA-IR) and insulin secretion (evaluated by ACR and AIR) in T2D patients. More interestingly, these associations are manifested in a gender-specific manner, which is in line with the previous findings that C-IMT is associated with insulin sensitivity in men, but with fasting plasma glucose in women, respectively [13]. This discrepancy might reflect a gender-specific mechanism involved in the development of atherosclerosis in T2D patients. However, the explanation behind is not fully understood, and therefore, future studies focus on exploring the causal relationship between impaired glucose metabolism and atherosclerosis in different gender groups are needed.

The progression of C-IMT is determined by a plethora of risk factors, such as age, blood pressure, lipids, smoking, obesity, and CRP [22, 23], though we found the C-IMT value is significantly correlated with insulin resistance and insulin secretion in a gender-specific manner. It is noteworthy that diet, exercise, parental history of premature death from coronary heart disease are also associated with carotid atherosclerotic plaques [24], which may confound the interpretation of our results. Therefore, in the present study, we employed stepwise multivariate linear regression analysis to explore the independent C-IMT-correlated parameters in gender-divided subgroups to adjust the potential confounding factors. After excluding the traditional risk factors (waist circumference, systolic BP, LDL-cholesterol, and current smoking), our multivariate regression model revealed the true independent determinants of C-IMT in T2D population in a gender-specific manner. Taken together, these suggest that the risk factors of carotid plaques as well as the mechanisms underlying may differ in gender in T2D individuals. Further studies are needed to explore the more comprehensive relationships between C-IMT and arginine-stimulated insulin secretion and insulin resistance after excluding all the possible variables.

HOMA-IR and HOMA-β are widely used for evaluating insulin resistance and insulin secretion, respectively [18, 25, 26]. It is noteworthy that these two measurements derived from fasting samples are limited to merely reflecting fasting nondynamic conditions in clinical settings. In addition, oral glucose tolerance test (OGTT) and euglycemic-hyperinsulinemia clamp have also been applied to evaluate insulin sensitivity [11–13]. In our study, AST was employed to examine the first phase insulin secretion and the reserved function of β cells. It has been established that β cell dysfunction plays a key role in the pathogenesis of diabetes development that leads to defects in glucose-stimulated insulin secretion. However, β cells still retain the act to stimuli by non-sugar substances such as arginine [27]. Arginine is more potent to trigger secretion in β cells than glucose, and hence, can be used to evaluate the reserved function of islet β cells. In fact, hyperglycemic clamp technique is a more accurate application for evaluating islet β cell secretory function [14]. Nonetheless, the high technique requirement and long operative time limit its utility in clinical settings. In contrast, AST is far less technically demanding and can bring reproducible and complementary measures of β cell function [28]. More importantly, in young T2D individuals, AST is beneficial to reflect β cell reserve regardless of disease duration and treatment [29]. At last, it has also been demonstrated that arginine is preferred to glucagon for the assessment of β cell function [30].

T2D is suggested to be linked with atherosclerotic cardiovascular diseases through multifactorial pathways. For instance, HDL-cholesterol levels or the HDL-based makers have been established to be associated with various disorders, including hypertension [31], hepatosteatosis [32], thyroiditis [33], and in particular, diabetes [34, 35]. Importantly, these conditions are also tightly connected with high burden of inflammation that is well known to play a key role in the development of atherosclerosis in T2D patients [36]. In addition, there is evidence that women generally have higher HDL-cholesterol levels than men [37, 38], which is also confirmed by the baseline data in our study population. These together strengthen our results that atherosclerotic cardiovascular disease is gender-specifically associated with T2D.

Clinically, the gender-specific C-IMT values demonstrated by our findings provide a novel means of risk assessment for T2D patients, which may shed light on the personalized T2D treatment. Moreover, our data also highlight the significance of usage of AST-based insulin secretion in clinical settings to evaluate the relationship between T2D and development of atherosclerosis.

Limitations

The current study cannot determine the causal relationship between arginine-stimulated insulin secretion and C-IMT due to its cross-sectional and unpaired design. In addition, the relatively small sample size may result in reduced statistical power. A larger population from more clinical study sites is warranted in the future. Notably, patients with diabetes may develop various severe vascular complications, which cannot be merely reflected by C-IMT with ultrasound detection. In addition, C-IMT values may also be affected by many other factors, such as premature deaths in family, diet, and exercise, which may confound the explanation of the results. Therefore, more parameters correlated with C-IMT value and/or atherosclerosis occurrence such as percentage of stenosis and peak systolic velocity (PSV) of carotid that indicating vascular plaque formation are of necessity to be included in the analysis model to establish a more comprehensive explanation.

Conclusion

Early insulin response stimulated by arginine is independently associated with C-IMT in women T2D individuals, whereas insulin resistance is positively correlated with C-IMT in men T2D subjects. These gender-specific findings provide prognostic and therapeutic implications in the personalized management of patients with T2D. Furthermore, AST has the potential to be used as a reliable parameter in evaluating the relationship between impaired glucose metabolism and development of atherosclerosis.

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Author contribution Conception and design of the study: C.L., N.C., and W.L.; collection of data: W.L., K.W, X.L., S.Z., J.Z., and S.L.; data analysis: W.L., K.W, and X.L.; manuscript writing: C.L., N.C., and W.L.; all authors revised the manuscript and approved the final version.

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Data Availability All data collected, generated and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Declarations

Ethical approval In accordance with ethical guidelines of the 1975 Declaration of Helsinki, this study was approved by the ethics committee of Zhongshan Hospital (Xiamen), Fudan University.

Competing interests The authors declare no competing interests.

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Such research proposals will be carried out in only centres with research capabilities across India.

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Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmachari Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh