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EDITORIAL

GLP1 agonists beyond glycemic control—redefining their role

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The "incretin effect" was discovered in 1964 when it was found that greater and more sustained insulin release occurs after oral glucose load compared to an equivalent intravenous load [1]. While the discovery of glucose-dependent insulinotropic peptide (GIP) provided the first etiological explanation for the "incretin effect," it was only later in the 1980s after the discovery of another incretin hormone—glucagon-like peptide 1 (GLP1)—that the entero-insular axis came into scientific limelight [2]. GLP1, released from the distal ileum and colon within minutes of a meal, was found to be more effective than GIP in effecting glucose-dependent insulin production and secretion. Subsequent studies showed that GLP1 also decreases glucagon secretion, increases glucose uptake and glycogen synthesis in peripheral tissues independent of insulin/ glucagon, delays gastric emptying, and increases satiety making scientists sit up and take notice of its therapeutic potential for diabetes management [3]. However, endogenous GLP1 is rapidly broken down by dipeptidyl peptidase-4 (DPP-4) and has a half-life of only 2 min, making it unsuitable as a pharmacotherapy for diabetes. The discovery of exendin-4 from the venom of the gila monster, *Heloderma suspectum,* turned things around as it acted like native GLP1 but was resistant to DPP-4 inhibition and possessed a much longer half-life [4]. Exenatide, a synthetic peptide identical to exendin-4, was the first GLP1 agonist to be approved by the US Food and Drug Administration (USFDA) in 2005 for the treatment of type 2 diabetes mellitus (T2DM) following which several others of this drug class were introduced given their unique profile of effective glucose lowering with low risk of hypoglycemia and the additional benefit of weight reduction.

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However, the beauty of the GLP1 agonist group of agents lies in their role beyond blood glucose lowering; this was subsequently discovered with the design and publication of several trials, most prominent of them being the USFDA-mandated cardiovascular outcome trials (CVOT) [5]. All the seven GLP1 agonist CVOTs completed thus far have shown non-inferiority to placebo, while liraglutide, subcutaneous semaglutide, albiglutide, and dulaglutide have also demonstrated significant reduction in composite cardiovascular (CV) outcomes. Three of the GLP1 agonist CVOTs also found favorable renal outcomes—in the LEADER trial, there were significantly fewer nephropathy events in the liraglutide group compared with placebo; in the SUSTAIN-6 trial, there were significantly fewer new or worsening nephropathy events using semaglutide compared with placebo; and the REWIND trial also showed significantly fewer adverse renal outcomes in the dulaglutide group compared with placebo [6]. Although the PIONEER 6 trial, the first CVOT for an oral GLP1 agonist (semaglutide), did not show cardiovascular benefit, this study was of a shorter duration with fewer participants compared to other trials so that fewer overall events were observed. A larger CVOT with oral semaglutide called "A Heart Disease Study of Semaglutide in Patients with Type 2 Diabetes" (SOUL) is currently in trial phase III [6]. The CVOTs were designed to maintain glycemic equipoise, and subgroup analysis revealed that the beneficial cardiovascular and renal effects noted with these agents occurred independent of blood glucose reduction. A systematic review and meta-analysis published in 2019 concluded that GLP1 agonists have beneficial effects on cardiovascular, kidney, and mortality outcomes in patients with T2DM. A more recent meta-analysis in 2020 showed significant reduction in major adverse CV events, CV and total mortality, stroke, and hospitalization for heart failure, with a trend for reduction of myocardial infarction in patients with T2DM with and without established CV disease [7, 8]. These findings have led to a paradigm shift in the management of T2DM with guidelines recommending that those with established cardiovascular disease should use either a GLP1 agonist (or SGLT2 inhibitor) with established cardiovascular benefit as the first add-on therapy to metformin *irrespective* of their glycated hemoglobin level [9].

Obesity pharmacotherapy is an area where GLP1 agonists have made their mark as well. GLP1 acts on the paraventricular and arcuate nuclei of the hypothalamus to suppress appetite [10]. They also act on other areas of the brain such as the mesolimbic system to diminish food-induced reward signals, thereby reducing food-seeking behavior [11]. GLP1 also acts on peripheral nervous system pathways to slow gastric emptying, and the resultant gastric stretch stimulates vagal afferent signals to the solitary nucleus of the medulla and onto the appetite centers of the hypothalamus to induce satiety or the area postrema to induce nausea [12]. An initial dose finding study with liraglutide showed the 3-mg dose to be most effective for inducing weight loss at 20 weeks and beyond [13]. This led to the Satiety and Clinical Adiposity–Liraglutide Evidence (SCALE) series of four trials which tested the effect of 3 mg liraglutide on weight loss as an adjunct to diet and physical exercise. In composite data analysis of the SCALE trials, 3.0 mg liraglutide led to a 7.5% weight loss over 1 year compared with 2.3% for placebo which was slightly better than naltrexone/ bupropion and lorcaserin and somewhat lesser than phentermine/topiramate [14]. In December 2014, 3 mg liraglutide was approved by the USFDA as an adjunct to a reduced calorie diet and increased physical activity for chronic weight management in adults with obesity (BMI of ≥ 30 kg/m2) or who are overweight (BMI of ≥ 27 kg/m2) in the presence of at least one weight-related comorbid condition (e.g., hypertension, dyslipidemia, T2DM) [14].

GLP1 has also been attracting attention as a link between metabolic and neurodegenerative diseases on account of its influence on neurogenesis, neurodegeneration, retinal repair, and energy homeostasis [15]. GLP1 in animal models has been shown to improve learning and memory by modulating synaptic plasticity and reducing hippocampal neurodegeneration. Modulation of GLP1 activity has been demonstrated to influence amyloid b peptide aggregation in Alzheimer's disease (AD) and dopamine levels in Parkinson's disease (PD) in animal experiments. In animal PD models, GLP1 agonists were able to protect motor activity and dopaminergic neurons, whereas in AD models, they seemed to improve nearly all neuropathological features and cognitive functions. In a human trial with patients having moderate PD, exenatide had a positive and sustained effect on clinically assessed motor function [16]. In animal models, GLP1 agonists have shown beneficial actions on brain ischemia, such as the reduction of cerebral infarct area and improvement of neurological deficit. Despite the substantial amount of animal data in this area, human trials are scanty although the possibilities are huge and the future promising.

In recent times, the domain where GLP1 agonists have generated considerable interest and evidence is that of nonalcoholic fatty liver disease (NAFLD). NAFLD is the most common chronic liver disease with a global prevalence of 25.2%, and has a higher prevalence of 55.5% in T2DM [17]. NAFLD is divided into two histological subtypes of (a) nonalcoholic fatty liver (NAFL), characterized by isolated hepatic steatosis, often with mild nonspecific inflammation, and (b) nonalcoholic steatohepatitis (NASH), characterized by the presence of hepatic steatosis and hepatocellular injury with or without fibrosis [18]. NASH is the more severe form of NAFLD from where 20% can progress to cirrhosis, liver failure, and hepatocellular carcinoma. NASH in T2DM patients is associated with a higher risk of progressing to cirrhosis and hepatocellular carcinoma and the coexistence of NAFLD and T2DM is also related to increased risk of extrahepatic diseases like cardiovascular disease and chronic kidney disease [19]. Lifestyle modification leading to 5–10% decrease in body weight has shown significant improvement in hepatic steatosis, necroinflammation, and even fibrosis in NAFLD; but weight reduction rarely achieves complete resolution of NASH and is difficult to maintain over a long period of time [20]. NAFLD is closely associated with both hepatic and adipose tissue insulin resistance and GLP1 agonists have been shown to reduce insulin resistance [21]. GLP1 improves insulin signal transduction in adipocytes by upregulating Akt phosphorylation and protein expression of cyclins A, D1, and E and has been shown to activate genes involved in hepatic fatty acid oxidation and insulin sensitivity in hepatocytes isolated from rats with NASH [22]. The earliest clinical evidence of GLP1 agonist in NAFLD comes from studies where exenatide showed an improvement in hepatic enzymes [23]. Subsequently, there have been several studies with GLP1 agonists showing an improvement in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) in NAFLD making it the most commonly observed index in such studies [18]. Until date, seven studies with GLP1 agonists have shown significant reduction in liver fat content, the most recent of them being a study with dulaglutide in Indian patients with T2DM [18, 24]. Although a handful of studies have shown significant improvement in noninvasive assessment of liver fibrosis in NAFLD with GLP1 agonists, their role for fibrosis regression and preventing progression to cirrhosis remains unclear [18]. Similarly, limited studies with GLP1 agonists have shown promise in improving histological features of NASH, but its role in management of NASH still remains inconclusive on account of scarcity of human studies with histological assessment [18].

The study by Zhang X et al. published in the current issue of the journal adds to the evidence on the role of GLP1 agonists in the management of NAFLD [25]. The authors looked at the effect of 3 months of liraglutide therapy on hepatic fat content in NAFLD in thirty-two patients with T2DM in China. The authors used fatty liver index (FLI) as a marker of hepatic fat on account of its ease of use and reliability which gives this study practical relevance [26]. None of the patients were on any hepatoprotective or lipid-regulating drugs and the dose of antidiabetic drugs was unchanged during the duration of the trial to avoid confounding with the exception of liraglutide whose dose was uptitrated. There was a significant reduction in FLI by 26% and was accompanied by a significant decrease in AST and GGT as well by 7% and 39%, respectively. Liraglutide also significantly improved body weight, serum triglyceride, insulin resistance, and blood glucose

parameters in these patients but did not significantly improve ALT and other lipid parameters. While the finding of reduction in FLI which suggests a decrease in hepatic fat with liraglutide is interesting and provides further evidence of its potential to be a therapeutic option in NAFLD, these findings need to be replicated in appropriately powered larger randomized controlled trials looking at histological endpoints as well.

GLP1 agonists have come a long way since they were first made commercially available 15 years ago. Although initially launched as glucose lowering agents, their benefits beyond glycemic control have taken centerstage at present. They provide cardiorenal benefits and are effective agents to treat obesity. They have shown potential for the management of neurodegenerative disorders and more so in the management of NAFLD. If the evidence accumulated so far is a sign of things to come, the path ahead of GLP1 agonists for extra glycemic benefits looks bright and promising.

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REVIEW ARTICLE

Economic menace of diabetes in India: a systematic review

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Abstract

Aim Diabetes mellitus is recognised as a major chronic pandemic disease that does not consider any ethnic and monetary background. There is a dearth of literature on the cost of diabetes in the Indian context. Therefore, the present study aims to capture the evidence from the literature on the cost of diabetes mellitus in India.

Methods An extensive literature was reviewed from ACADEMIA, NCBI, PubMed, ProQuest, EBSCO, Springer, JSTOR, Scopus and Google Scholar. The eligibility criterion is based on 'PICOS' procedure, and only those studies which are available in the English language, published between 1999 and February 2019, indexed in ABDC, EBSCO, ProQuest, Scopus and peer-reviewed journals are included.

Results A total of thirty-two studies were included in the present study. The result indicates that the median direct cost of diabetes was estimated to be ₹18,890/- p.a. for the north zone, ₹10,585/- p.a. for the south zone, ₹45,792/- p.a. for the north-east zone and ₹8822/- p.a. for the west zone. Similarly, the median indirect cost of diabetes was ₹18,146/- p.a. for the north zone, ₹1198/- p.a. for the south zone, ₹18,707/- p.a. for the north-east and ₹3949/- p.a. for the west zone.

Conclusion The present study highlighted that diabetes poses a high economic burden on individuals/households. The study directed the need to arrange awareness campaign regarding diabetes and associated risk factors in order to minimise the burden of diabetes.

Keywords Diabetes · India · Economic menace · Costs and complications

Introduction

'Diabetes is a metabolic disease characterised by hyperglycemia resulting from defects in insulin secretion, insulin action or both' [1]. With rising pervasiveness globally, diabetes is conceded as a major chronic pandemic disease which does not consider any ethnic background and monetary levels both in developing and developed economies and has also been designated with the status of 'public health priority' in the majority of the countries [2, 3]. Individuals with diabetes are more susceptible to develop any of the associated complications, viz. macrovascular or microvascular. As a consequence, people experience frequent and exhaustive confrontation with the health care systems [4]. The treatment cost for diabetes and its associated complications exert an enormous economic burden both at the household and national levels [5–9].

In a developing nation like India, the majority of diabetes patients experience a substantial cost burden from out-of-pocket (OOP). Also, the dearth of insurance schemes and policies escalate the cost of diabetes care [2]. Instantaneous urbanisation and socio-economic transitions, viz. rural to urban migration, low exercise regimen, lifestyle disorder, etc., have resulted in an escalation of diabetes prevalence in India over the last couple of decades [10–14]. According to the International Diabetes Federation [15], 'India is the epicentre of diabetes mellitus and it was found that in 2017 India had the second-largest populace of 73 million diabetic patients, after China. And the figure is expected to be just double 134 million by 2045'. Considering that fact, the epidemiologic transition of diabetes has a colossal economic burden [16]. The estimated country-level health care expenditure on diabetes mellitus

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in India after amending purchasing power difference was 31 billion US dollars in 2017, pushing India in fourth place globally after the USA, China and Germany. Looking at the economic burden, in India, diabetes alone exhausts 5 to 25% share of an average Indian household earning [17–19].

Chronic nature and the rising epidemic of diabetes have everlasting consequences on the nation's economy and health status [20]. Therefore, managing diabetes and its comorbidities is a massive challenge in India due to several issues and stumbling blocks, viz. dearth of awareness regarding diabetes, its risk factors, prevention strategies, health care systems, poverty-stricken economy, non-adherence to medicines, etc. Altogether, these issues and problems remarkably contribute to the economic menace of diabetes in India [20–24].

After a perspicuous representation of the economic menace of diabetes in India, policymakers and health experts should provide healthier prospects to enhance the quality of life of millions [19]. Thus, the present study aims at capturing the evidence from the literature on the cost of diabetes mellitus in India, reviewing the materials and methods used to estimate the costs and, lastly, exploring future research area. For the accomplishment of the objective, the paper has been divided into five sections. The 'Introduction' section of the study discusses diabetes and its economic burden. The 'Materials and methods' section deals with materials and methods applied for data extraction and quality assessment. The 'Results' section of the present study reports the results of the study. The 'Discussion' section concludes the discussion along with policy implications and limitations.

Materials and methods

A comprehensive literature review was carried out by following the 'Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines' [25]. The article suggests a minimum set of guidelines and procedures of writing items to enhance the quality of the systematic review. A search was performed between February and March 2019 for the accumulation and review of studies published up to January 2019.

Literature search

An extensive desk search was executed for all published articles and book chapters in relevant databases such as ACADEMIA, NCBI, PubMed, ProQuest, EBSCO, Springer, ResearchGate, Google Scholar, JSTOR and Scopus. For better insight, a literature search was performed on the World Health Organization (WHO) and International Diabetes Federation (IDF) libraries available online. Additional articles were investigated by scrutinising the backward referencing lists or references of the included articles. The search terms and keywords were adjusted by following different databases using words or phrases, viz. 'India', 'Diabetes Mellitus or Diabetes', 'Economic Burden', 'Economic Menace', 'Costs of Diabetes', 'Health Care Utilization', 'Cost of Illness', 'Out-of-Pocket Expenditure', 'Diabetes Care', 'Health Economics', 'Direct/Indirect Costs', 'Cost Analysis', 'Hospitalization', 'Diabetic Complications', 'Developing Countries', 'Lifestyle Modification', 'Non-communicable diseases', 'Expenses by patients', 'Comorbidity Burden' and 'Treatment Costs' were utilised to attain expected results. A total of 412 studies were acquired including duplicates by exercising the desk search criteria. Further, a comprehensive analysis of the studies was performed as per the recommendations suggested by Moher et al. [25]. Later, 187 articles were identified to be duplicate and removed immediately.

Inclusion criterion

Of the total 225 articles, limited studies managed to clear the eligibility criterion based upon the significant elements of the 'Patient Intervention Comparison Outcome Study (PICOS)' procedure [26]. Title, abstract and keywords of the remaining 225 studies were assessed to determine their relevance. Those articles which have been included (a) were available in English language; (b) were published between 1999 and February 2019; (c) were indexed under ABDC, EBSCO, ProQuest and Scopus; (d) were under journals that are to be peer-reviewed in nature; (e) highlighted unprecedented research outcomes on costs; and (f) were comprising at least one or more demographic zones. Thus, the screening procedure facilitated the selection of 32 articles. Majority of research publications were excluded on the grounds if they (a) did not provide the detailed analysis of how costs were estimated; (b) were conference articles or posters; (c) only presented the costs of diabetes prevention; and (d) were published in non-peer-reviewed journals.

Data extraction and quality assessment of included studies

The exploration includes those articles which highlight the cost burden of diabetes in India. Whilst performing the analysis, two interdependent excel spreadsheets were developed for data to be summarised. In the very first spreadsheet, a predefined category was used, viz. publication title/year, study type, location, diabetes type, methodology and findings. Relevant information is drawn out and presented in Table 1, highlighting the study characteristics of the included articles. The second excel spreadsheet focuses its attention on the list of technical criteria applied to assess the quality of the articles incorporated in the review process. Copious checklist has been put forward for the quality assessment of the included studies and majority of them emphasise on the economic

Table 1 Profile of the studies included for review

Characteristics of the study	Number of studies (%)
Year of publication	
1999–2009	11 (34)
2010–2019	21 (66)
Year of costing	
1999–2003	05 (16)
2004–2008	05 (16)
2009–2013	10 (31)
2014–2019	12 (37)
Location	
North zone	08 (25)
East zone	-
West zone	01 (3.5)
South zone	11 (34)
Central zone	-
North-east zone	01 (3.5)
India	11 (34)
Indicators of cost	
Direct cost	17 (53)
Indirect cost	-
Direct and indirect cost	15 (47)
Others (not specified)	-
Study perspective	
Household	06 (19)
Patient	19 (61)
Societal	09 (29)
Government	07 (22)
Others (not specified)	-
Type of diabetes	
Type 1	02 (07)
Type 2	09 (28)
Type 1 and type 2	08 (25)
Gestational/foot ulcer	01 (03)
Not defined	12 (37)
Complications	
With complication	10 (31)
Without complication	22 (69)
Sample size	
Not defined	03 (10)
> 100 respondents	02 (07)
≤ 100 respondents	27 (83)
Study interest	
General cost	30 (94)
Foot ulcer	01 (03)
Others (not specified)	01 (03)
Source of cost data	
Medical institute	11 (34)
Patients	16 (50)
Publications	05 (16)
Others (not specified)	-

*Multiple responses possible

Source: Based on author's calculation

assessment, viz. cost analysis, cost-benefit analysis (CBA), health care utility analysis, etc. [27, 28]. Therefore, the quality indicators developed for the present study were grounded on the criterions suggested by prior literature [29–32].

A symbol of (√) yes, (×) no and (±) moderately available was assigned to individual quality indicator. Each symbol was allocated with a score of 1, which leads to a maximum attainable score of 10 for each study reviewed. Hence, a complete detailed analysis of the parameters utilised is presented in Table 2.

Results

Study characteristics

The characteristics of the included thirty-two studies are presented in Table 1. A majority of 66% (21) of the studies were published between 2010 and 2019 and the remaining 11 studies (34%) were published in 1999–2009. Year of costing was 1999–2003 for 5 studies; between 2009 and 2013, 10 studies (31%) were included; and for 2014–2019, 12 studies (37%) were included. The cost of diabetes was estimated from various locations such as the south zone ($n = 11$), followed by the north zone ($n = 8$), the north-east zone ($n = 1$) and the west zone ($n = 1$). A large proportion of 11 studies (34%) were defined under India as a whole.

Whilst conducting review studies, it is imperative to initially define the type, study interest, sample size, data source and outlook of the study. The included studies majorly focus on type 2 diabetes ($n = 9$), followed by both type 1 and type 2 studies ($n = 8$), 2 studies were identified under type 1 diabetes and only 1 study was acknowledged under gestational/foot ulcer category, whilst the remaining 12 studies did not define any diabetes type (Table 1). Of the total 32 studies, 94% of studies focus on general costs and the remaining 2 studies emphasise on foot ulcers and others. Whilst discussing the cost interests, the complications associated with diabetes were estimated by merely10 studies and the remaining 22 studies (69%) estimated the diabetes cost without any complications. Defining sample size is the utmost priority of the study, 27 studies (83%) of the total 32 studies have properly identified the sample size to be ≤ 100 respondents, only 2 studies specified the population size to be > 100 respondents and 3 studies (10%) did not define or provide the sample size.

Under the source of the cost data section, 16 studies (50%) retrieved data on cost from the patients themselves; for 11 studies (34%), source of cost data was obtained from medical institutes; and the remaining 5 studies (16%) acquired the data on cost from publications. Studies on the economic burden of illness could be done through several perspectives, viz. household, patient, societal and governmental. In the particular study, the patient's perspective was most commonly

Table 2 Quality index of the included studies

Reference no.	[2]	[33]	[34]	[35]	[36]	[37]	[7]	[38]	[8]	[20]	[39]	[40]	[22]	[41]	[42]	[43]	[44]	[23]	[6]	[45]	[9]	[46]	[5]	[47]	[48]	[49]	[50]	[19]	[51]	[52]	[53]	[24]
Questions																																
1. A comprehensive definition of diabetes was given?	×	×	×	×	×	√	×	±	×	×	×	×	√	×	×	±	×	×	×	×	×	×	×	±	×	×	×	±	×	×	×	±
2. The research question of the study was mentioned?	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3. Epidemiological definition such as type of diabetes (1 and 2) studied was provided?	√	×	√	√	×	×	√	√	×	√	×	√	√	√	√	√	√	√	×	√	×	√	×	×	×	√	√	√	√	×	×	×
4. Complications associated with diabetes were clearly stated?	√	√	×	×	√	×	√	×	×	×	√	×	×	×	×	√	×	×	×	×	√	√	×	√	×	×	×	×	√	×	×	×
5. The location of the study respondent was clearly defined?	√	√	±	√	√	√	√	±	√	√	√	√	√	√	√	√	±	√	±	√	√	±	√	√	√	√	√	√	±	±	±	×
6. The sampling technique for data collection was well-defined?	√	±	√	√	±	×	√	√	√	√	±	√	√	√	√	√	±	±	√	±	±	√	√	√	√	±	±	√	√	√	√	±
7. The sample size of the study was adequate?	±	±	√	√	±	±	√	±	±	±	±	±	±	√	±	√	±	±	√	±	√	±	√	±	√	±	√	±	√	√	√	×
8. Tools and techniques of the study	√	√	±	√	±	±	±	±	√	√	±	±	√	±	±	±	√	√	√	√	±	√	√	±	√	√	√	±	√	√	√	±

Table 2 (continued)

Reference no.	[2]	[33]	[34]	[35]	[36]	[37]	[7]	[38]	[8]	[20]	[39]	[40]	[22]	[41]	[42]	[43]	[44]	[23]	[6]	[45]	[9]	[46]	[5]	[47]	[48]	[49]	[50]	[19]	[51]	[52]	[53]	[24]
were lucidly defined?																																
9. Cost of diabetes was properly classified?	√	√	√	√	±	√	±	√	±	√	√	√	√	√	√	√	√	√	±	√	±	√	√	√	√	√	√	√	√	√	√	√
10. The findings of the study were clearly discussed?	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Total score of the studies reviewed																																
Yes (√)	8	6	6	8	4	5	7	5	5	7	5	6	8	7	6	8	5	6	5	6	5	7	7	6	7	6	7	6	8	6	6	3
No (×)	1	2	2	2	2	2	1	1	3	2	2	2	1	2	2	0	2	2	3	2	2	1	3	1	3	2	2	1	1	3	3	4
Moderately available (±)	1	2	2	0	4	2	2	4	2	1	3	2	1	1	2	2	3	2	2	2	3	2	0	3	0	2	1	3	1	1	1	3

Source: Authors' compilation established on reviewed articles

acknowledged by 19 studies (61%), 9 studies considered societal perspective, followed by government perspective for 7 studies and lastly, household perspective was adopted by 6 studies as highlighted in Table 1.

Quality of the reviewed articles

The quality of the included studies is broadly presented in Table 2. For all 32 studies, research questions and findings were discussed and explained in a very well-defined manner. The presentation of the results was completely in synchronisation with the aim and conclusions derived from the reviewed articles. It was found that 60% (19) of the studies have comprehensively defined the epidemiological definition such as type of diabetes (type 1 and type 2). Limitations experienced by the majority of studies that hampered the quality of the reviewed articles were the absence of a broad definition of diabetes and a lack of adequate sample size. A major proportion of 25 studies (78%) did not extensively define diabetes and 18 studies (56%) moderately considered the sample size.

For most of the reviewed articles, the sampling technique for data collection was addressed and only 1 study did not define the sampling technique. However, 56% (18) of studies lucidly defined the tools and technique employed in the reviewed articles and the remaining 14 studies moderately describe the tools and technique. A majority of 27 studies (84%) have properly classified the cost of diabetes and the remaining 5 studies defined moderately. Hence, based on quality index scores, the majority of the studies ($n = 11$) scored '6 Yes' on a 10-point scale. Interestingly, 5 studies attained a marginally higher score of '8 Yes' of the total 32 studies as presented in Table 2.

Cost of diabetes

The economic burden of diabetes mellitus has led to numerous studies on the cost of illness. The cost exerted by diabetes can be categorised into three groups: direct cost, indirect cost and intangible cost [55, 56]. Direct cost includes both direct health care costs (diagnosis, treatment, care and prevention) and direct non-health care costs (transport, housekeeping, social service and legal cost) [1, 57]. Indirect cost includes cost for absenteeism, loss of productivity and disability [58, 59]. Lastly, intangible costs embrace cost for social isolation and dependence, low socio-economic status, mental health and behavioral disorder and loss of quality of life [56, 60, 61]. All twenty-one reviewed studies put forward data and statistics to evaluate per capita cost of individual/household at zone level and the remaining eleven studies highlighted the cost of diabetes at the national level (Table 3). To have a clear insight on cost, the reviewed articles have been categorised into four different zones, viz. north zone, west zone, south zone and north-east zone.

Table 3 Cost profile of the reviewed studies

Ref. no.	Author	Publication year	Cost of individual/household (without complications)	Cost of individual/household (with complications)
[8]	Acharya et al.	2016	The total direct cost without complication was ₹21, 258/- p.a. The total indirect cost without complication was ₹1198/- p.a.	The total direct cost with complication ₹28,888/- p.a. The total indirect cost with complication ₹1746/- p.a. The cost of illness (COI) with complication was 1.4 times higher.
[7]	Akari et al.	2013	The average total direct medical and non-medical cost was 15,588/- p.a. and The average total indirect cost was ₹ 1079/- p.a.	The average cost with diabetic complications was ₹6633/- p.a. for macrovascular complications and ₹4798/- p.a. for microvascular complications
[51]	Bjork et al.	2000	The estimated annual direct cost was ₹ 7070/individual and indirect cost was ₹12,756 including productivity and income loss through illness.	----
[43]	Bjork et al.	2003	The mean total cost of diabetes in India accounts to ₹7159/- p.a. The mean direct cost of diabetes was ₹4724/-- and indirect cost, viz. hospitalisation, was 2435/- p.a. (Some regional differences in patterns of expenditure exist, with patients in the west of India likely to spend 26% more on laboratory fees, check-ups and medicines than any other region.)	----
[5]	Cavanagh et al.	2012	----	Results of the study found India to be most expensive country for a patient with a complex diabetic foot ulcer, where 68.8 months of income was required to pay for treatment. The average direct and indirect monthly cost was ₹5258 (63,096/- annually).
[41]	Chandra et al.	2014	The mean annual direct cost of treatment was ₹8822/- and 52% of amount is spent on drugs and medicines. The mean annual indirect cost of treatment was ₹3949/- of which 91.3% was wage loss.	----
[9]	Eshwari et al.	2018	The total cost for diabetes management was ₹5041/- p.a. of which ₹4282/- was direct cost for the treatment of diabetes and ₹462/- was spent on indirect cost.	The total cost for treatment of diabetes with comorbidities was ₹9133/- p.a. The direct cost with complications was ₹8185/- p.a. and indirect cost amounts to be ₹508/- p.a.
[35]	Grover et al.	2005	The total annual cost of care for diabetes was ₹14,508/-. The biggest proportion was made up of direct cost of ₹9865/- p.a. and remaining ₹4642/- p.a. cost burden was adding up by indirect cost.	----
[39]	Joshi et al.	2013	Majority of the respondents spend ₹ 999/- p.a. on direct cost of care for diabetes.	----
[19]	Kansra	2018	The mean direct cost of diabetes for consultation, lab investigation, medicines etc. was ₹9112/- monthly, whereas indirect cost for outpatient care was ₹1166/- monthly and indirect cost for inpatient care was ₹7068 per month.	----
[38]	Kapur	2007	The total average yearly direct cost was observed to be ₹7158/-. However, the mean direct cost for all patients with diabetes was ₹4724/- p.a.	Individuals with three or more comorbidities encountered 48% more cost of care, amounting to ₹10,593/- annually.
[50]	Katam et al.	2016	The average total direct cost per patient annually was amounted to be ₹27,915/-. The highest portion of direct cost was spent on insulin and glucose test strips (40%).	----
[47]	Khongrangjem et al.	2018	The total median cost of illness per month was ₹5375/-. Total cost was made up of ₹3816/- direct cost and ₹1559/- indirect cost.	----
[44]	Kumar et al.	2008	The total mean evaluation of annual direct spending on ambulatory diabetes care was ₹6000/-.	----
[49]	Kumar and Mukherjee	2014	The total direct expenditure incurred on diabetes was ₹76,779/- p.a. and total indirect expenditure was ₹30,670/- p.a.	----
[2]	Kumpatla et al.	2013	The total direct cost estimates without any complication were observed to be ₹4493/-.	The total cost of expenditure with complication was ₹15,280/-. (cost for patients with foot complication was ₹19,020/-, also average cost for renal patients

Table 3 (continued)

Ref. no.	Author	Publication year	Cost of individual/household (without complications)	Cost of individual/household (with complications)
				was ₹12,690/- followed by 13,135/- for cardiovascular disease.)
[36]	Ramachandran	2007	The average inpatient and outpatient cost of diabetes is ₹7505/- p.a. and ₹3310/- p.a.	----
[34]	Ramachandran et al.	2007	The total median direct expenditure on health care was ₹8130/- p.a.	----
[23]	Rao et al.	2011	The mean cost per hospitalizations was ₹5925/- p.a. for diabetes.	----
[33]	Rayappa et al.	1999	The direct annual cost (incl. hospital, test, monitoring etc.) was ₹15,460/- and indirect annual cost was ₹3572/-.	----
[42]	Sachidanandaa et al.	2010	The annual medical cost spent on diabetes was ₹10,584.7/-.	The annual medicine (direct) cost spent by complicated non-hospitalised was ₹19,326.91/- and ₹25,960.2/- by complicated hospitalised patients
[53]	Satyavani et al.	2014	----	Monthly diabetic patients with chronic kidney disease spend ₹12,664/- on treatment.
[37]	Sharma et al.	2016	The direct annual cost was maximum for private clinics ₹19,552/- and Indirect cost was ₹2462/-.	----
[6]	Shivaprakash et al.	2012	The average cost per visit (direct cost) was ₹377/- in 2010 in comparison to ₹363/- in 2005.	The average cost per visit (direct cost) for patients with complications was ₹464/- in 2010.
[40]	Shobhana et al.	2000	The total direct cost (incl. drugs, tests, consultation, hospital, surgery, transport) was ₹4510/- half yearly.	
[45]	Shobhana et al.	2002	₹13,980/- was spent annually on direct costs of diabetes by the patients.	----
[48]	Singla et al.	2019	The total direct cost (drugs and medicine) for diabetes patients was ₹3241 p.m.	----
[46]	Thakur et al.	2017	The mean annual direct expenditure for diabetes care was ₹9832 and indirect cost was ₹5622.	----
[52]	Tharkar et al.	2009	The total direct cost for hospitalisation was ₹14,000 p.a.	The total direct cost for hospitalisation with comorbidities was ₹19,000/- p.a.
[22]	Tharkar et al.	2010	The median annual direct cost associated with diabetes care was ₹25,391 and indirect cost was ₹4970, respectively.	----
[24]	Tripathy and Prasad	2018	The annual median out-of-pocket household expenditure because of hospitalisation due to diabetes was ₹9996.20/-.	----
[20]	Viswanathan and Rao	2013	The annual direct and indirect cost to treat diabetes was ₹16,756 and ₹5504/-	----

Source: Authors' compilation established on reviewed articles

Under the north zone, 8 studies were included to calculate both direct and indirect costs of diabetes at the individual/household level (Fig. 1). The median direct cost of diabetes is estimated to be ₹18,890/- per annum, ranging from ₹999/- to ₹1,09,344/- [19, 35, 39, 44, 46, 48–50]. The most commonly measured costing items under direct cost were expenditure on medicines (7 studies), diagnostic expenses (2 studies), transportation cost (1 study), hospitalisation (2 studies) and consultation fee (3 studies). The median indirect cost of diabetes for the north zone was evaluated to be ₹18,146/- per annum, ranging from ₹4642/- to ₹98,808/- [19, 35, 46, 49]. For all indirect cost studies, costing items, viz. wage loss and leisure time forgone, were used majorly.

South zone includes 11 studies, majorly from Karnataka state (6 studies), followed by Tamil Nadu (4 studies) and Andhra Pradesh (1 study). The median direct cost was assessed to be ₹10,585/– per annum (Fig. 1), ranging from ₹377/- to ₹21,258/- per annum [2, 6–9, 33, 37, 38, 40, 42, 45]. Direct costing items, viz. medicine cost (9 studies), consultation fees (4 studies) and hospitalisation (3 studies), were used in the reviewed article. The median indirect cost of diabetes was ₹1198/- per annum, ranging from ₹462/- to ₹3572/- per annum with major cost items such as monitoring cost (1 study), absenteeism (3 studies) and impairment (1 study) [7–9, 33, 37].

Under the north-east and west zone, only one-one study was observed, to evaluate the direct and indirect cost of

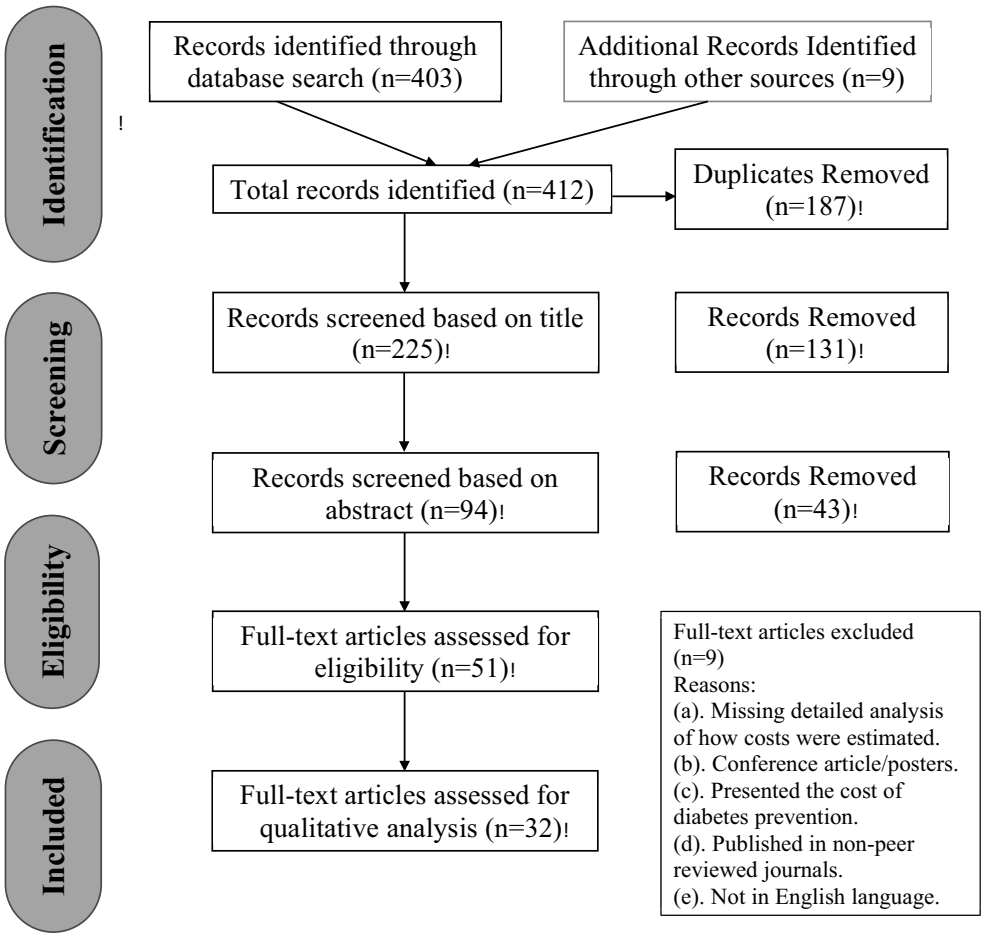

Fig. 1 PRISMA Framework for detailed inclusion criterion. Source: Based on Oberoi and Kansra [54], as suggested by Moher et al. [25]

diabetes at the individual/household level [47, 51]. The median direct cost of diabetes for north-east was evaluated to be ₹45,792/- per annum and ₹8822/- per annum was observed for the west zone (Fig. 1). Commonly estimated costing items were surgical procedures, expenditure on drugs/medicines, clinical fees, etc. The median indirect cost estimated for the north-east zone was ₹18,707/- per annum and ₹3949/- per annum was analysed for the west zone. Indirect costing items identified for both reviewed studies were loss of wage, spendings on health class, travelling expenditure and spendings on diet control. Lastly, 11 studies were incorporated to estimate the cost of diabetes for India as a whole at the individual/household level [5, 20, 22–24, 34, 36, 43, 51–53]. The median direct cost of diabetes for India as a whole was ₹9996/- per annum, ranging from ₹4724/- to ₹25,391/- per annum. Also, the median indirect cost of diabetes at the individual/household level was estimated to be ₹5237/- per annum, ranging from ₹2435/- to ₹12,756/- annually (Figs. 1 and 2).

Complications

Diabetes mellitus is associated with a large number of serious and chronic complications, which act as a major cause of hospitalisation, morbidity and premature mortality in diabetic patients [2, 7, 8, 42]. Diabetes mellitus is commonly associated with chronic complications both macrovascular and microvascular origin [2, 3]. Microvascular complications of diabetes mellitus include retinopathy, autonomic neuropathy, peripheral neuropathy and nephropathy [3, 53]. The macrovascular complication of diabetes mellitus broadly includes coronary and peripheral arterial disease [2, 7]. Of the total reviewed studies, only 10 studies estimated the cost of complications associated with diabetes (Table 3). A couple of studies on diabetes assessed the cost of illness to be 1.4 times higher for individuals with complications as exhibited in Table 3 [8, 52]. A similar study by Sachidananda et al. [42] concluded that the cost of diabetes is 1.8 times higher for complicated non-hospitalised patients and 2.4 times higher for complicated hospitalised patients. Kapur [38] inferred that individuals with three or more comorbidities encounter 48% more cost of care, amounting to ₹10,593/- annually. According to Cavanagh et al. [5], India is the most expensive country for a patient with a complex diabetic foot ulcer, where 68.8 months of income was required to pay for treatment. Three reviewed studies incorporated in the study estimated the cost of individual/household with both macrovascular and microvascular complications [2, 7, 53]. Of these 3 reviewed articles, a couple of them primarily concentrate on

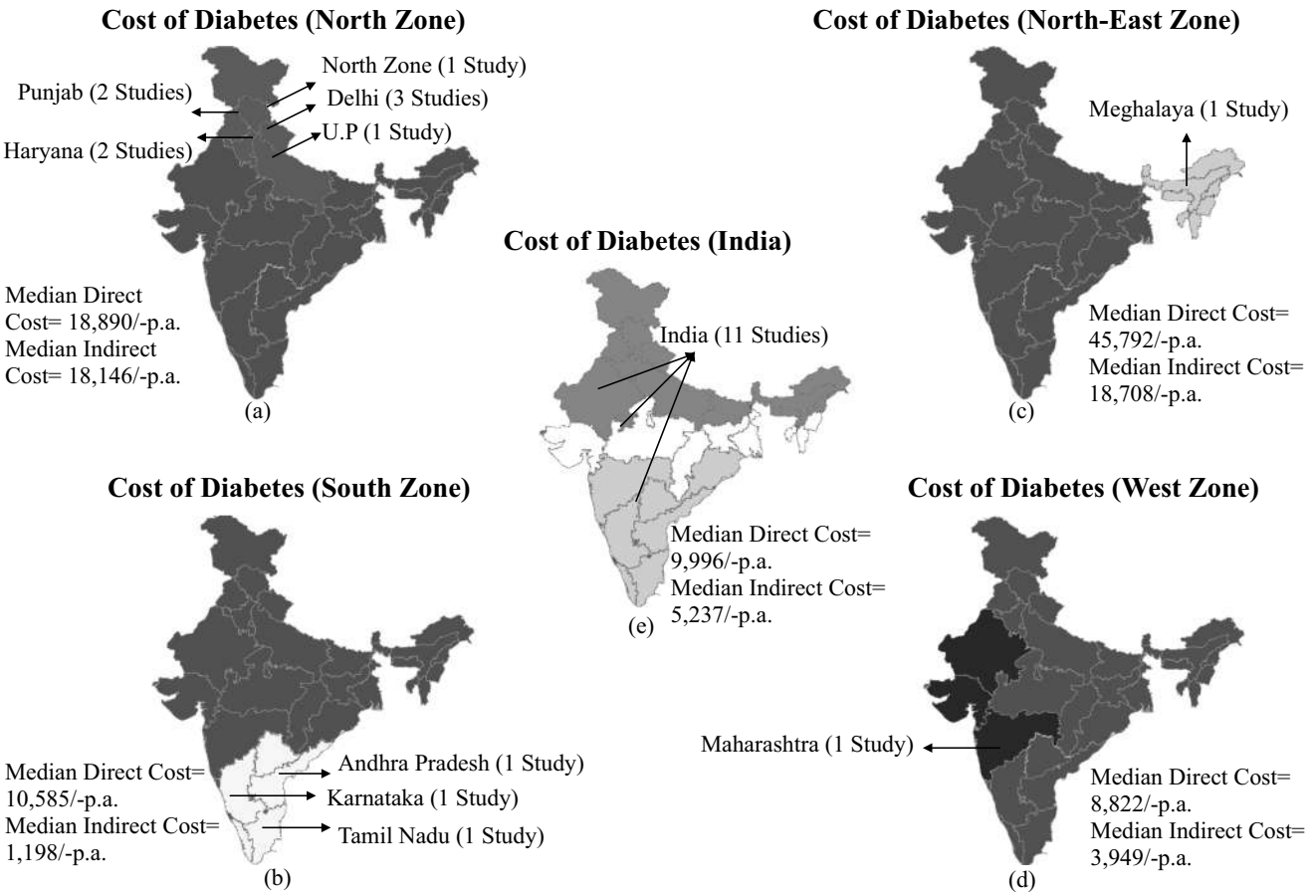

Fig. 2 Cost estimates of India and zone-wise cost profile. Source: Based on the author's compilation and reviewed studies

the cost of illness prompted by renal (kidney) complication [2, 53]. Lastly, Eshwari et al. [9] estimated the total cost for the treatment of diabetes with comorbidities was ₹9133/- annually. Direct cost with complications was ₹8185/- per annum and indirect cost amounts to be ₹508/- annually.

Discussion

Rising menace of diabetes has been a major concern for India. With a frightening increase in population with diabetes, India is soon going to be crowned as 'diabetes capital' of the world. A swift cultural and social alteration, viz. rising age, diet modification, rapid urbanisation, lack of regular exercise regimen, obesity and a sedentary lifestyle, will result in the continuous incidence of diabetes in India. The primary objective of this article is to detect and capture the evidence from published literature on the per capita cost at the individual/household level for both direct and indirect costs of diabetes in India which are available and published since 1999. Of the total 412 records, 32 studies were identified to meet the inclusion criterion. Therefore, the findings of the present study suggest that per annum median direct and indirect cost of diabetes at the individual/household level is very colossal in India.

A large proportion of health care cost is confronted by the patients themselves, which affects the fulfilment of health care because of financial restraints [62]. The proportion of public health expenditure by the Indian government is the lowest in the world. As a consequence, out-of-pocket (OOP) spending constitutes to be 70% of the total health expenditure. Hence, financing and delivering health care facilities in India is majorly catered by the private sector for more than 70% of diseases in both rural and urban areas [24].

Direct cost items (expenditure on medicines, diagnostic expenses, transportation cost, hospitalisation and consultation fee) and indirect cost items (loss of wage, spendings on health class and travelling expenditure) were most commonly reported costing items in the present study [8, 9, 19, 37, 46, 48]. Most of the reviewed studies on the cost of diabetes highlighted expenditure on drugs/medicine as the foremost costing item which accounts for a significant share of all direct costs. The finding of the present study is consistent with Yesudian et al. [62], 'cost on drugs constitutes 50% of the total direct costs'. The majority of the reviewed articles included in the study justify that the primary cause for such abnormal costs of medicines is the

common practice adopted by physicians to prescribe brand-named medicines, rather than generic medicines.

In context to the quality of tools and techniques incorporated by the included studies, a large number of articles (56%) witnessed to acknowledge the standards of tools and techniques. Similarly, the classification of the cost of diabetes was also determined by the majority of reviewed articles (27 articles). But the absence of a comprehensive definition of diabetes and a small size of individuals/households produce dubiousness about the standards or quality of the study. Hence, the limitations experienced by the majority of reviewed articles hampered the quality of the present study. Thus, it is beneficial to develop and suggest standard procedures and framework to conduct a comprehensive and exhaustive study on the cost of diabetes.

Limitations of the study

The present study holds few limitations. Primarily the exclusion of the relevant articles presented as conference papers and those studies published under non-peer-reviewed journals. With the omission of the above literature, some biasness might have been introduced into the review process. Furthermore, the major limitation of the present study is the non-availability of published articles under the central and east zone of India. Also, the studies published under the north-east zone and west zone were only one. Lastly, the heterogeneity in material and methodology used in cost estimation are not analogous. As a consequence, conducting a meta-analysis is not feasible.

Conclusion

The above discussion highlighted a huge economic burden of diabetes in India and variations were recorded in the different zones. It was observed that the cost of drugs/medicines accounts for a major burden of the cost of diabetes. The study suggested few policy interventions to cope with the high economic burden of diabetes. There is a dire need in the country to arrange awareness programmes on diabetes and associated risk factors. The menace of diabetes can be controlled by devising new health care policies, introducing new generic medicines and taxing alcohol/tobacco. Diabetes is a lifestyle disease so along with the above measures, a change in dietary habits, physical activity, beliefs and behavior can reduce its economic burden.

Compliance with ethical standards

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

Ethical approval The study is a review-based study, so it does not contain any studies with animals. The present study only reviews those studies which contain individual's performance.

Informed consent For the present study, it is not necessary to obtain any consent.

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ORIGINAL ARTICLE

Improved glycemic control amongst people with long-standing diabetes during COVID-19 lockdown: a prospective, observational, nested cohort study

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Abstract

Background and aims COVID-19 is likely to affect the lives of individuals with type 2 diabetes. However, the effect of COVID-19 lockdown on physical activity and glycemic control in such individuals is not known. We studied the physical activity and glycemic control during lockdown in comparison to pre-lockdown parameters in individuals with long-standing type 2 diabetes.

Methods This prospective, observational study includes 2240 people with T2DM regularly attending diabetes clinic prior to lockdown. Glycemic record, HbA1c, and physical activity assessed with Global Physical Activity Questionnaire (GPAQ) as metabolic equivalents (MetS min/week) were obtained during lockdown (minimum duration of 3 months).

Results A total of 422 out of 750 participants (nest) responded. The median (IQR) for age was 58 (52 to 64) years, duration of diabetes 11 (6 to 16) years, prevalent foot complications in 59.7%, and atherosclerotic cardiovascular disease in 21.3% of participants. There was a decrease in HbA1c from 7.8% (6.9 to 9.4) prior lockdown to 7.4% (6.6 to8.7) during lockdown [ΔHbA1c $-0.41 \pm 0.27\%$ ($p = 0.005$)] and postprandial blood glucose 200.0 mg/dl (152.0 to 252.0) to 158.0 (140.0 to 200.0) mg/dl ($p < 0.001$). The physical activity increased during lockdown from a GPAQ score 140 (0.0 to 1260) MetS to 840 (0.0 to 1680) MetS ($p = 0.014$). The improvement of glycemic control was observed in either gender and independent of the presence of foot complications or increase in physical activity.

Conclusions There is an overall improvement of glycemic control during COVID-19 lockdown independent of increase in physical activity in people with long duration of diabetes.

Keywords COVID-19 · Glycemic control · HbA1c · Global Physical Activity Questionnaire (GPAQ) · Physical activity

Introduction

COVID-19 due to SARS-CoV-2 infection was declared as global pandemic by WHO on 11 March 2020. It was suggested that the transmission may be significantly curbed by limitation of outdoor activities through the imposition of strict lockdown [1]. Subsequently, complete lockdown was enforced in India on March 25, 2020, until May 4, and partial lockdown is in place limiting daily activities at the time of writing the manuscript. A significant restriction of outdoor physical activity during lockdown may have perpetuating influence on lifestyle disorders including obesity, hypertension, and diabetes. Sedentary lifestyles, poor dietary habits, and sleep deprivation are known potentially modifiable risk factors for poor glycemic control in people with diabetes. Hence, lockdown during COVID-19 pandemic may be associated with poor glycemic control in people with diabetes.

However, there is no evidence set forth for this presumption except for the experiences from the past natural disasters which mimic the similar difficulties and limitations of daily activities [2, 3]. Isolated studies in type 1 diabetes individuals have conflicting reports of worsening or no impact of lockdown period on glycemic control [4–6]. It is also evident that glycemic control may worsen due to the direct effect of SARS-CoV-2 infection in individuals affected, and people with diabetes are likely to have poorer outcomes from SARS-CoV2 infection [7]. Therefore, we prospectively

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studied the effect of lockdown on physical activity and glycemic control in people with pre-existing type 2 diabetes mellitus.

Materials and methods

We invited 750 participants out of 2240 people with pre-existing type 2 diabetes who were regularly attending diabetes clinic at PGIMER, Chandigarh, prior to COVID-19 lockdown and had access to home-based capillary glucose monitoring by glucometer during the lockdown period. We have complete demographic and disease-related detail in electronic case record system. Patients with type 1 diabetes, incomplete records, or not having facility for self-monitoring of blood glucose during the lockdown period or not accessible for telemedicine counselling or consultation and COVID-positive patients were excluded from the study.

Their demographic characteristics, duration of diabetes, physical activity, microvascular and macrovascular complications, and glycemic parameters including HbA1c were evaluated and entered in the electronic database at each follow-up visits. Subsequently, the scheduled visits to the hospital were not possible due to lockdown; therefore, they were approached telephonically for consultation and guidance for titration of the medication doses including oral anti-diabetic drugs and/or insulin. They were requested to share glycemic records of fasting (FBG) and postprandial (1–2 h after major meal) blood glucose (PPBG) by home available glucometers and obtain HbA1c at the nearest available laboratory facility after a minimum of 3-month duration of lockdown.

The physical activity pattern of the participants during lockdown was enquired telephonically by Global Physical Activity Questionnaire (GPAQ) that has been validated earlier in Indian population [8, 9] and represented as metabolic equivalents (MetS min/week). Body weight prior to lockdown was obtained from the electronic repository, and weight during lockdown was recorded from the home-based weighing scales or at nearest available health facility. The primary outcome was change in HbA1c, FBG, and PPBG compared to the last observed value before the lockdown in the electronic database. The other outcome measure was the change in GPAQ scores (MetS min/week). The evaluation for micro- and macrovascular complications was performed as per existing protocol of the institute that included annual (more frequently, if needed) fundus examination, neuropathy and vascular assessment, urine protein, creatinine (eGFR) estimation, and fasting lipids.

Statistical analysis

Data analysis was performed using the Statistical Package of Social Sciences (SPSS) version 23 (IBMCorp, Armonk, NY). Normality was examined using the Shapiro-Wilk test. The data is expressed as the median and interquartile range (IQR) as most of the data variables were non-parametric. The pre- and post-lockdown glycemic variables were compared using Wilcoxon signed-rank T test and Fischer Exact test/Chi-square test for categorical variables. A sub-group analysis by stratifying data according to gender and the presence or absence of foot complications (active pedal ulcer or foot with deformities limiting physical activity) was performed. The correlation between change in glycemic control (HbA1c) with the modification of weight, BMI, and physical activity (GPAQ) during the lockdown period was analysed. The change in HbA1c during the lockdown was considered as dependent variable with the change in FBG, PPBG, weight, BMI, and physical activity (GPAQ) as independent variables. $p < 0.05$ was considered significant for the study.

Results

A total of 422 of the 750 individuals (303 male and 119 female) with diabetes responded with the requisite glycemic parameters within the stipulated duration. The median age of the participants was 58 (52 to 64) years, duration of diabetes of 11(6 to 16) years, and body mass index of 25.6 (22.7 to 28.7). Prevalent microvascular complications include neuropathy in 58.3%, retinopathy in 30.1%, and nephropathy in 27.0% of participants (Table 1). Foot complications were prevailing in 59.7% and atherosclerotic cardiovascular disease in 21.3% of participants. Overall, 22.7% of participants are on insulin, and the rest are on oral anti-diabetic drugs (Fig. 1).

Table 1 Demographic characteristics and diabetes complication of the studied cohort prior to COVID-19 lockdown

Parameters	Values ($n = 422$)
Age (years)	58.0 (52.0 to 64.0)
Duration of diabetes (years)	11.0 (6.0 to 16.0)
Creatinine (mg/dl)	1.03 (0.83 to 1.37)
BMI (kg/m^2)	25.6 (22.7 to 28.7)
Hypertension, n (%)	194 (45.97)
Retinopathy, n (%)	127 (30.09)
Neuropathy, n (%)	246 (58.29)
Nephropathy, n (%)	114 (27.01)
ASCVD, n (%)	90 (21.32)
Foot complication, n (%)	252 (59.71)

Normality of data was checked by Shapiro-Wilk test

Categorical data represented as n (%) and numerical data as median (interquartile range)

ASCVD atherosclerotic cardiovascular disease; *BMI* body mass index

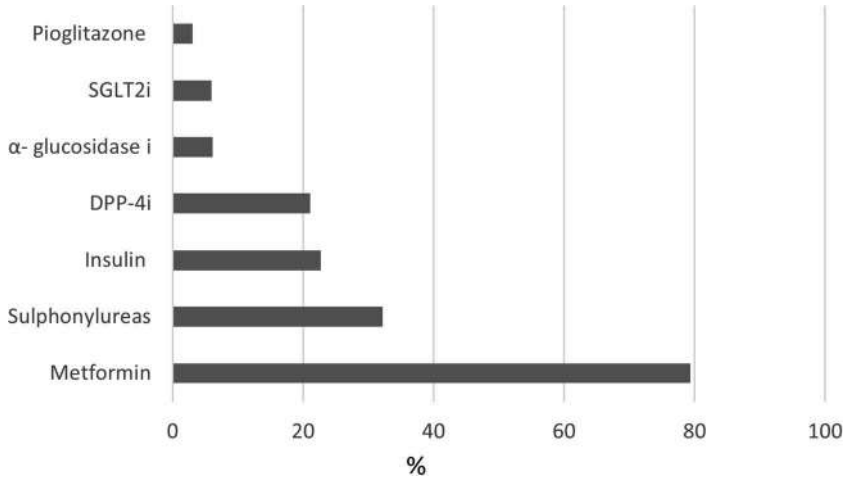

Fig. 1 Various anti-diabetic received by the patients

Last observed HbA1c before COVID-19 lockdown is 7.8 (6.9 to 9.4%), and a HbA1c of 7.4 (6.6 to 8.7) after 3 months of lockdown, with an overall HbA1c reduction of 0.41 ± 0.27% ($p = 0.005$) (Table 2). Overall, 35.1% participants had HbA1c < 7%, prior to lockdown as compared to 38.1% 3 months during lockdown ($p = 0.102$). Fasting blood glucose was 135.0 (112 to 175.0) mg/dl and 150.0 (120.0 to 180.0) mg/dl ($p = 0.02$) and postprandial blood glucose of 200.0 (152.0 to 252.0) mg/dl and 158.0 (140.0 to 200.0) mg/dl ($p < 0.001$) before and after 3 months of lockdown, respectively.

We observed a decrease in weight from 72.0 (61.4 to 78.4) kg to 71.0 (62.0 to 80.0) kg ($p = 0.536$) with an increase in physical activity with GPAQ score of 140 (0.0 to 1260) MetS to 840 (0.0 to1680) MetS ($p = 0.014$). We find no difference in reduction of HbA1c between male and female [ΔHbA1c − 0.6 (− 1.5 to 1.0)% in male and − 1.1 (− 2.2 to 0.4)% in female ($p = 0.39$)] or physical activity GPAQ [Δ GPAQ 0.00 (0.00 to 840) MetS in male and 0.0 (0.0 to 1680) MetS in female ($p = 0.080$)] as detailed in Table 3. Participants with foot complications constituted more than half (59.7%) of the respondents. Participants with foot complications had a higher baseline HbA1c 7.9% (6.9 to 9.4) compared to those without foot complications 7.3% (6.6 to 8.3) ($p = 0.180$) with a decrease in HbA1c of − 0.4% (− 1.7 to 0.9) and − 0.3% (− 1.0 to 0.5) ($p = 0.341$) in the two groups, respectively (Table 4). We did not find significant correlation between change in glycemic control (ΔHbA1c) with either age ($p = 0.549$), duration of diabetes ($p = 0.416$), change in weight ($p = 0.597$), or physical activity by GPAQ scores ($p = 0.128$).

Discussion

We observed an overall improvement of glycemic parameter in people with long-standing type 2 diabetes associated with an increase in physical activity as assessed with GPAQ score during the lockdown period unlike the conventional belief of worsening of glycemic control and limitation of physical activity. The decline in HbA1c was independent of the increase in physical activity and was observed in either gender and irrespective of the presence or absence of diabetic foot complications.

Table 2 Alterations in glycemic parameters and physical activity of the studied cohort during the lockdown

Parameters	Pre-lockdown	During lockdown	*p* value
Weight (kg)	72.0 (61.4 to 78.4)	71.0 (62.0 to 80.0)	0.536
Body mass index (kg/m^2)	25.8 (22.8–28.9)	25.8 (22.8–28.9)	0.810
HbA1c (%)	7.8 (6.9 to 9.4)	7.4 (6.5 to 8.7)	0.005
mmol/mol	61.7 (51.9 to 79.2)	57.4 (47.5 to 71.6)	
Fasting blood glucose (mg/dl)	135.0 (112.0 to 175.0)	150.0 (120.0 to 180.0)	0.002
Postprandial blood glucose (mg/dl)	200 (152 to 252.0)	158.0 (140.0 to 200.0)	< 0.001
GPAQ score (MetS)	140 (0.0 to 1260)	840 (0.0 to 1680)	0.014

$p < 0.05$ was considered significant. *GPAQ* Global Physical Activity Questionnaire (GPAQ). *MetS (min/week)* metabolic equivalents

Table 3 Alterations in glycemic parameters and physical activity of the studied cohort during the lockdown stratified by gender

Parameters	Male $n = 303$	Female $n = 119$	*p value
Weight-PL (kg)	73.2 (63.0 to 83.0)	65.0 (60.0 to 75.6)	0.000
Weight-DL (kg)	72.0 (64.0 to 82.0)	68.0 (60.8 to 75.0)	0.003
p value	0.170	0.805	
Δ weight	0.0 (− 5.7 to 3.2)	1.00 (− 1.5 to 11.4)	0.020
HbA1C-PL (%)	7.7 (6.8 to 8.9)	7.6 (6.7 to 9.1)	0.819
mmol/mol	60.7 (50.8 to 73.8)	59.6 (49.7 to 76.7)	
HbA1C-DL (%)	7.3 (6.5 to 8.4)	7.5 (6.6 to 9.0)	
mmol/mol	56.3 (47.5 to 68.3)	58.5 (48.6 to 74.9)	0.420
p value	0.134	0.789	
ΔHbA1C (%)	− 0.6 (− 1.6 to 1.0)	− 1.1 (− 2.2 to 0.4)	0.390
FBG-PL (mg/dl)	130.0 (105.5 to 175.0)	137.4 (110.0 to 187.5)	0.315
FBG-DL (mg/dl)	150.0 (120.0 to 187.0)	152.50(152.5 to 198.5)	0.768
p value	0.004	0.039	
ΔFBG (mg/dl)	23.0 (− 17.1 to 68.0)	− 20.0 (− 37.5 to 49.0)	0.487
PPBG-PL (mg/dl)	191.0 (148.0 to 258.0)	234.5 (157.5 to 250.5)	0.292
PPBG-DL (mg/dl)	155.0 (140.0 to 195.0)	155.0 (131.5 to 242.5)	0.336
p value	0.000	0.160	
ΔPPBG (mg/dl)	− 20.0 (− 78.50 to 44.00)	− 22.0 (− 90.0 to 99.00)	0.751
GPAQ-PL (MetS)	420.0 (0.0 to1680)	780.0 (0.0 to 1680)	0.524
GPAQ-DL(MetS)	840.0 (0.0 to1680)	840.0 (0.0 to 1680)	0.362
p value	0.196	0.000	
Δ GPAQ	0.0 (0.0 to 840)	0.0 (0.0 to 560)	0.080

Data represented as median (IQR) and comparison done by Mann-Whitney U test. *PL* pre-lockdown; *DL* during lockdown; *FBG* fasting blood glucose; *PPBG* postprandial blood glucose *GPAQ* Global Physical Activity Questionnaire (GPAQ); *MetS* metabolic equivalents; Δ last observed value prior to lockdown—value during lockdown p value: intragroup comparison; *p value, intergroup comparison

COVID-19 pandemic has necessitated lockdown to limit the SARS-CoV2 infection and shown to be effective in reducing the R0, i.e. number of people infected by each infected person [10]. While lockdown slows the spread of infection, it is likely to have adverse influence on lifestyle patterns contributing to weight gain. A failure to adhere to lifestyle recommendations for diabetes during lockdown due to a significant curb of outdoor physical activity along with psychological stress related to pandemic may be associated with worsening of glycemic control. The stress of acquiring COVID has also been ascribed as one of the reasons for poor glycemic control. A predictive modelling using a simulation model created with the aid of a multivariate regression analysis has shown that the predicted increment in HBA1c from baseline at the end of 30 days and 45 days lockdown could be 2.26 and 3.68%, respectively [11]. However, this prediction was based on data from similar natural disasters but not exactly the same scenario as COVID-19 lockdown and is likely to overestimate the risk because of model-based risk prediction. A cross-sectional study in type 1diabetes individuals observed an increase in average blood glucose 276.9 ± 64.7 mg/dl as compared to 212.3 ± 57.9 mg/dl and HbA1c of 10 ± 1.5% compared to 8.8 ± 1.3%) ($p < 0.05$) during and before lockdown, respectively [4]. The major reason attributed to worsening of glycemic control was the non-availability of insulin in rural and semi-urban areas.

We prospectively studied glycemic parameters in people with diabetes along and a change in their physical activity consequent to lockdown. Unlike the belief, we observed an improvement in glycemic parameters compared to the last available pre-lockdown with a significant reduction in HbA1c and postprandial blood glucose after a minimum of 3 months of lockdown. There was an increase in fasting blood glucose but an overall decrease in HbA1c that was likely contributed by a considerable decrease in postprandial blood glucose during the lockdown phase. Our results are consistent with recent studies predominantly in type 1 diabetes people that noticed no effect of lockdown on glycemic control [5, 6]. Italian authors observed a decrease in time spent in hypoglycemia (time below range) during lockdown in insulin-treated people [5]. The possible reasons for better glycemic control in our study could be a decrease in work-related stress, adequate time for self-care, better compliance to medications, adherence to dietary recommendations (home cooked food), lack of availability of outside calorie-dense diet, and an increase in physical activity though indoors. Though Ghosh et al. observed an increase in carbohydrate consumption and snacking

Table 4 Alterations in glycemic parameters and physical activity of the studied cohort during the lockdown stratified by the presence or absence of foot complications

Parameter	With foot complications $n = 252$	Without foot complications $n = 170$	*p value
Weight-PL (kg)	72.6 (61.5 to 81.7)	67.7 (62.0 to 78.5)	0.187
Weight-DL (kg)	70.9 (61.7 to 80.0)	68.0 (62.0 to 77.5)	0.579
P value	0.339	0.863	
Δ weight	0.0 (− 3.6 to 2.0)	0.0 (− 1.1 to 1.3)	0.490
HbA1C-PL (%)	7.9 (6.9 to 9.4)	7.3 (6.6 to 8.3)	0.180
mmol/mol	62.8 (51.9 to 79.2)	56.3 (48.6 to 67.2)	
HbA1C-DL (%)	7.6 (6.5 to 9.6)	7.1 (6.4 to 8.0)	0.020
mmol/mol	59.6 (47.5 to 81.4)	54.1 (46.4 to 63.9)	
p value	0.164	0.211	
ΔHbA1C	− 0.4 (− 1.7 to 0.9)	− 0.3 (− 1.0 to 0.5)	0.341
FBG-PL (mg/dl)	131.0 (106.2 to175.7)	135.5 (105.2 to 176.5)	0.965
FBS-DL (mg/dl)	150.0 (120.0 to188.2)	132.5 (104.0 to 157.7)	0.587
p value	0.000	0.000	
ΔFBG(mg/dl)	16.0 (− 29.75 to 64.0)	− 10.5 (− 36.7 to 22.5)	0.287
PPG-PL (mg/dl)	196.5 (151.2 to254.2)	221.0 (144.7 to304.2)	0.579
PPBG-DL (mg/dl)	157.5 (140.0 to200.0)	154.5 (127.5 to171.7)	0.000
p value	0.000	0.099	
ΔPPBG (mg/dl)	− 21.5 (87.5 to 36.5)	− 68.5(139.7 to 8.2)	0.889
GPAQ PL(MetS)	2.0 (0.0 to 1260)	840 (141.0 to 1680)	0.000
GPAQ-DL(MetS)	420 (0.0 to 1680)	1200 (490 to 1680)	0.001
P value	0.004	0.488	
Δ GPAQ	0.0 (0.0 to 720)	0.0 (− 560 to 560)	0.864

Data represented as median (IQR) and intergroup comparison performed by Mann-Whitney *U* test. *PL* pre-lockdown; *DL* during lockdown *FBG* fasting blood glucose; *PPBG* postprandial blood glucose; *GPAQ* Global Physical Activity Questionnaire (GPAQ); *MetS* metabolic equivalents; Δ last observed value prior to lockdown—value during lockdown *p* value, intragroup comparison; **p* value, intergroup comparison

in people with type 2 diabetes from north India [12], recurrent contact through teleconsultations may have helped in allaying fear and stress of acquiring COVID in the present cohort.

Excessive sedentary behavior and lack of exercise are a problem area in management of diabetes due to lack of adherence which is likely to be further worsened by COVID-19 pandemic. However, we observed that most of the respondents engaged themselves in physical activity doing household chores and indoor exercise consequent upon availability of time that was reflected in a significant increase in GPAQ scores during the lockdown. All the respondents were motivated individuals having long duration of diabetes, attending diabetes clinic regularly, and were knowledgeable of lifestyle recommendations and glycemic targets. Moreover, they were regularly counselled telephonically and encouraged to limit calorie intake and sedentary behavior during lockdown. It has been observed that unstructured physical activity like performing household chores is known to help in weight management, controlling postprandial hyperglycemia, and overall improved glycemic control by reducing the total sedentary time, increasing the energy expenditure that may [13, 14]. Thus, despite a significant limitation of outdoor activities during lockdown, an increase in GPAQ scores suggests that increasing indoor activities and limiting sedentary time are also beneficial for people with diabetes in improving glycemic control.

Our results also suggest that people with significant comorbidities of diabetes that limit outdoor activities like foot complications are also able to achieve good glycemic control. Knowing that people with foot complications like neuropathic foot ulcers or Charcot neuroarthropathy and foot deformities are likely to have higher mortality as compared to individuals with diabetes without foot complications [15, 16], good glycemic control in this cohort is more desirable. The improvement in glycemic parameters associated with an increase in physical activity and weight loss was observed irrespective of gender. COVID-19 is associated with significant psychosocial impact on people with type 2 diabetes related to concerns about worsening of glycemic control. However, improvement noticed in glycemic control in the present study will help to counsel the patients for better self-care during COVID-19 pandemic [17].

This is the first large, prospective study amongst people with long-standing type 2 diabetes to assess the effect of more

than 3 months duration of lockdown on glycemic control. However, certain potential biases cannot be ruled out in the present study including that all the respondents in our study were self-motivated, had long duration of diabetes (> 10 years), were under clinic follow-up for long duration, and aware of lifestyle recommendations and glycemic goals. Moreover, only the motivated patients are likely to respond with glycemic parameters that might have contributed to most patients having improved glycemic control.

During lockdown, GPAQ survey was conducted telephonically; various kinds of glucometers were used for capillary glucose that might have an inherent bias. The reliability and reproducibility of the home-based weighing scales cannot be vouched, but it helped us in understanding the trend of weight change in real life pandemic situation. The dietary change, macronutrient composition, and calorie intake were not recorded. The results of our study may not be generalized to those with shorter duration of diabetes or with limited healthcare teleconsultation access.

In conclusion, the present study assures that lockdown period may not be associated with worsening of glycemic control in people with long-standing diabetes. Limiting sedentary time and increasing indoor activities also help in achieving better glycemic control during COVID-19 lockdown. Awareness of glycemic goals, access to self-monitoring of blood glucose, and ability to cope with restrictions of lockdown by rigorously following lifestyle recommendations and engagement in some form of physical activity are beneficial.

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Compliance with ethical standards

Conflict of interest None.

Informed consent A written informed consent was obtained from all participants (signed digitally) and the study was approved by the institute Ethics Committee.

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ORIGINAL ARTICLE

Clinical features of critically ill patients infected with SARS-CoV-2 outside Wuhan with and without diabetes

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Abstract

Aim Some patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rapidly develop to critical condition. Here, we investigated the clinical features of critically ill SARS-CoV-2 patients with and without diabetes and identified risk factors for death of these patients.

Methods The medical records including epidemiological, demographic, clinical, and laboratory data from 49 critically ill SARS-CoV-2 patients were collected and analyzed in Huanggang City and Xiaogan City, Hubei Province, outside Wuhan.

Results Sixty-seven percent (33) of patients survived and 33% (16) of patients died in 49 critically ill patients (32 men, 17 women), with a median age of 63 years (IQR 53–73). Univariate analyses indicated that the deceased patients were more often associated with two or more comorbidities, one or more gastrointestinal symptoms, high neutrophil percentage, low lymphocytes and lymphocyte percentage, high C-reactive protein, high procalcitonin, high fasting blood glucose (FBG), and high lactate dehydrogenase (LDH) compared with the survivors; moreover, the patients with T2DM had the higher neutrophil percentage, the lower lymphocyte percentage, and the higher levels of FBG and LDH compared with the patients without T2DM. Multivariable logistic regression analyses indicated that gastrointestinal symptoms (≥ 1 symptoms), decreased lymphocytes ($< 1.1 \times 10^9$/L), and increased FBG (≥ 7.0 mmol/L) were the independent risk factors for death of critically ill patients.

Conclusions Critically ill COVID patients with T2DM had more severe damages of the lymphocytes, islet cells, and heart function, and gastrointestinal symptoms, lymphopenia, and increased FBG may be early predictors for poor prognosis.

Keywords SARS-CoV-2 · Critically ill patients · Clinical features · T2DM · Independent risk factors

Introduction

Coronaviruses (CoVs) are enveloped, positive-sense, single-stranded RNA viruses and belong to the family Coronaviridae and the order Nidovirales, of which two coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) [1, 2] and Middle East respiratory syndrome coronavirus (MERS-CoV) [3], have caused recent pandemics of respiratory

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infectious diseases with high mortality rates of 10% for SARS-CoV [4] and 37% for MERS-CoV [5].

On Jan 7, 2020, a novel coronavirus was identified by the Chinese Center for Disease Control and Prevention (CDC) from the lower respiratory tract sample of a patient, and subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the World Health Organization (WHO) [6]. Since the first case was reported in December 2019 in Wuhan, China, the outbreak of the disease is currently continuously spreading all over the world.

The disease caused by SARS-CoV-2 that is named as coronavirus disease 2019 (COVID-19) by the WHO could induce symptoms including fever, dry, cough, dyspnea, fatigue, and lymphopenia in infected patients, with some patients rapidly developing acute respiratory distress syndrome (ARDS), acute respiratory failure, or multiple organ failure and even death [7]. Mechanical ventilation is usually required in critically ill patients with ARDS or acute respiratory failure. At present, although many articles have established the epidemiology and clinical features of patients with SARS-CoV-2 infection [7–10], the information regarding clinical manifestations and laboratory findings in critically ill patients with COVID-19 has not been reported yet. In the present study, we investigated the clinical features of critically ill patients with COVID-19 and identified the independent risk factors for death of these patients in Huanggang City and Xiaogan City, Hubei Province, outside Wuhan.

Methods

Patients

We retrospectively analyzed the medical records from critically ill patients with COVID-19 admitted to Dabie Mountain Medical Center and the First People's Hospital of Xiaochang County, Hubei Province, from February 1 to March 25, 2020. The two hospitals, which are located in Huanggang City and Xiaogan City, respectively, Hubei Province, about 80–90 km away from Wuhan, China, are the designated hospitals for the hospitalization of patients with COVID-19. SARS-CoV-2 pneumonia was diagnosed based on clinical symptoms with typical changes in chest CT and positive for the nucleic acids of SARS-CoV-2. Severity of COVID-19 was defined according to the diagnostic and treatment guideline for SARS-CoV-2 issued by the Chinese National Health Committee (version 3-6). Critically ill patients with COVID-19 were designated when the patients had one of the following criteria: (a) respiratory failure with mechanical ventilation; (b) shock; (c) combination with other organ failures, with ICU monitoring and treatment.

Data collection

Epidemiological, demographic, clinical, laboratory, and medical imaging data from patients' medical records were collected by Qi Liu and Liangfei Deng who is a doctor of critical medicine and rush to the rescue of Dabie Mountain Medical Center on February 1, 2020. If data were missing from the records or clarification was needed, we obtained data by direct communication with attending doctors and patients or their families. The nucleic acid testing for SARS-CoV-2 was performed using quantitative RT-PCR on samples from the respiratory tract by Huanggang CDC and Xiaogan CDC, which are the designated laboratory for SARS-CoV-2 test.

Statistical analysis

Categorical data were expressed as number (%) and evaluated by $\chi 2$ or Fisher's exact test; continuous data were expressed as median (interquartile range (IQR)) and evaluated by Mann-Whitney U test. To explore the risk factors associated with death of critically ill patients, a multivariable logistic regression model was used. A two-sided α of less than 0.05 was considered statistically significant. All the statistical analyses were performed with SPSS (version 26.0).

Results

Demographics and clinical characteristics

A total of 49 critically ill patients with COVID-19, who all were admitted to intensive care unit (ICU) and required oxygen therapy, were included in this study (32 men, 17 women), with a median age of 63 years (IQR 53–73). Thirty-three patients were discharged and 16 patients were deceased. Among them, there were 26 patients with familiar/cluster exposure history and 23 cases with community exposure history. Seventy-three percent of patients had comorbid chronic systemic diseases, including hypertension (45%), chronic heart disease (33%), type 2 diabetes mellitus (T2DM) (22%), chronic obstructive pulmonary disease (22%), cerebrovascular disease (18%), chronic liver disease (10%), chronic renal disease (4%), and malignant tumor (4%), of whom 57% of patients had two or more of the comorbidities. The median intervals from disease onset to admission and from admission to critical condition were 7.0 days (IQR 4.5–10.0) and 4.0 days (IQR 2.5–8.0), respectively. The deceased patients were more often associated with two or more of comorbidities and shorter time from admission to critical condition compared with the survivors, whereas there were no significant differences between the survivors and deceased groups with respect to age, sex, exposure history, occupation, smokers, etc. (Table 1).

Table 1 Demographics and baseline characteristics of critically ill patients with COVID-19

Items	All patients ($n = 49$)	Survivors ($n = 33$)	Deceased ($n = 16$)	p value
Age, years	63 (53–73)	58 (50–74)	67 (60–72)	0.267
Sex, *n* (%)				
Female	17 (35)	12 (36)	5 (31)	0.724
Male	32 (65)	21 (64)	11 (69)	
Exposure history, *n* (%)				
Familiar/cluster infections	26 (53)	16 (48)	10 (63)	0.357
Community infections	23 (47)	17 (52)	6 (37)	0.357
Occupation, *n* (%)				
Agricultural worker	13 (27)	9 (27)	4 (25)	0.867
Employee	11 (22)	8 (24)	3 (19)	0.669
Retired	25 (51)	16 (49)	9 (56)	0.610
Smokers, *n* (%)	18 (37)	11 (33)	7 (44)	0.478
Chronic systemic diseases, *n* (%)	36 (73)	22 (67)	14 (88)	0.174
Hypertension	22 (45)	13 (39)	9 (56)	0.266
Chronic heart disease	16 (33)	10 (30)	6 (38)	0.614
T2DM	11 (22)	6 (18)	5 (31)	0.456
Chronic obstructive pulmonary disease	11 (22)	8 (24)	3 (19)	1.000
Cerebrovascular disease	9 (18)	4 (12)	5 (31)	0.130
Chronic liver disease	5 (10)	4 (12)	1 (6)	1.000
Chronic renal disease	2 (4)	1 (3)	1 (6)	1.000
Malignancy	2 (4)	1 (3)	1 (6)	1.000
Two or more of the above diseases	28 (57)	15 (45)	13 (81)	0.018
Days from disease onset to admission, days	7.0 (4.5–10.0)	6.0 (4.0–10.0)	8.0 (6.3–10.8)	0.534
Days from admission to critical condition, days	4.0 (2.5–8.0)	6.0 (3.5–8.5)	2.5 (1.0–4.0)	0.001

Data are shown as median (IQR) or *n* (%). *p* values comparing survivors and deceased are from χ^2 test, Fisher's exact test, or Mann-Whitney *U* test.
COVID-19, coronavirus disease 2019; *IQR*, interquartile range

The most common symptoms at disease onset were fever (82%), cough (76%), fatigue (57%), gastrointestinal symptoms (47%), and chest tightness/dyspnea (45%). Gastrointestinal symptoms included nausea or vomiting (31%), diarrhea (22%), anorexia (16%), and abdominal pain (6%). The gastrointestinal symptoms were more common in the deceased patients compared with the survivors, whereas there were no significant differences between the survivors and deceased groups about the other symptoms (Table 2).

Laboratory and imaging findings

On admission, leucocytes were within the normal range in most of the patients (59%), with 35% increased and 6% decreased numbers. Neutrophils were above the normal range in 39% of patients, and nearly half of the patients (47%) had increased neutrophil percentage. Lymphopenia was common, and lymphocytes and lymphocyte percentage were below the normal range in 71% and 65% of the patients, respectively. Platelets and hemoglobin were below the normal range in 20% of patients and 45% of patients, respectively. Most of the patients had increased levels of C-reactive protein (CRP) (86% cases) and procalcitonin (PCT) (55% cases), with a median CRP level of 37.4 mg/L (IQR 16.0–62.4) and median PCT level of 0.6 ng/mL (IQR 0.1–3.8) (Table 3).

On admission, most patients showed normal prothrombin time, and all patients showed normal activated partial thromboplastin time. D-dimer level was above the normal range in 59% of patients, with a median D-dimer level of 0.6 μg /mL (IQR 0.2–1.3) (Table 3).

The median fasting blood glucose (FBG) level of the patients was 6.9 mmol/L (IQR 5.7–9.7), with FBG ≥ 7.0 mmol/L in 49% of patients on admission. The patients had liver function abnormality, with 57% decreased albumin level, 8% increased alanine aminotransferase (ALT) or 14% increased aspartate aminotransferase (AST) level, and 22% increased total bilirubin level. The patients had abnormal myocardial zymogram, which showed the elevation of creatine kinase (CK) in 31% of patients, the elevation of MB isoenzyme of creatine kinase (CKMB) in 16% of patients, and the elevation of lactate dehydrogenase (LDH) in 76% of patients. The patients had renal function damage, with elevated blood urea nitrogen

Table 2 Clinical manifestations of critically ill patients with 2019-nCoV pneumonia

Items	All patients ($n = 49$)	Survivors ($n = 33$)	Deceased ($n = 16$)	p value
Fever, *n* (%)	40 (82)	28 (85)	12 (75)	0.449
Cough, *n* (%)	39 (80)	27 (82)	12 (75)	0.709
Fatigue, *n* (%)	28 (57)	21 (64)	7 (44)	0.187
Muscle ache, *n* (%)	9 (18)	5 (15)	4 (25)	0.449
Headache, *n* (%)	5 (10)	2 (6)	3 (19)	0.313
Sore throat, *n* (%)t	4 (8)	2 (6)	2 (13)	0.588
Chill, *n* (%)	3 (6)	1 (3)	2 (13)	0.245
Chest tightness/dyspnea, *n* (%)	22 (45)	13 (39)	9 (56)	0.266
Gastrointestinal symptoms, *n* (%)	23 (47)	10 (30)	12 (75)	0.003
Nausea or vomiting	15 (31)	8 (24)	7 (44)	0.198
Diarrhea	11 (22)	5 (15)	6 (38)	0.141
Anorexia	8 (16)	3 (9)	5 (31)	0.094
Abdominal pain	3 (6)	1 (3)	2 (13)	0.245

Data are shown as *n* (%). p values comparing survivors and deceased are from χ^2 test or Fisher's exact test. *COVID-19*, coronavirus disease 2019; *IQR*, interquartile range

in 22% of patients and elevated serum creatinine in 22% of patients (Table 3).

On admission, all patients showed bilateral viral pneumonia in chest CT images. The representative chest CT findings of a deceased patient and a discharged patient showed bilateral ground glass opacity (Table 3; Supplementary Fig. 1).

The deceased patients had higher median neutrophil percentage, lower median lymphocytes and lymphocyte percentage, higher median C-reactive protein level and procalcitonin level, higher median fasting blood glucose level, and higher median lactate dehydrogenase level compared with the survivors, whereas there were no significant differences between the survivors and deceased groups concerning other blood routine, coagulation function, and other blood biochemistry parameters (Table 3).

Treatment regimen

According to the diagnostic and treatment guideline for COVID-19 issued by the Chinese National Health Committee (version 3-6), all patients received antiviral therapy, including combination of interferon-α (5 million U, twice daily, inhalationally) and lopinavir/ritonavir tablets (500 mg, twice daily, orally) or combination of interferon-α (5 million U, twice daily, inhalationally) and abidol (200 mg, three times daily, orally). The duration of treatment was 4–10 days. Sixty-one percent of patients were given corticosteroid treatment (methylprednisolone or dexamethasone) for 4–7 days. Forty-nine percent of patients were administered with empirical antibiotic treatment (cephalosporins, quinolones, or carbapenems) for 5–12 days. Four (8.2%) patients were also treated with antifungal drugs (voriconazole) (Supplementary Table 1).

All patients received respiratory support for 5–18 days, with 45% non-invasive ventilation and 55% invasive ventilation, of whom a patient was given combination of invasive ventilation and extracorporeal membrane oxygenation (ECMO). Four patients were treated with continuous renal replacement therapy (CRRT) in deceased patients. Moreover, 90% of patients were administered with traditional Chinese medicine (Lianhua Qingwen granules or capsules). There were no significant differences between the survivors and deceased groups about the above treatment regimens except for CRRT (Supplementary Table 1).

Demographics and clinical and laboratory characteristics of 11 critically ill COVID patients with T2DM

In 49 critically ill patients with COVID-19, 11 patients had history of T2DM (7 men, 4 women), with a median age of 58 years (IQR 51–62). The median duration of T2DM and the median HbA1c level of the patients were 15 years and 8.2%, respectively. All 11 patients had complications, including diabetic peripheral neuropathy (64%), diabetic nephropathy (36%), diabetic retinopathy (36%), and diabetic macroangiopathy (9%), of whom 36% of patients had two or more of the above complications. They received medications including sulfonylureas, biguanide, alpha-glucosidase inhibitors, glinides, DPP-IV inhibitors, and long-acting insulin analogs (Table 4). We compared the demographics and laboratory characteristics between 11 diabetic and 38 nondiabetic critically ill COVID patients according to the laboratory

Table 3 Laboratory characteristics of critically ill patients with COVID-19

Items	All patients ($n = 49$)	Survivors ($n = 33$)	Deceased ($n = 16$)	p value
Blood routine				
Leucocytes ($\times 10^9$/L; normal range 3.5–9.8)	5.8 (4.1–10.1)	5.5 (4.2–9.8)	10.3 (3.7–14.7)	0.277
Increased, *n* (%)	17 (35)	9 (27)	8 (50)	0.117
Decreased, *n* (%)	3 (6)	2 (6)	1 (6)	1.000
Neutrophils (× 109/L; normal range 1.8–6.3)	4.1 (2.8–9.5)	3.9 (2.7–6.0)	9.5 (3.0–13.5)	0.050
Increased, *n* (%)	19 (39)	10 (30)	9 (56)	0.08
Neutrophil percentage, (%) (normal range 40–75)	74.6 (65.6–87.6)	70.3 (61.4–78.7)	88.8 (82.3–93.0)	0.000
Increased, *n* (%)	23 (47)	10 (30)	13 (81)	0.001
Lymphocytes ($\times 10^9$/L; normal range 1.1–3.2)	0.8 (0.6–1.4)	1.0 (0.7–1.6)	0.6 (0.4–0.8)	0.002
Decreased, *n* (%)	35 (71)	20 (61)	15 (94)	0.019
Lymphocyte percentage, (%) (normal range 20–50)	15.6(8.7–25.1)	19.8 (11.3–26.6)	8.7 (3.4–11.7)	0.000
Decreased, *n* (%)	32 (65)	17 (52)	15 (94)	0.004
Platelets ($\times 10^9$/L; normal range 125–350)	178 (131–226)	130 (115–145)	147 (120–201)	0.150
Decreased, *n* (%)	10 (20)	6 (18)	4 (25)	0.709
Hemoglobin (normal range 115–150 g/L)	131 (115–145)	130 (115–145)	134 (113–146)	0.991
Decreased, *n* (%)	22 (45)	16 (48)	6 (38)	0.468
Infection biomarkers				
C-reactive protein (mg/L; normal range 0.0–8.0)	37.4 (16.0–62.4)	31.2 (10.0–56.0)	58.3 (26.7–90.0)	0.047
Increased, *n* (%)	42 (86)	27 (82)	15 (94)	0.402
Procalcitonin (ng/mL; normal range 0.0–0.5)	0.6 (0.1–3.8)	0.2 (0.1–1.2)	3.0 (0.6–7.5)	0.004
Increased, *n* (%)	27 (55)	14 (42)	13 (81)	0.010
Coagulation function				
Prothrombin time (s; normal range 9.0–15.0)	13.2 (12.2–14.2)	13.2 (11.8–13.9)	13.7 (13.0–14.5)	0.088
Increased, *n* (%)	7 (14)	4 (12)	3 (19)	0.668
Activated partial thromboplastin time (s; normal range 22.0–45.0)	30.3 (28.2–33.0)	30.3 (28.6–32.4)	31.2 (26.1–35.6)	0.749
D-dimer (μg/mL; normal range 0.0–0.5)	0.6 (0.2–1.3)	0.6 (0.2–1.1)	0.8 (0.1–2.2)	0.654
Increased, *n* (%)	29 (59)	19 (58)	10 (63)	0.742
Blood biochemistry				
Fasting blood glucose (mmol/L; normal range 3.9–6.1)	6.9 (5.7–9.7)	6.1 (5.3–7.9)	8.3 (7.1–12.7)	0.003
Increased (≥ 7.0 mmol/L), *n* (%)	24 (49)	11(33)	13 (81)	0.002
Albumin (g/L; normal range 35.0–52.0)	34.1(29.5–37.7)	34.7 (29.2–39.2)	33.7 (29.8–36.8)	0.587
Decreased, *n* (%)	28 (57)	17 (52)	11 (69)	0.253
Alanine aminotransferase (U/L; normal range 9–50)	20.0 (14.5–33.0)	19.0 (1.5–32.5)	21.5 (14.0–36.8)	0.579
Increased, *n* (%)	4 (8)	3 (9)	1 (6)	1.000
Aspartate aminotransferase (U/L; normal range 15–40)	23.0 (1.08–34.9)	22.0 (18.0–34.5)	26.5 (17.7–35.3)	0.685
Increased, *n* (%)	7 (14)	5 (15)	2 (13)	1.000
Total bilirubin (μmol/L; normal range 5.1–23.0)	14.4 (9.3–22.1)	13.5 (8.8–19.0)	15.2 (10.8–32.8)	0.365
Increased, *n* (%)	11(22)	5 (15)	6 (38)	0.141
Creatine kinase (U/L; normal range 26–174)	113.0 (58.5–196.0)	108.0 (58.5–191.0)	118.5 (58.3–225.8)	0.550
Increased, *n* (%)	15 (31)	10 (30)	5 (31)	0.946
Creatine kinase-MB (U/L; normal range 3–25)	13.5(9.0–23.2)	12.1 (8.4–27.0)	16.4 (10.1–22.5)	0.354
Increased, *n* (%)	8 (16)	5 (15)	3 (19)	1.000
Lactate dehydrogenase (U/L; normal range 109–245)	315.6 (216.5–449.0)	268.6 (201.6–367.6)	443.4 (279.7–528.5)	0.016
Increased, *n* (%)	37 (76)	23 (70)	14 (88)	0.290
Serum creatinine (μmol/L; normal range 32–106)	69.5(61.0–96.4)	68.9 (57.7–88.0)	86.6 (64.3–112.5)	0.147
Increased, *n* (%)	11 (22)	6 (18)	5 (31)	0.466
Blood urea nitrogen (mmol/L; normal range 1.5–7.5)	5.5 (4.4–7.1)	5.5 (4.4–7.1)	5.4 (4.5–9.7)	0.815
Increased, *n* (%)	11 (22)	7 (21)	4 (25)	1.000
Bilateral involvement of chest CT images, *n* (%)	49 (100)	33 (100)	16 (100)	–

Data are shown as median (IQR) or *n* (%). *p* values comparing survivors and deceased are from χ^2 test, Fisher's exact test, or Mann-Whitney *U* test. *COVID-19*, coronavirus disease 2019; *IQR*, interquartile range

parameters which showed significant differences between the survivors and deceased groups. The result indicated the patients with T2DM had the higher neutrophil percentage, the lower lymphocyte percentage, and the higher levels of FBG and LDH compared with the patients without T2DM; but there were no significant differences between the diabetic and nondiabetic patients concerning age, sex, infection biomarkers, and mortality rate (Table 5).

The independent risk factors for poor prognosis

To investigate the risk factors for poor prognosis in our cohort of 49 critically ill patients with SARS-CoV-2 infection, we performed a multivariable logistic regression analysis by the forward method using clinical manifestations and laboratory parameters which showed significant differences between the survivors and deceased groups. The result indicated that

gastrointestinal symptoms, decreased lymphocytes, and increased fasting blood glucose were the independent risk factors for death of critically ill patients (Table 6).

Discussion

According to the diagnostic and treatment guideline for COVID-19 issued by the Chinese National Health Committee (version 3-6) (http://www.nhc.gov.cn/), the clinical classification of severity of COVID-19 includes four types: mild, common, severe, and critical. In this study, we reported clinical features of 49 critically ill patients with COVID-19 confirmed by clinical and laboratory results, who needed mechanical ventilation therapy in ICU. These patients came from Huanggang City and Xiaogan City, Hubei Province, 80–90 km away from Wuhan, who may be second- or third-generation cases by human-to-human transmission of SARS-CoV-2.

Table 4 Demographics and baseline data of 11 critically ill COVID patients with T2DM

Items	Values
Age, years	58 (51–62)
Sex, *n* (%)	
Female	4 (36)
Male	7 (64)
Duration of T2DM, years	15.0 (11.0–20.0)
HbA1c, %	8.2 (7.6–8.8)
Complications, *n* (%)	11(100)
Diabetic peripheral neuropathy	7 (64)
Diabetic nephropathy	4 (36)
Diabetic retinopathy	4 (36)
Diabetic macroangiopathy	1 (9)
Two or more of the above complications	4 (36)
Treatment regimen, *n* (%)	
Sulfonylureas	2 (18)
Biguanide	1 (9)
Alpha-glucosidase inhibitors	1 (9)
Combination of sulfonylureas and biguanide	1 (9)
Combination of long-acting insulin analogs and biguanide	1 (9)
Combination of alpha-glucosidase inhibitors and biguanide	1 (9)
Combination of alpha-glucosidase inhibitors and glinides	2 (18)
Combination of biguanide and glinides	1 (9)
Combination of DPP-IV inhibitors and glinides	1 (9)

Data are shown as *n* (%) or median (IQR). *COVID-19*, coronavirus disease 2019; *IQR*, interquartile range; *T2DM*, type 2 diabetes mellitus. Sulfonylureas: glimepiride (2 mg qd po) or gliclazide (80 mg bid po); biguanide: metformin (0.5 g bid po); alpha-glucosidase inhibitors: acarbose (50 mg tid po); glinides: repaglinide (1 mg tid po); DPP-IV inhibitors: sitagliptin (100 mg qd po); long-acting insulin analogs: lantus (16 U IH)

In our cohort, 33% (16) of patients were deceased, and the mortality was lower than that of the first-generation cases reported by Huang et al. in Wuhan, who showed that 38% (5 cases) died in 13 ICU patients with SARS-CoV-2 pneumonia. The median time from admission to critical condition was 4 days, which was longer than that (2 days) between hospital admission and ARDS reported by Huang et al. [8]. The deceased patients had shorter median time from admission to critical condition compared with the survivors. These findings suggest that pathogenicity of SARS-CoV-2 seems to decrease with the increase of its generations, and the shorter the time from admission to critical condition, the more serious the illness. Sixty-five percent of the patients were male, and this percentage was also lower than that of ICU patients (85%) reported by Huang et al. [8]. The median age of all patients was 63 years, which was older than those reported by Chen et al. (55.5 years) [7], Huang et al. (49 years) [8], Wang et al. (56 years) [9], Liu et al. (57 years) [11], and Zhang et al. (57 years) [12]. In our study, 73% (36) of patients had comorbid chronic systemic diseases, and hypertension (45%), chronic heart disease (33%), diabetes (22%), and chronic obstructive pulmonary disease (22%) were the most common comorbidities, whose percentages were higher than those of other reports [7–9, 11, 12]. These discrepancies may be due to 100% of critical COVID-19 patients in our series. Importantly, we found that the percentage of two or more of comorbid chronic systemic diseases in the deceased patients was higher than that in the survivors, which was consistent with the report by Guan et al., who have verified the significantly escalated risk of poor prognosis in patients with two or more comorbidities as compared with those who had no or only a single comorbidity [13].

In the present study, the most common symptoms included fever (82%), cough (76%), fatigue (57%), and chest tightness/dyspnea (45%), which was in accordance with the previous reports [10–15]. Moreover, it was noteworthy that the incidence of gastrointestinal symptoms was 47% in our cohort, including nausea or vomiting (31%), diarrhea (22%), anorexia (16%), and abdominal pain (6%), and gastrointestinal symptoms were more common in the deceased patients than in the survivors. Consistent with our result, Zhang et al. showed that gastrointestinal symptoms were observed in 39.6% of the patients, and 42.1% of the severe patients with COVID-19 [12]; Wang et al. found that more than 60% of the patients with COVID-19 had gastrointestinal symptoms, and the gastrointestinal symptoms were more common in the ICU patients than those in the non-ICU patients [9]; of patients with COVID-19 with gastrointestinal symptoms, 30% had severe/critical types, significantly higher than those without gastrointestinal symptoms (8%) [16]. These results suggest that

Table 5 Comparison of outcomes between 11 diabetic and 38 nondiabetic critically ill COVID patients

Items	Diabetic ($n = 11$)	Nondiabetic ($n = 38$)	p value
Age, years	58 (51–62)	68 (53–75)	0.116
Sex, *n* (%)			
Female	4 (36)	13 (34)	1.000
Male	7 (64)	25 (66)	1.000
Blood routine			
Neutrophil percentage, (%) (normal range 40–75)	85.8 (71.5–91.5)	73.8(64.2–85.6)	0.045
Lymphocytes ($\times 10^9$/L; normal range 1.1–3.2)	0.7 (0.5–0.9)	0.9 (0.7–1.4)	0.168
Lymphocyte percentage, (%) (normal range 20–50)	8.5 (3.7–19.7)	16.9 (9.6–26.1)	0.042
Infection biomarkers			
C-reactive protein (mg/L; normal range 0.0–8.0)	27.8 (12.9–40.8)	40.7 (16.6–65.4)	0.297
Procalcitonin (ng/mL; normal range 0.0–0.5)	4.1 (0.2–7.9)	0.5 (0.1–1.6)	0.106
Blood biochemistry			
Fasting blood glucose (mmol/L; normal range 3.9–6.1)	10.7 (7.4–14.5)	6.1 (5.5–8.1)	0.001
Lactate dehydrogenase (U/L; normal range 109–245)	419.0 (326.0–523.8)	268.3 (203.3–431.2)	0.042
Prognosis			
Discharge, *n* (%)	6 (55)	27(71)	0.466
Death, *n* (%)	5 (45)	11 (29)	0.466

Data are shown as median (IQR) or *n* (%). *p* values comparing diabetic and nondiabetic COVID patients are from χ^2 test, Fisher's exact test, or Mann-Whitney *U* test. *COVID-19*, coronavirus disease 2019; *IQR*, interquartile range

gastrointestinal symptoms are a potential indicator for severity of COVID-19.

In our cohort, abnormal blood routine results mainly included decreased lymphocytes and lymphocyte percentage, and increased neutrophil percentage. Noticeably, abnormalities of the above parameters were more prominent in the deceased patients than those in the survivors. Infection biomarkers showed that increased level of CRP and PCT was found in 86% and 55% of the patients, respectively, and the level of CRP and PCT was higher in the deceased patients compared with that in the survivors. These changes of CRP and PCT may represent more prominent inflammation, whereas higher neutrophil percentage and PCT may be due to more significant secondary bacterial infection in the deceased patients. Numerous studies have shown that lymphopenia is common in patients with COVID-19 [7–12, 14]. Huang et al. showed that lymphopenia was more prominent in the ICU patients than that in the non-ICU patients [8]; Zhang et al. reported that lymphocyte percentage was lower in severe patients compared with that in nonsevere patients [12].

Table 6 Multivariable logistic regression analysis

Items	p value	OR	95% CI
Gastrointestinal symptoms (≥ 1 symptoms)	0.009	13.4	1.9–94.8
Lymphocytes ($< 1.1 \times 109$/L)	0.020	25.5	1.6–394
Fasting blood glucose (≥ 7.0 mmol/L)	0.016	11.7	1.6–85.5

Increased neutrophil has been rarely reported in patients with COVID-19. Huang et al. showed that the median neutrophil count was significantly higher in the ICU patients than that in the non-ICU patients [8]; Chen et al. indicated that increased neutrophils were found in 38% of the patients with COVID-19 [7]. Increased level of CRP is often reported in recent studies about COVID-19 [7, 11, 12], whereas increased PCT is rarely observed. Zhang et al. [12] reported CRP and PCT concentrations were significantly higher in severe patients with COVID-19 compared with those in nonsevere patients with COVID-19; Chen et al. found that PCT level > 0.5 ng/mL was one of the independent risk factors associated with fatal outcome in patients with COVID-19 [14]. The above findings were roughly consistent with our results.

Significant abnormal blood biochemistry findings were increased FBG ≥ 7.0 mmol/L, decreased albumin, and increased LDH levels, whereas other liver functions, myocardial zymogram, and renal function indexes were within the normal range in most of the patients. In addition, the level of FBG and LDH was higher in the deceased patients than that in the survivors. Increased LDH level is common in patients with COVID-19 [7–10, 17]. Two reports indicated that LDH level was higher in the ICU patients than that in the non-ICU patients [8, 9]. Few studies have reported hyperglycemia in patients with SARS-CoV-2 infection. Chen et al. showed that increased FBG was found in 52% of the patients with 2019-

nCoV infection [7]; Li et al. reported that hyperglycemia (> 7.1 mmol/L under fasting state) was detected in 56.5% (13/23) of severe cases with COVID-19 and 21.4% (9/42) of mild cases with COVID-19 [18]. The above results suggest that higher FBG and LDH levels are the important laboratory indexes for patients with COVID-19.

Moreover, in this cohort of 49 critically ill patients with COVID-19, we found that the neutrophil percentage and levels of FBG and LDH were higher and the lymphocyte percentage was lower in the patients with T2DM than those in the patients without T2DM, suggesting the more significant secondary bacterial infection and more severe damages of the lymphocytes, islet cells, and heart function in diabetic critically ill COVID patients.

In our cohort of 49 critically ill patients with COVID-19, all patients showed bilateral viral pneumonia in chest CT images, and there were no significant differences in treatment regimens between the deceased patients and the survivors, including antiviral treatment, corticosteroid treatment, antibacterial treatment, antifungal treatment, respiratory support, and traditional Chinese medicine, which were consistent with the other reports [7–12].

In our study, some independent risk factors for fatal outcome were found by using a multivariable logistic regression analysis. Gastrointestinal symptoms, decreased lymphocytes, and increased FBG were the independent predictive factors for death of critically ill patients with COVID-19.

The multi-organ nature of COVID-19 has been demonstrated in a latest autopsy study [19]. The sequence of SARS-CoV-2 receptor-binding domain is similar to SARS-CoV, and angiotensin-converting enzyme 2 (ACE2) is its receptor. ACE2 is highly expressed not only in the lung but also in other organs including the heart, the kidney, and the gastrointestinal tract [20–25]. SARS-CoV-2 may mediate the invasion into gastrointestinal epithelium cells by binding to ACE2 receptor, leading to malabsorption, unbalanced intestinal secretion, and activated enteric nervous system, in turn resulting in gastrointestinal symptoms and electrolyte disturbance [16, 20, 22]. In this way, the patients with gastrointestinal symptoms trend towards the critical type of the disease and a poor prognosis [16].

SARS-CoV-2 might mainly act on lymphocytes, especially T lymphocytes like SARS-CoV. The virus induces a cytokine storm, generates a series of immune responses, and consumes many immune cells that result in the decrease in lymphocytes and cellular immune deficiency [7, 9]. Damage to T lymphocytes might be an important factor leading to exacerbations of the patients [26].

Hyperglycemia caused by respiratory pathogenic virus infection has been reported. SARS-CoV causes acute pancreatic islet injury by binding to ACE2 receptor, resulting in hyperglycemia in patients with SARS-CoV infection [27]; the report by Wang et al. indicated that high FBG is an independent predictor for severity of H1N1 pneumonia [28]. Therefore, it is reasonable to think that SARS-CoV-2 may invade islet cells through ACE2 receptor and causes hyperglycemia. Increased glucose level inhibits T lymphocyte proliferation, which aggravates lymphopenia and cellular immune dysfunction leading to the deterioration of the disease [29, 30]. Indeed, Jin et al. showed that increased glucose level was the independent risk factor for severe/critical COVID-19 in patients with gastrointestinal symptoms [16], which is similar to our result. Thus, high attention should be paid to rescue this process to prevent the further deterioration of COVID-19.

Our study has several limitations. First, the sample size of this study is small. Second, although the risk factors for death of critically ill patients with COVID-19 were identified according to the data on admission, there is still a lack of a predictive model for disease progression. Third, cytokine storm is found in the disease [8]; thus, it would be better if cytokine changes were detected in this study.

Conclusion

In this study, we reported for the first time that critically ill COVID patients with T2DM had more severe damages of the lymphocytes, islet cells, and heart function, and gastrointestinal symptoms, decreased lymphocytes, and increased FBG are the independent risk factors for death of critically ill patients with COVID-19. The early identification of these risk factors is urgently necessary to facilitate appropriate intensive care.

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Authors' contributions Xiaojuan Peng, Yanfang Chen, Liangfei Deng, Qi Liu, and Qing Li contributed equally to this paper. Xiaojuan Peng, Yanfang Chen, and Qing Li helped design the study, analyzed the study data, helped draft the manuscript, made critical revisions of the manuscript, and provided final approval of the version. Liangfei Deng and Qi Liu helped design the study; acquired epidemiological, demographic, clinical, and laboratory data; analyzed the study data; made critical revisions of the manuscript; and provided final approval of the version. Jie Xiong and Ying Shi analyzed the study data, helped draft the manuscript, and provided final approval of the version. Shaohui Tang designed the study, analyzed study data, drafted the manuscript, made critical revisions of the manuscript, and provided final approval of the version.

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Data availability Data sharing will be considered only on a collaborative basis with the principal investigators, after evaluation of the proposed study protocol and statistical analysis plan.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

The effect of liraglutide on nonalcoholic fatty liver disease in type 2 diabetes mellitus

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Abstract

Aims The objective is to investigate the effects of liraglutide on nonalcoholic fatty liver disease in type 2 diabetes mellitus.

Materials and methods Thirty-two patients with T2DM and NAFLD admitted to the Third Affiliated Hospital of Dalian Medical University from December 2014 to December 2016 were selected, including 11 females and 21 males, aged 39.34 ± 8.54 years old. The patients were given liraglutide on the basis of their original hypoglycemic regimen.

Results After 3 months treatment of liraglutide, FPG was reduced from 8.54 ± 2.21 mmol/L to 6.90 ± 1.73 mmol/L. HbA1c was reduced from 9.72 ± 1.95 to 7.78 ± 1.99. WC was reduced from 103.27 ± 9.92 kg to 93.97 ± 8.35 kg. BMI was reduced from 30.56 ± 4.06 kg/m^2 to 28.01 ± 3.12 kg/m^2. FLI was reduced from 79.23 ± 16.56 to 58.83 ± 19.75. The differences were statistically significant ($p < 0.001$). TG was reduced from 2.95 ± 2.13 mmol/L to 2.27 ± 1.31 mmol/L. The difference was significant ($p < 0.01$). Meanwhile, HOMA-IR was reduced from 1.504 ± 0.002 to 1.503 ± 0.002. GGT was reduced from 62.63 ± 71.61 U/L to 38.13 ± 30.13 U/L. AST was reduced from 27.25 ± 13.74 U/L to 25.44 ± 16.69 U/L. The differences were statistically significant ($p < 0.05$). After treatment, FCP, TC, HDL-C, LDL-C, ALT, and HOMA-β were also improved compared with before treatment, but the difference was not statistically significant ($p > 0.05$).

Conclusion In addition to effectively lowering glucose and improving islet resistance, liraglutide could also improve obesity and adjust blood lipids. However, the improvement of islet function might not be significant after 3 months of treatment. Liraglutide could reduce liver fat accumulation in patients with T2DM and NAFLD.

Keywords Liraglutide · Nonalcoholic fatty liver disease · Type 2 diabetes

Introduction

The prevalence of diabetes mellitus (DM), especially type 2 diabetes (T2DM), is increasing markedly worldwide, including in China [1]. In 2013, the overall prevalence of DM in Chinese adult population was 10.4% [2]. Insulin resistance is a metabolic feature of T2DM. Obesity and insulin resistance are key factors in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). The progression of NAFLD is due to the accumulation of triglycerides (TG) in the liver. At the same time, there was evidence that the accumulation of TGs in the liver increased systemic insulin antagonism of cytokine level and also increased insulin resistance in the liver [3]. In addition, metabolic changes accompanying diabetes are also included as a causative factor in nonalcoholic liver disease. Studies have shown that 18 to 33% of patients with impaired glucose tolerance or impaired fasting glucose (> 6 mmol/L) had NAFLD and 62% of patients with T2DM had fatty liver. It can be seen that both T2DM and NAFLD are complications of each other, affect each other, and promote the progress of the disease [4, 5].

Patients with T2DM and NAFLD, compared with those only with T2DM, resulted in more severe hyperlipidemia and high levels of inflammatory markers, as well as more severe insulin resistance and metabolic disorders of visceral

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obesity [6]. The mortality of patients with T2DM and NAFLD was increased compared with the one with T2DM without NAFLD. The most common causes of death were ischemic heart disease and liver-related disease [7]. Patients with NAFLD and diabetes were more likely to develop nonalcoholic steatohepatitis (NASH), compared with patients with NAFLD alone [8]. There was increasing evidence that NAFLD was one of the most common causes of death in diabetic patients with vascular disease. Cross-sectional study showed that NAFLD was positively correlated with carotid intima-media thickness, carotid plaque formation, and cardiovascular and cerebrovascular disease prevalence [9].

The treatment of T2DM with NAFLD focuses on reducing body weight and improving insulin resistance. The common drugs in clinical practice are antioxidants, lipid-lowering drugs, cell protective agents, insulin sensitizers, tumor necrosis factor inhibitors, and intestinal microecological preparations. But it is hard to say which drug works better. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are widely used as novel hypoglycemic drugs. The extra benefits of hypoglycemic agents are slowly discovered, significant weight loss caused by reducing gastrointestinal motility, inhibiting the feeding center and losing appetite [10, 11]. Thus, it has been proposed to have a therapeutic effect on fatty liver. Dutch scholars firstly reported in 2006 that GLP-1RAs had improved liver fat content.

Liraglutide is an analog of glucagon-like peptide-1 (GLP-1), which was approved for marketing in the USA in 2010 and is marketed in China the following year. The hypoglycemic effect of liraglutide is undoubted, and its treatment of NAFLD has also been confirmed by research. Liraglutide reduced intrahepatic fat content, alanine aminotransferase (ALT), and TG in 87 T2DM patients with NAFLD [12]. At the same time, it has been confirmed that liraglutide has the effect of improving liver lipid content and then treating NAFLD in the animal model [13]. However, there are few studies on the efficacy of liraglutide in the treatment of T2DM with NAFLD.

Liver biopsy is the golden standard for quantitative measurement of fat content of NAFLD [14]. But it is an invasive test with significant sampling error. Hydrogen proton magnetic resonance spectrum is a noninvasive golden standard for detecting fatty liver. But it is often as a scientific research tool. Due to its complicated operation and high cost, it is difficult to apply to clinical practice. At present, the common fatty liver tests are liver/spleen CT ratio, liver and kidney ultrasound index, and abdominal ultrasound. However, there are still many factors such as radiation, special operation, inconvenient detection, and high price. Therefore, it is clinically necessary to have an easy and accurate evaluation method. The fatty liver index (FLI) was proposed in 2005 and widely accepted. FLI is an indicator to evaluate fatty liver, based on body mass index (BMI), waist circumference (WC), TG, and glutamyl transpeptidase (GGT). Fatty liver is excluded when FLI is lower than 30. Fatty liver is considered when FLI is greater or equal to 60. Studies have shown that FLI, liver/spleen CT ratio, and liver and kidney ultrasound index have a good correlation with the evaluation of liver fat accumulation in NAFLD. FLI as a noninvasive blood test method is cheap, no radiation, easy to operate, and widely screened [15]. In this study, we observed the effects of liraglutide on liver fat metabolism after observing the changes of FLI and blood lipid of patients of T2DM combined with NAFLD after 3 months treatment, in order to understand the effects of liraglutide on nonalcoholic fatty liver disease in type 2 diabetes mellitus. It may provide evidence for clinically applying liraglutide to treat patients of T2DM with NAFLD.

Materials and methods

Objects

Thirty-three patients with T2DM and NAFLD who were inpatients in the third department of The First Affiliated Hospital of Dalian Medical University from December 2014 to December 2016 were selected. The sample size was estimated based on published data [16].

The inclusion criteria were as follows: (1) aged 28–68 years; (2) BMI $\geq$ 25 kg/m^2 and/or male WC > 90 cm, female WC > 85 cm; (3) complying with the 1999 World Health Organization (WHO) diabetes diagnosis and classification criteria; (4) not adjusting the hypoglycemic regimen and dose of hypoglycemic drugs 2 months before admission; (5) meeting the diagnostic criteria of the 2006 NAFLD diagnosis and treatment guidelines; (6) no history of alcohol consumption or alcohol equivalent to alcohol for men < 140 g and women < 70 g per week.

The exclusion criteria were as follows: (1) type 1 diabetes, including adult late-onset autoimmune diabetes (LADA); (2) secondary diabetes; (3) severe acute complications of diabetes and stress state; (4) impaired liver function, transaminase > 3 times; (5) severe renal insufficiency; (6) TG < 9 mmol/L; (7) oral dipeptidyl peptidase IV (DPP-4) inhibitors or secretagogues before or during the observation period; (8) subcutaneous injection of premixed insulin hypoglycemic before or during the observation period; (9) women who are pregnant or breastfeeding or plan to become pregnant within half a year; (10) presence of infection and malignancy; (11) a history of severe cardiovascular and cerebrovascular disease in the past 3 months; (12) various diseases that significantly affect blood sugar; (13) previous pancreatitis; (14) previous medullary thyroid carcinoma and related family history of disease.

The withdrawal criteria were as follows: (1) applying liver-protective drugs and/or lipid-lowering drugs during enrollment; (2) serious adverse drug reactions occur; (3) allergic reactions to the drug; (4) not tolerating the drug; (5) poor compliance; (6) fasting blood glucose (FBG) > 13.3 mmol/L after half a month of treatment.

Methods

Testing methods and testing indicators

Liraglutide injection (3 mL: 18 mg/piece) is produced by Novo Nordisk, Denmark. It can be injected subcutaneously once a day and can be injected into the upper arm, abdomen, or thigh. It is recommended to take the medicine at the same time every day.

Patients with T2DM and NAFLD who met the enrollment criteria were given normal diet and exercise education, and were treated with liraglutide based on their original hypoglycemic regimen (metformin, glycosidase inhibitor, long-acting insulin). The medical history, age, and sex were recorded as baseline information. Height, weight, and WC were measured to calculate BMI. The patients fasted for 12 h, and the elbow median venous blood was drawn in the fasting state in the early morning the next day. The biochemical instrument was used to determine fasting plasma glucose (FPG), total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), hemoglobin A1c (HbA1c), fasting C peptide (FCP), ALT, aspartate transaminase (AST), and glutamyl transpeptidase (GGT). Insulin resistance index (homeostasis model assessment for insulin resistance, HOMA-IR), islet function (homeostasis model assessment for beta cell, HOMA-β), and FLI were calculated.

Related calculation formulas are as follows:

$$\mathrm{BMI}\left(\mathrm{kg/m^2}\right) = \mathrm{weight(kg)} \div \mathrm{height(m)}^2,$$

$$\mathrm{HOMA\text{-}IR} = 1.5 + \mathrm{FPG(mmol/L)} \times \mathrm{FCP(pmol/L)} \div 2800,$$

$$\mathrm{HOMA\text{-}\beta} = 0.27 \times \mathrm{FCP(pmol/L)} \div [\mathrm{FPG(mmol/L)} - 3.5];$$

$$\mathrm{FLI} = \left(e^{(0.953\times\log e(\mathrm{TG})+0.139\times\mathrm{BMI}+0.718\times\log e(\mathrm{GGT})+0.053\times\mathrm{WC}-15.745)}\right) \div \left(1+e^{(0.953\times\log e(\mathrm{TG})+0.139\times\mathrm{BMI}+0.718\times\log e(\mathrm{GGT})+0.053\times\mathrm{WC}-15.745)}\right) \times 100$$

The TG unit is mg/dL, the GGT unit is U/L, and the WC unit is cm.

Experiment procedure

The baseline data of the enrolled patients were recorded and checked the next day. The patients were injected subcutaneously once daily with a starting dose of 0.6 mg. After 3–7 days of observation, if the patient had no obvious adverse reactions (diarrhea, vomiting, nausea, and other gastrointestinal reactions), the liraglutide dose was adjusted to 1.2 mg/day. If the above adverse drug reactions occurred, the dose was reduced back to 0.6 mg. The patients were increased the dose, until the adverse reactions were completely relieved. Patients were followed up for blood glucose control at the second week and the sixth week, respectively. If FBG was higher than 7 mmol/L, and/or 2-h postprandial blood glucose (2hPBG) was higher than 10 mmol/L, other oral drugs and long-acting insulin were not adjusted. Liraglutide's dose was increased to 1.8 mg. Patients were withdrawn from the trial if FBG was higher than 13.3 mmol/L at the second week. In order to avoid the influence of the drug on the experimental results, no lipid-lowering drugs and/or liver-protective drugs were used and the dose of other hypoglycemic drugs except liraglutide was not adjusted in this study. Patients were followed up for 3 months after BMI, HbA1c, FPG, FCP, AST, ALT, GGT, TC, TG, HDL-C, and LDL-C. HOMA-IR, HOMA-β, and FLI were calculated. We observed whether the patient had obvious adverse drug reactions during the whole process. The patient discontinued the drug if the adverse was serious.

Statistical analyses

All data were analyzed using statistical software SPSS21.0. Data are described using mean ± standard deviation ($\bar{x} \pm SD$). When performing hypothesis tests, the comparison of the indicators before and after the intervention was performed using paired sample *t* tests.

Results

Baseline data and follow-up

A total of 33 subjects were enrolled. One patient withdrew from the trial due to poor glycemic control. A total of 32 subjects were analyzed, including 21 males (65.6%) and 11 females (34.38%). The average age was 39.34 ± 8.54 years.

The average duration of disease was 3.59 ± 2.22 years. Eleven patients had comorbidities (10 patients with hypertension, 5 patients with coronary heart disease, 4 both), and 7 with chronic complications of diabetes. After using liraglutide, all the patients experienced nausea and decreased appetite to varying degrees, but they tolerated and symptoms gradually disappeared after 1to 3 days. There was no allergy at the injection site, and no patients had severe hypoglycemia (Table 1).

Effects of liraglutide on blood glucose, islet function, and insulin resistance

FPG decreased from 8.54 ± 2.21 mmol/L before treatment to 6.90 ± 1.73 mmol/L after treatment. The difference was statistically significant ($p < 0.001$). With FPG < 7 mmol/L as the standard, the standard compliance rate was 62.5%. The HbA1c was 9.72 ± 1.95% before treatment and 7.78 ± 1.99% after treatment. The difference was statistically significant ($p < 0.001$). With HbA1c < 7% as the compliance standard, the compliance rate was 31.3%. HOMA-IR decreased from 1.504 ± 0.002 before treatment to 1.503 ± 0.002 after treatment. The difference was statistically significant ($p < 0.05$). The HOMA-β difference was not statistically significant ($p > 0.05$), but it was improved to a certain extent compared with before treatment (Table 2).

Effect of liraglutide on obesity

WC decreased 9%, from 103.27 ± 9.92 cm before treatment to 93.97 ± 8.35 cm after treatment. BMI decreased 8%, from 30.56 ± 4.06 kg/m^2 before treatment to 28.01 ± 3.12 kg/m^2 after treatment. The differences between the two indexes were statistically significant ($p < 0.001$). Liraglutide can significantly reduce BMI and WC. It has the effect of improving obesity (Table 3).

Effects of liraglutide on fatty liver and blood lipids

TG decreased from 2.95 ± 2.13 mmol/L before treatment to 2.27 ± 1.31 mmol/L after treatment, a decrease of 22%, and the difference was statistically significant ($p < 0.01$). Comparing before and after treatment, LDL-C and TC decreased by 3% and 0.2% respectively, and HDL-C increased

Table 1 Baseline and follow-up data

Index		$\bar{x} \pm s$	(min, max)
Gender (male/female)		21/11	
Age (years)		39.34 ± 8.54	(18, 56)
Course of disease (years)		3.59 ± 2.22	(1, 10)
WC (cm)		103.27 ± 9.92	(91, 124.5)
BMI (kg/m^2)		30.56 ± 4.06	(24.29, 38.10)
FPG (mmol/L)		8.54 ± 2.21	(5.29, 16.80)
FCP (pmol/L)		1.27 ± 0.54	(0.55, 3.30)
TG (mmol/L)		2.95 ± 2.13	(1.04, 9.87)
TC (mmol/L)		4.92 ± 1.07	(3.07, 7.41)
HDL-C (mmol/L)		1.12 ± 0.31	(0.64, 1.85)
LDL-C (mmol/L)		3.01 ± 0.75	(1.58, 4.16)
AST (U/L)		27.25 ± 13.74	(12, 59)
ALT (U/L)		48.09 ± 29.21	(8, 113)
GGT (U/L)		62.63 ± 71.61	(11, 287)
HbA1c (%)		9.72 ± 1.95	(6.4, 13.2)
Comorbidities		11	
Chronic complications		7	
Adverse effects of liraglutide	Gastrointestinal reaction	32	
	Hypoglycemia response	2	
	Allergic reaction	0	
Exit group	Poor glycemic control	1	

Demographic parameters and clinical and treatment history data were collected from medical records. Data are shown as mean ± SD

WC, waist circumference; *BMI*, body mass index = body weight (kg)/height (m)^2 ; *FPG*, fasting plasma glucose; *FCP*, fasting C-peptide; *TG*, total triglycerides; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *ALT*, alanine aminotransferase; *AST*, aspartate transaminase; *GGT*, γ-glutamyl transpeptidase; *HbA1c*, hemoglobin A1c

Table 2 Effects of liraglutide on FPG, HbA1c, islet function, and insulin resistance

Index	Before treatment ($n = 32$)	After treatment ($n = 32$)	Compliance rate	p	95% CI
FPG (mmol/L)	8.54 ± 2.21	6.90 ± 1.73	62.5%	< 0.001	(0.12, 1.66)
HbA1c (%)	9.72 ± 1.95	7.78 ± 1.99	31.3%	< 0.001	(0.86, 2.34)
FCP (pmol/L)	1.27 ± 0.54	1.25 ± 0.74		0.838	(− 0.18, 0.20)
HOMA-IR	1.504 ± 0.002	1.503 ± 0.002		0.008	(0.00, 0.001)
HOMA-β	0.082 ± 0.048	0.134 ± 0.120		0.472	(− 0.06, 0.01)

Clinical history and data from most recent laboratory investigations within the past year were collected from medical records. Data are shown as mean ± SD. Data are presented as odds ratios (95% CI)

FPG, fasting plasma glucose; *HbA1c*, hemoglobin A1c; *FCP*, fasting C-peptide;

HOMA-IR = 1.5 + FPG (mmo/L) × FCP (pmol/L) ÷ 2800;

HOMA-β = 0.27 × FCP (pmol/L) ÷ [FPG (mmo/L) − 3.5];

by 5%. They had no statistical significance ($p > 0.05$) (Table 4).

GGT decreased from 62.63 ± 71.61 U/L before treatment to 38.13 ± 30.13 U/L after treatment, a decrease of 39%. AST decreased from 27.25 ± 13.74 U/L before treatment to 25.44 ± 16.69 U/L after treatment, a decrease of 7%. The differences were statistically significant ($p < 0.05$). Although ALT decreased by 9% compared with that before treatment, the difference was not statistically significant ($p > 0.05$) (Table 5). Analysis of FLI showed a decrease of 26% from 79.23 ± 16.56 before treatment to 58.83 ± 19.75 after treatment. The effect of liraglutide on FLI was statistically significant ($p < 0.001$). Liraglutide can significantly reduce FLI (Table 5).

Discussion

Epidemiology shows that the number of people with diabetes is increasing day by day. The detection rate of NAFLD is increasing with the wide application of ultrasound. In a study in the USA, the prevalence of NAFLD in T2DM population was as high as 70 to 80%, compared with 10 to 24% in the general population [17]. Therefore, there is an urgent need for a drug that simultaneously lowers blood sugar and blood lipids, and has a therapeutic effect on NAFLD. Liraglutide is widely used as a new type of hypoglycemic drug, and its benefits other than hypoglycemic are slowly being explored. Its role in the treatment of fatty liver has been widely concerned.

FPG is the basic sugar, and good FPG level is more conducive to smoothly control of blood sugar. This trial showed that FPG decreased from 8.54 ± 2.21 mmol/L to 6.90 ± 1.73 mmol/L (a decrease of 19%) after 3 months of treatment with liraglutide. The result was consistent with the results of LEAD confirming its hypoglycemic effect [18, 19]. GLP-1 is a kind of incretin, and its blood glucose effect after meal reduction is more significant. Its effect mainly manifests in the stimulation of islet β-cell secretion of insulin after oral administration of glucose, and it has the effect of delaying gastric emptying. Since this study mainly observed the effect of liraglutide on NAFLD, postprandial blood glucose changes were not detected.

HbA1c responds to blood glucose levels for nearly 2–3 months. Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (2017 Edition) presented HbA1c should remain < 7% for adult nongestational age T2DM patients of expected long-term survival, short history, and no associated complications [20]. This test showed that the pretreatment HbA1c was 9.72 ± 1.95% and HbA1c was 7.78 ± 1.73% after 3 months of treatment with liraglutide, a decrease of 20%, consistent with the results of the LEAD series of tests. The compliance rate of HbA1c in this trial was 31.3%, which was considered to be related to the higher level of HbA1c before application of liraglutide and the shorter follow-up time. HbA1c is clinically used to guide and evaluate hypoglycemic therapy. Recently, clinical studies have shown that HbA1c is an important risk factor for NAFLD, may alert the occurrence of NAFLD. Bae et al. observed 7849 participants of NAFLD and the metabolic syndrome for 4 years. Four hundred thirty-five (5.5%) participants developed diabetes.

Table 3 Effects of liraglutide on obesity

Index	Before treatment ($n = 32$)	After treatment ($n = 32$)	p	95% CI
WC (cm)	103.27 ± 9.92	93.97 ± 8.35	< 0.001	(7.61, 11.05)
BMI (kg/m^2)	30.56 ± 4.06	28.01 ± 3.12	< 0.001	(1.99, 3.10)

History data were collected from medical records. Data are shown as mean ± SD. Data are presented as odds ratios (95% CI)

WC, waist circumference; *BMI*, body mass index = body weight (kg)/height (m)^2

Table 4 Effects of liraglutide on blood lipids

Index	Before treatment ($n = 32$)	After treatment ($n = 32$)	p	95% CI
TG (mmol/L)	2.95 ± 2.13	2.27 ± 1.31	0.010	(0.06,1.05)
TC (mmol/L)	4.92 ± 1.07	4.91 ± 1.00	0.958	(− 0.42,0.47)
HDL-C (mmol/L)	1.11 ± 0.31	1.17 ± 0.28	0.481	(− 0.21,0.10)
LDL-C (mmol/L)	3.01 ± 0.75	2.91 ± 0.79	0.465	(− 0.15,0.34)

Clinical history and data from most recent laboratory investigations within the past year were collected from medical records. Data are shown as mean ± SD. Data are presented as odds ratios (95% CI)

TG, total triglycerides; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol

NAFLD had an additive effect on the development of diabetes in patients with MetS [21].

Insulin resistance can promote the development of NAFLD [22]. The first hit of the classic "second strike theory" has been confirmed to be related to insulin resistance, which shows that insulin resistance is closely related to the onset of NAFLD. In the LEAD 2, 3, and 4 studies, after 24, 52, and 26 weeks of treatment with liraglutide, the results showed that liraglutide could significantly increase islet β-cell function and improve insulin resistance compared with glimepiride and improve islet function compared with gliclazide [18, 23, 24]. This test uses the C-peptide fitting HOMA-IR formula, which has been shown to be useful for determining insulin resistance in an individual. And the HOMA-IR index is positively correlated with the degree of insulin resistance [25]. Before and after treatment, HOMA-IR decreased from 1.504 ± 0.002 to 1.503 ± 0.002. The difference was statistically significant ($p < 0.05$). HOMA-β difference was not statistically significant ($p > 0.05$), but there is a certain degree of improvement compared with before treatment. Studies have shown that human islet beta cells produce new cells at a limited rate, requiring 6 to 12 months of treatment to alter islet beta cell architecture [26]. However, the treatment time of this experiment was only 3 months and only 8 patients in this experiment used a dose of liraglutide to 1.8 mg, which may cause inconsistency with the LEAD test results.

Studies have suggested that obese and the age of diabetic patients are risk factors for advanced liver fibrosis in NAFLD. And obesity is a common risk factor for T2DM and NAFLD, especially central obesity [27, 28]. WC and BMI can initially reflect central obesity. Central obesity increases the risk of cardiovascular and metabolic diseases, can aggravate insulin resistance and impair beta cell function, and form a vicious circle with T2DM and NAFLD. At present insulin, sulfonylureas, and thiazolidinediones in the treatment of T2DM hypoglycemic drugs can cause weight gain. Glycosidase inhibitors and DPP-4 inhibitors have no significant effect on body weight. Although metformin has a weight-reducing effect, it has limited effect and is related to the gastrointestinal reaction in the initial stage of medication. The GLP-1RA can directly act on the hypothalamus to suppress appetite, and also acts on the autonomic nervous system to delay gastric emptying, and finally achieve the effect of reducing body weight. At the same time, it has the effect of regulating lipid distribution and improving central obesity [29]. The WC of patients in this trial decreased from pre-treatment (103.27 ± 9.92 cm) to post-treatment (93.97 ± 8.35 cm). BMI decreased from pre-treatment 30.56 ± 4.06 kg/m^2 to 28.01 ± 3.12 kg/m^2 after treatment. The differences between the two groups were

Table 5 Effects of liraglutide on liver enzymes and FLI

Index	Before treatment ($n = 32$)	After treatment ($n = 32$)	p	95% CI
FLI	78.40 ± 16.96	57.96 ± 20.08	< 0.001	(15.33, 25.55)
GGT (U/L)	62.63 ± 71.61	38.13 ± 30.13	0.015	(2.79, 45.14)
ALT (U/L)	48.09 ± 29.21	43.63 ± 29.87	0.138	(− 1.64, 11.21)
AST (U/L)	27.25 ± 13.74	25.44 ± 16.69	0.009	(− 1.08, 5.69)

Clinical history and data from most recent laboratory investigations within the past year were collected from medical records. Data are shown as mean ± SD. Data are presented as odds ratios (95% CI)

FLI, fatty liver index; *GGT*, γ-glutamyl transpeptidase; *ALT*, alanine aminotransferase; *AST*, aspartate transaminase

$FLI = (e(0.953 \times \log e(TG) + 0.139 \times BMI + 0.718 \times \log e(GGT) + 0.053 \times WC - 15.745)) \div (1 + e(0.953 \times \log e(TG) + 0.139 \times BMI + 0.718 \times \log e(GGT) + 0.053 \times WC - 15.745)) \times 100$

The TG unit is mg/dL, the GGT unit is U/L, and the WC unit is cm

statistically significant ($p < 0.001$), confirming that liraglutide can improve the WC and BMI of patients, which was consistent with many studies. LEAD 1 suggests that liraglutide can reduce body fat content without reducing muscle mass. In the LEAD 1 to 5 series of studies, increasing the dose of liraglutide to 1.8 mg significantly reduced body weight and the reduction in body weight was positively correlated with dose size and also positively correlated with treatment duration. Liraglutide not only reduces subcutaneous fat but also reduces visceral fat content. It was confirmed in the LEAD 2 and 3 studies using CT analysis of body composition before and after treatment [18, 23, 24, 30, 31].

During the trial, no liver-protecting drugs were used in the patients. The data of ALT, GGT, and AST before and after treatment showed that GGT and AST decreased 39% and 7%. Although the difference between GGT and AST was statistically significant ($p < 0.05$), only a few of them showed abnormalities, so the difference was not clinically significant. ALT decreased by 9% after treatment compared with before treatment. The difference was not statistically significant ($p > 0.05$). At present, there is still a lack of research on the treatment of nonalcoholic steatohepatitis with liraglutide and relevant research is needed. During the trial, no lipid-regulating drugs were used in the patients. The statistical analysis before and after the application of liraglutide showed that TG had statistically significant difference. There were no significant differences in HDL-C, TC, and LDL-C, while the levels after treatment were improved compared with the pre-treatment levels. However, liraglutide reduced TC, LDL-C, TG, HDL-C, and FFAs levels in LEAD 4. This might be related to the application of liraglutide in this test being small (0.6 to 1.8 mg), the application time being short (only 3 months), and the test sample size being small (only 32 cases).

This test uses the FLI to measure the degree of fat accumulation before and after liver treatment. FLI is an indicator based on BMI, WC, TC, and GGT, used to assess liver fat accumulation. When FLI is lower than 30, fatty liver is excluded. While FLI is more than or equal to 60, fatty liver is considered. Studies support the predictive validity of FLI [32, 33]. At present, FLI has not been tested with liver biopsy. FLI, liver/spleen CT ratio and liver and kidney ultrasound index have a good correlation with the evaluation of NAFLD liver fat accumulation. At the same time, FLI assessed liver fat accumulation with quantitative indicators in several studies. Analysis of FLI before and after treatment showed that FLI decreased 26%, from pre-treatment (79.23 ± 16.56) to post-treatment (58.83 ± 19.75). And the difference of liraglutide on FLI was statistically significant ($p < 0.01$), showing that liraglutide can significantly reduce liver fat accumulation. And the difference of liraglutide on FLI was statistically significant ($p < 0.01$), showing that liraglutide can significantly reduce liver fat accumulation.

The mechanism by which liraglutide improves liver fat accumulation has not been elucidated. The possible mechanism is to play a role by improving insulin resistance and regulating liver lipid metabolism. Fibroblast growth factor 21 (FGF-21) is a regulator of insulin regulating sugar and lipid metabolism. It is produced mainly by the liver and to a lesser extent by adipose tissue. The study found that FGF-21 concentration was significantly increased in the plasma of NAFLD patients. At the same time, some studies have shown that the concentration of FGF-21 in T2DM combined with NAFLD plasma is significantly higher than that in the healthy group and the fatty liver group alone [34–36]. The above research suggests that the increase of FGF-21 level may be a compensation mechanism to improve insulin resistance and impaired insulin function. Studies have shown that the plasma FGF-21 concentration level in the GLP-1Ra intervention group is significantly higher than that in the placebo control group, suggesting that it can participate in regulating liver lipid metabolism by regulating FGF-12 levels. At the same time, studies have shown that cAMP-responsive element-binding protein H (CREBH) and peroxisome proliferator activated receptor α (PPARα) in the liver mutual coordination plays an important role in regulating lipid metabolism [37, 38]. The CREBH-PPARα-FGF21 axis is an indispensable part of the liver involved in mediating lipid and sugar metabolism. Some research results show that the mRNA levels of CREBH and PPARα transcripts and related proteins in the liver of diabetic rats are significantly reduced. However, the use of GLP-1Ra significantly increased the expression of CREBH and PPARα.

Hepatic lipid balance is maintained through the β-oxidation of fatty acids after entering the liver, the production of fat from scratch, and secretion of very low-density lipoprotein (VLDL) [39]. Defects on either side can cause liver lipid accumulation. Lipid breakdown causes the concentration of free fatty acids in the blood to rise, leading to impaired insulin signaling, reducing the metabolic clearance of glucose, and increasing the glucose content. Excessive sugar accumulation further increases insulin secretion and eventually unbalances the accumulation and breakdown of lipids. Insulin resistance outside the liver triggers the mobilization of peripheral fats, inhibits the use of free fatty acids, increases the concentration of triglycerides after esterification, and decreases the concentration of TGs secreted by the liver, resulting in liver cells fat accumulation inside. It can be seen that blood glucose, lipid metabolism disorders, and insulin resistance all directly or indirectly promote the occurrence and development of NAFLD.

In this study, we found that liraglutide can well control blood glucose, lipids, and body weight, improve insulin resistance, and combined with in vitro and in vivo, research data show that GLP-1 can directly affect liver cell lipid metabolism by binding to GLP-1 receptors. In summary, liraglutide has the

potential to be used as a new drug to treat NAFLD. Nevertheless, there are still several limitations of our study. Firstly, the number of patients included in the study was small, so the results obtained, to some extent, were not representative. Maybe we should expand the sample size as much as possible in the future studies, in order to get more convincing results. Secondly, due to the cross-sectional, retrospective design of the study, it was not possible to draw conclusions on the cause-and-effect relationship between risk factors and various diabetes-related complications.

Conclusion

In addition to effectively lowering glucose and improving islet resistance, liraglutide could also improve obesity and adjust blood lipids. However, the improvement of islet function might not be significant after 3 months of treatment. Liraglutide could reduce liver fat accumulation in patients with T2DM and NAFLD.

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Compliance with ethical standards All procedures of the study were approved by the Ethics Committee of The First Affiliated Hospital of Dalian Medical University (YJ-KY-SB-2019-86). All procedures of the study were approved by The First Affiliated Hospital of Dalian Medical University (YJ-KY-SB-2019-86).

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

Abbreviations DM, diabetes mellitus; T2DM, type 2 diabetes; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; GLP-1, glucagon-like peptide 1; FLI, fatty liver index; BMI, body mass index; WC, waist circumference; WHO, World Health Organization; LADA, adult late-onset autoimmune diabetes; FPG, fasting plasma glucose; FBG, fasting blood glucose; 2hPBG, 2-h postprandial blood glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FCP, fasting C-peptide; ALT, alanine transaminase; AST, aspartate transaminase; GGT, glutamyl transpeptidase; DPP-4, dipeptidyl peptidase IV; LEAD, liraglutide effect and action diabetes; FGF-21, fibroblast growth factor 21; IHL, intrahepatic lipids; CREBH, cAMP-responsive element-binding protein H; PPARα, peroxisome proliferator activated receptor α; VLDL, very low-density lipoprotein

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ORIGINAL ARTICLE

Effect of liraglutide on epicardial adipose tissue thickness with echocardiography in patients with obese type 2 diabetes mellitus

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Abstract

Background Liraglutide, an analogue of glucagon-like peptide 1 (GLP-1), indicated for the treatment of type 2 diabetes mellitus (T2DM), has shown the effect on loss of weight and adipose deposits. The study was to evaluate the effect of liraglutide on epicardial adipose tissue (EAT) with echocardiography in patients with obese T2DM controlled on liraglutide monotherapy.

Methods A single-arm follow-up research was performed in our study. Thirty-six subjects with obese T2DM were enrolled in the study, who were administered with liraglutide monotheraphy daily. The study period was 3 months. EAT thickness was measured with echocardiography both before and after treatment in each subject; meanwhile, anthropometrics and biochemicals (fasting blood glucose and hemoglobinA1c) were also conducted.

Results EAT thickness decreased significantly after three-month treatment, from 9.14 ± 2.39 to 6.42 ± 1.48 mm ($p < 0.05$). Anthropometrics including body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), and some biochemicals including fasting blood glucose (FBG), hemoglobinA1c (HbA1c), C-peptide, insulin, HOMA-IR, AST, ALT, and urinary albumin significantly improved (totally $p < 0.05$). Interestingly, the variation of EAT thickness had positive correlation with the variations of WC, HC, and BMI.

Conclusions Liraglutide is beneficial to the decrease of EAT. EAT thickness can be measured with echocardiography to evaluate the effect of liraglutide in obese T2DM patients.

Keywords Obese type 2 diabetes mellitus · Liraglutide · Epicardial adipose tissue · Echocardiography

Introduction

According to World Health Organization, obesity is associated with diabetes by 44%. In recent years, the morbidity of diabetes is gradually increasing, especially obese type 2 diabetes mellitus (T2DM). It is worth highlighting that visceral fat accumulation is a noticeable risk factor for cardio-metabolic and coronary artery events [1]. Recently, the close affiliation between anatomy and function of ectopic visceral fat distribution has been paid more and more attention. Epicardial adipose tissue (EAT) is an organ special visceral fat deposit, surrounding the myocardium and coronary vessels without fibrous fascial tissue [2]. EAT thickness in groups with T2DM and obesity is thicker than that of normal population [3]. Thicken EAT secretes varieties of cytokines including inflammatory factors, adipokines, and reactive oxidative species which have underlying adverse effects on the proximate myocardium and coronary arteries [4]. Several studies have affirmed that EAT thickness is inseparably related with diabetes, cardio-metabolic diseases [5, 6]. Clinically, EAT has been evaluated with computed tomography (CT) and magnetic resonance imaging (MRI), which could accurately quantify EAT [7, 8]. CT and MRI are superior for measuring overall EAT volume. Echocardiography technique, firstly proposed and validated by Iacobellis et al. [9], could be used as a new way to measure EAT deposit.

Glucagon-like peptide 1 (GLP-1) is a polypeptide hormone secreted by intestinal-type L cells. GLP-1, secreted by the

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body itself or injected subcutaneously, is not suitable for clinical application due to its too short half-life (just few minutes) and rapid deactivation. Liraglutide, glucagon-like peptide 1 receptor agnoists (GLP-1RA), which can perfectly improve hyperglycemia through increasing insulin secretion by pancreatic β cells, suppressing glucagon production and liver glycogen output, promoting transdifferentiation from pancreatic α to β cells and other ways [10–12], is clinically used for the treatment of T2DM. Several studies [13, 14] have demonstrated that liraglutide not only has glucose-lowering effect but also decreases the body weight and adipose accumulation. In addition, it has been demonstrated that liraglutide could provide cardiovascular protective effect [15], though it is not definitely clear whether the effect was attributed to decrease of visceral fat.

So far, there are several preliminary studies which have demonstrated that GLP-1 RA could significantly reduce EAT deposit in patients with T2DM [16, 17]. In our study, the aim was to further clarify the effect of liraglutide on EAT based on echocardiography evaluation, and analyse the correlation between the variation of EAT thickness and the variation of anthropometric parameters.

Materials and methods

Study subjects

This was a 3-month single-arm follow-up study. Thirty-six subjects (28~63 years of age, mean age 46.2 ± 10.4 years), diagnosed with obese T2DM by Department of Endocrinology, The Second Affiliated Hospital of Dalian Medical University, according to American Diabetes Association (ADA), between March 2016 and May 2018, were recruited for the study.

All enrolled subjects must meet the following inclusion criteria: obese T2DM; hemoglobinA1c (HbA1c) levels > 6.5%; body mass index (BMI) levels 25.2~37.5 kg/m^2; waist circumference (WC) levels >80 cm (female) or > 90 cm (male), without serious chronic diseases. Exclusion criteria were the following: severe allergies or gastrointestinal reactions for the use of liraglutide; women who were pregnant or breastfeeding; subjects with heart, hepatic, renal, and other important organic injuries or lesions; some endocrine diseases, such as Graves' disease, Cushing syndrome; T2DM with some emergencies (such as diabetic ketoacidosis), or severe benign and malignant tumors.

Study design

All subjects followed rational dietary and exercise administration during the study period. Subjects were treated with liraglutide (Novo Nordisk, Denmark, specification: 3 ml/branch) monotherapy. Liraglutide was administered with initial dosage of 0.6 mg through subcutaneous injection once daily, and added to 1.2 mg once daily after 1 week if subjects could tolerate the medication, without performing serious adverse reactions (allergies, severe nausea, and vomiting). If blood glucose levels in some subjects were poorly controlled at the dosage of 1.2 mg once daily, we would consider subsequent increments to 1.8 mg once daily. Subjects who were intolerant of the dosage of 1.8 mg were suggested to drop up to 1.2 mg. Each subject underwent a transthoracic echocardiography, examinations of anthropometric, and biochemical parameters at baseline. The above procedures were repeated after continuous 3-month treatment.

Methodologies

Anthropometrics

Height (in centimeter) and weight (in kilogram) with as little clothing as possible, without shoes and hats were measured. WC (in centimeter) was measured at the level of midpoint between the lowest margin of rib arch and the superior margin of the iliac crest, midpoint between the xiphoid process and the umbilicus at the end of quite exhalation. Hip circumference (HC, in centimeter) was measured at the level of pubic symphysis and the widest part of the hip. BMI was equal to weight in kilograms divided by the square of height in meters (kg/m^2). All the above parameters were measured after 12-h fasting, and each measurement was taken three times and the average was obtained.

Biochemical characteristics

Median elbow venous blood samples for biochemicals were drawn after 12-h fasting. Fasting blood glucose (FBG), HbA1c, C-peptide, insulin, urinary albumin, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), spartate aminotransferases (AST), alanine aminotransferases (ALT), were undertaken using an automatic biochemical analyzer. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated through the following equation:

HOMA-IR = fasting blood glucose (mg/dL) × fasting insulin (mU/mL)/22.5.

Echocardiographic EAT thickness

Echocardiographic examinations were carried out with GE Vivid E9 (GE Health Care, American, Vingmed Ultrasound AS) ultrasound apparatus equipped with a M5S probe (the frequency of 1.7~3.4 MHz). An experienced echocardiographer who was blinded to participants' clinical information performed echocardiographic scanning for all subjects. During acquisition, each subject was required in the left lateral

decubitus position, and an electrocardiogram was simultaneously recorded. The EAT thickness was measured in the parasternal left ventricle long-axis section, utilizing the method presented and confirmed by Iacobellis et al. [9]. EAT was identified as hypoechoic or hyperoechoic space between the visceral layer of pericardium and the outer wall of the myocardium. Maximum EAT thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus, at the end systole according to electrocardiogram. The average value of EAT thickness in three cardiac cycles was obtained. Intraobserver reproducibility of echocardiographic measurement of EAT was assessed by the intraclass correlation coefficient (ICC) [18]. The significant EAT thickness reduction could be visually appreciated in Fig. 1.

Statistical analysis

The data was analyzed with SPSS 17.0 software (SPSS, Chicago, IL, USA). Continuous variables were expressed as the mean ± standard ($\bar{x} \pm s$) (normal distribution) or median with quartile range (M (Q)) (skewed distribution), respectively, matched t test or Wilcoxon signed-rank test was used to evaluate the difference of the variables both before and after treatment. Pearson correlation was presented to analyse the correlation between the variation of EAT thickness (Δ_1) and the variation of anthropometric parameters (Δ_2). $p < 0.05$ was set for statistical significance.

Results

During the 3-month period, subjects well tolerated the medication and had neither serious allergies nor gastrointestinal adverse reactions. None dropped out of the study.

Biochemical characteristics

Table 1 summarizes the biochemical characteristics among the patients before and 3-month treatment. FBG obviously varied from 11.43 ± 2.46 to 8.54 ± 2.36 mmol/L ($p < 0.05$), as well as Hb1Ac, C-peptide, insulin, and HOMA-IR (all $p < 0.05$). Lipid profile variations were not evident, apart from a decrease in TC levels (from 5.10 ± 1.04 to 4.43 ± 0.60 mmol/L, $p = 0.030$). Also, there was significant improvement in the AST, ALT, and urinary albumin ($p < 0.05$ for all).

Adiposity markers

The main data in regard to anthropometric and EAT thickness parameters, before and after 3-month treatment, respectively, are summarized in Table 2. As shown, body weight, WC, HC, and BMI performed highly significant decreases ($p < 0.05$ for all). EAT thickness decreased significantly from 9.14 ± 2.39 to 6.42 ± 1.48 mm ($p = 0.001$). ICC of EAT thickness measurement was 0.87 indicating good reproducibility and small variability. Figures 2, 3, and 4, respectively, showed that there was positive correlation between Δ_1 and Δ_2-WC, Δ_2-HC, Δ_2-BMI ($r = 0.878$, $r = 0.899$, $r = 0.846$, totally $p < 0.05$).

Discussion

On the basis of adipose distributional position, global adipose tissue is categorized as both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). Visceral obesity is the main manifestation of obese T2DM [19]. VAT accumulation was identified as an important predictor of metabolic syndrome (MetS) [20] and is more closely associated with MetS risk than SAT due to its greater lipolytic activity and higher inflammatory profile [21]. EAT, like the abdominal VAT, originated from the splanchnopleuric mesoderm of embryo [22]. EAT has been recognized as the new symbol of VAT adiposity [23, 24]. Recently, EAT has been reported to be associated with fatal and nonfatal coronary arterial events beyond the conventional cardiovascular risk factors [25, 26]. Moreover, EAT has been affirmed to be a modifiable predictor of MetS. Studies performed by Drapkina et al. [27] and Kim et al. [28] have confirmed that EAT could be regarded as a new marker of risk for MetS and cardiovascular diseases. EAT

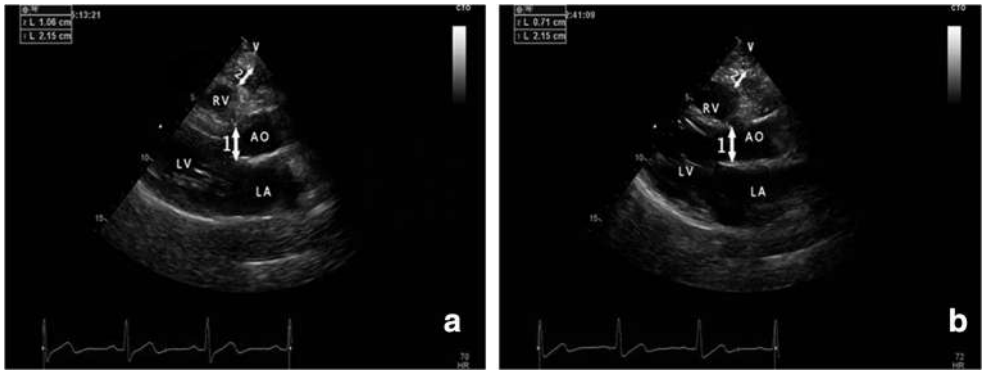

Fig. 1 The typical image shows EAT thickness in the parasternal left ventricle long-axis section before (Fig. 1**a** 10.60 mm) and after 3-month (Fig. 1**b** 7.10 mm) treatment in the same patient. EAT, epicardial adipose tissue; AO, aorta; LA, left atrium; LV, left ventricle; RV, right ventricle White arrow 1 stands for aortic annulus White arrow 2 stands for EAT

Table 1 Biochemical variation in subjects at baseline and after three-month treatment

Parameter	Baseline ($n = 36$)	After 3-month treatment ($n = 36$)	p
FBG (mmol/L)	11.43 ± 2.46	8.54 ± 2.36	0.000
Hb1Ac (%)	9.96 ± 2.84	7.48 ± 1.58	0.003
C-peptide (ng/ml)	1.77 ± 0.91	2.71 ± 0.94	0.020
Insulin (mU/L)	14.12 (10.21)	12.20 (8.14)	0.043
HOMA-IR	5.74 ± 1.79	3.89 ± 1.58	0.025
TC (mmol/L)	5.10 ± 1.04	4.43 ± 0.60	0.030
TG (mmol/L)	2.41 ± 2.23	1.84 ± 0.79	0.372
HDL-C (mmol/L)	1.20 ± 0.38	1.27 ± 0.30	0.354
LDL-C (mmol/L)	2.57 ± 1.06	2.44 ± 0.69	0.612
AST (U/L)	23.62 (18.87)	19.20 (15.12)	0.008
ALT (U/L)	31.62 (21.87)	20.02 (18.87)	0.006
Urinary albumin (mg/L)	37.12 (23.24)	41.00 (10.00)	0.021

Continuous variables are presented as ($\bar{x} \pm s$) (normal distribution) or (M (Q)) (skewed distribution). *FBG*, fasting blood glucose; *Hb1Ac*, hemoglobinA1c; *TC*, total cholesterol; *TG*, triglyceride; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *AST*, aspartate aminotransferases; *ALT*, alanine aminotransferases

thickness is known to be higher in patients with insulin resistance and impaired fasting glucose. Proinflammatory and adipose cytokines secreted by thicken EAT, such as resistin, interleukin-6, adiponectin, and tumor necrosis factor-α, can directly damage myocardial and coronary function [29, 30]. Therefore, it is conceivable that the shrinkage of EAT is important for subjects with obese T2DM to prevent cardiovascular diseases.

The effect of liraglutide on body weight manifests as restraint in food intake through the mediation of central nervous system [31], and delayed gastric emptying by inhibiting gastrointestinal peristalsis and gastric juice secretion [32]. Furthermore, gastrointestinal reaction (such as anorexia, nausea, and vomiting), one of the adverse effects of liraglutide, could also contribute to the weight loss. Recent studies have shown liraglutide attained an independent and marked role in reducing visceral fat (especially EAT), though the mechanisms are not definitely clear. GLP-1 receptors distribute in the adipose tissue, and corresponding mRNA and protein expressions are increased with obesity and insulin resistance [33]. It is helpful to turn white fat tissue into brown fat tissue and stimulate brown fat tissue thermogenesis induced by liraglutide [34]. There is a hypothesis that implies decrease of adipose depot caused by liraglutide is possible to be correlated with modifications in cardiac natriuretic peptides amounts in obese T2DM [35]. Iacobellis et al. [17] and Morano et al. [16] have demonstrated that liraglutide caused distinct effect of reducing EAT depot independently of the weight loss. Our result of EAT was in agreement with previous observations; consequently, it further illustrated liraglutide has a favorable, at least partially, effect on EAT and provided potential approval of cardio-protective function of liraglutide.

EAT, as a modifiable risk factor, can be tracked with imaging techniques. As the gold standard, CT and MRI have been applied to quantify EAT thickness and volume. However, they are not suitable for long-time follow-up studies because of long-time consuming, high cost, and radiation hazards. Recently, ultrasonography has become a valuable and reliable technique to investigate changes of EAT fat deposits over time, based on low-cost, easy performance, noninvasive, and well-repeatable advantages, which was used in clinical practice [36]. Recent evidence has been known that

Table 2 Anthropometric and EAT thickness variation in subjects at baseline and after 3-month treatment

Parameter	Baseline ($n = 36$)	After 3-month treatment ($n = 36$)	p
Body weight (kg)	87.81 ± 17.94	81.98 ± 16.96	0.000
WC (cm)	97.51 ± 6.79	91.58 ± 7.19	0.001
HC (cm)	104.24 ± 8.39	97.98 ± 7.09	0.001
BMI (kg/m^2)	30.70 ± 5.23	28.72 ± 4.95	0.002
EAT thickness (mm)	9.14 ± 2.39	6.42 ± 1.48	0.001

Continuous variables are presented as ($\bar{x} \pm s$) (normal distribution). *WC*, waist circumference; *HC*, hip circumference; *BMI*, body mass index

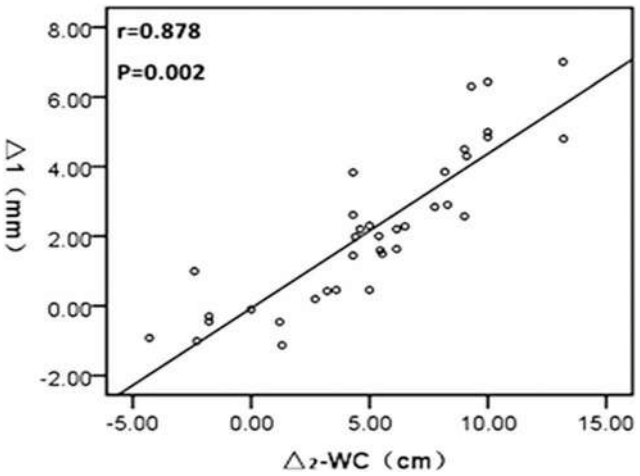

Fig. 2 Correlation between Δ_1 and Δ_2-WC as seen after 3-month treatment Δ_1: the variation of EAT thickness Δ_2-WC: the variation of waist circumference

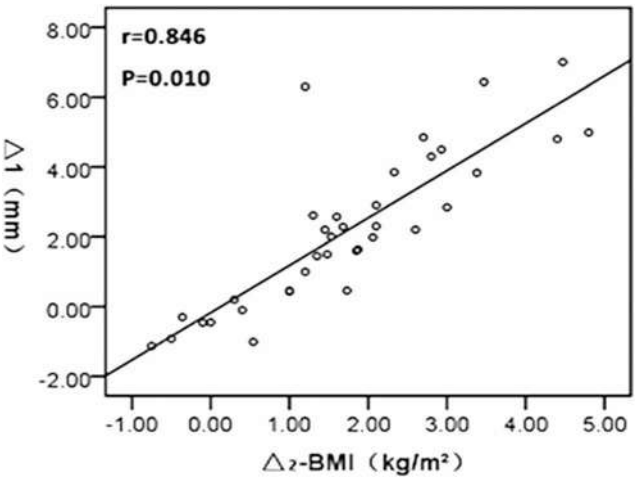

Fig. 4 Correlation between Δ_1 and Δ_2-BMI as seen after three-month treatment Δ_1: the variation of EAT thickness Δ_2-BMI: the variation of body mass index

ultrasonographic VAT can reflect visceral adiposity in common with CT and MRI. Koda et al. [37] indicated ultrasonographic visceral fat index was significantly correlated with visceral fat volume by MRI, and they thought visceral fat could be easily measured with ultrasonography, instead of CT or MRI. Study from Iacobellis et al. [9] indicated that EAT thickness measured by echocardiography had strong correlation with VAT areas measured by MRI, and they finally came up with the idea that transthoracic echocardiography could serve as a dependable and easy imaging tool for VAT prediction. Hence, in our study, EAT thickness obtained by echocardiography was relatively reliable. Our results of Pearson correlation analysis revealed that the variation of EAT thickness has significantly positive correlation with the variations of WC, HC, and BMI, before and after a 3-month treatment. The result illustrated that the effect of liraglutide on EAT was reasonably obvious, and the variation of EAT thickness was capable of reflecting general adipose variation. EAT thickness may become a therapeutic target for medications improving the adipose accumulation to prevent cardiovascular and metabolic diseases.

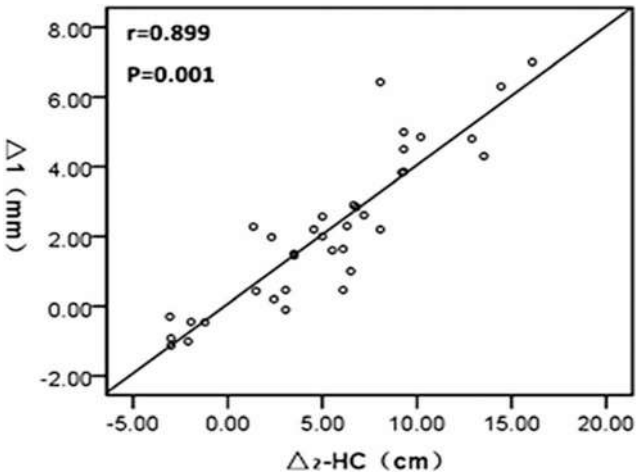

Fig. 3 Correlation between Δ_1 and Δ_2-HC as seen after three-month treatment Δ_1: the variation of EAT thickness Δ_2-HC: the variation of hip circumference

Limitations of the study

Although our research offers novel findings, it is necessary to admit some limitations. Firstly, the sample size is relatively small, statistical power may be reduced because of the subjects' heterogeneity. Secondly, this is only a single-arm follow-up study without control groups. Thirdly, in fact, all enrolled subjects received treatment with 1.2 mg liraglutide once daily in our study, there are two reasons: (1) The blood glucose levels in some subjects have been effectively controlled at the dosage of 1.2 mg; (2) others who were intolerant of the dosage of 1.8 mg had to drop up to 1.2 mg. It requires further study whether liraglutide at the dosage of 1.8 mg once daily has the same or more effective role on EAT. Fourthly, the follow-up period is relatively short. We should extend the study time to explore the long-term effect of liraglutide on EAT. Moreover, EAT thickness measured by echocardiography is just linear acquisition at a single position rather than total EAT volume like MRI and CT.

Conclusions

In conclusion, our study demonstrated that liraglutide could specifically reduce EAT thickness in patients with obese T2DM, independently of its lowering blood glucose effect. Due to its easy measurability and great reproducibility, EAT can be measured with echocardiography to evaluate the effect of liraglutide in patients with obese T2DM.

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Compliance with ethical standards

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

Ethical approval All the subjects submitted written informed consent after in detail understanding the study protocol and possible risks. The design proposal, methods of material collection, and data analysis were consistent with the declaration of Helsinki and were approved by the Ethics Committee of The Second Affiliated Hospital of Dalian Medical University (Dalian, China; Approval number, KY2015-128).

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ORIGINAL ARTICLE

Study on the expression of CD14 + CD16 + monocytes and VEGF in peripheral blood of patients with type 2 diabetes mellitus and diabetic macroangiopathy

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Abstract

Objectives To study the expression of CD14 + CD16 + monocytes and VEGF and the levels of serum adiponectin and MCP-1 in peripheral blood of patients with type 2 diabetes mellitus (T2DM) and diabetic macroangiopathy to understand the possible mechanism of inflammatory immune response in T2DM and diabetic macroangiopathy.

Methods Peripheral blood CD14 + CD16 + monocytes were detected by flow cytometry in 50 T2DM patients, 50 patients with diabetic macroangiopathy, and 20 healthy controls or normal controls who participated in outpatient physical examination, and used the Ficoll-Hypaque density gradient centrifugation isolated PBMC and quantitative PCR technology comparison between groups research object in the peripheral blood PBMC VEGF mRNA expression level. Serum levels of adiponectin and MCP-1 were measured by ELISA.

Results Compared with normal control group (NGT), the fluorescence intensity of proinflammatory CD14 + CD16 + monocytes in simple T2DM group and T2DM combined with macroangiopathy group were significantly increased ($p < 0.05$), and T2DM combined with macroangiopathy group was significantly higher than T2DM group, the difference was statistically significant ($p < 0.01$). Moreover, the expression of VEGF mRNA in peripheral blood PBMCs, T2DM combined with macroangiopathy> T2DM patients> healthy volunteers, the differences were significant ($p < 0.05$). Compared with NGT group, the levels of serum adiponectin in T2DM group and T2DM combined with macroangiopathy group were significantly lower than those in NGT group ($p < 0.01$), and the levels of serum adiponectin, simple T2DM Group were lower than T2DM combined with macroangiopathy group ($p < 0.05$). The level of MCP-1 in serum compared with simple T2DM group and NGT group, T2DM combined with macroangiopathy group had statistically significant difference ($p < 0.05$). The serum level of MCP-1 in T2DM group was also higher than that in NGT group ($p < 0.05$). At the same time, we also found that the increase of CD14 + CD16 + monocytes was positively correlated with serum MCP-1 levels.

Conclusions T2DM patients and T2DM combined with macroangiopathy patients have increased expression of VEGF and MCP-1 concentration in peripheral blood mononuclear cells. The increase of MCP-1 may increase the number of CD14 + CD16 + monocytes, which is involved in the chronic inflammation in patients with T2DM and T2DM combined with macroangiopathy, resulting in the occurrence and development of T2DM and its complications.

Keywords Type 2 diabetes · CD14 + CD16 + monocytes · VEGF · Cytokines

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CD14 + CD16 + monocytes are considered to be a subset of proinflammatory monocytes. Our previous studies have found that in cytokine-mediated chronic low-grade inflammatory response in patients with type 2 diabetes mellitus (T2DM) and diabetic kidney disease (DKD), non-specific immune system activation participates in the occurrence and development of T2DM and its complications [1, 2]. In patients with diabetes mellitus and macrovascular disease, the expression of CD14 + CD16 + in chronic low-grade inflammation is rarely reported. Our purpose is to study the expression of CD14 + CD16 + monocytes and VEGF and the concentration of MCP-1 in peripheral blood in patients with T2DM and diabetic macroangiopathy to understand the possible mechanism of nonspecific inflammatory immune response in T2DM and diabetic macroangiopathy.

Subjects and methods

Research objects

From September 2012 to October 2014, all the newly diagnosed T2DM patients and normal glucose-regulated patients in the Affiliated Hospital of Zunyi Medical University of Guizhou Province received 75 g oral glucose tolerance test (OGTT). A total of 120 patients were enrolled, including 50 T2DM patients, 50 T2DM patients complicated with macrovascular disease, and 20 healthy controls or normal controls who participated in outpatient physical examination. T2DM diagnosis is in line with the 1999 World Health Organization (WHO) Diabetes Diagnostic and Classification Criteria and excludes the following: (1) a history of diabetes and hypoglycemic agents, (2) type 1 diabetes, gestational diabetes, and special types of diabetes, (3) acute complications of diabetes (e.g., diabetic ketoacidosis, hyperglycemia, and hyperosmolarity, and lactic acidosis), (4) severe infection, stress, (5) cerebrovascular accident, (6) heart, liver, and kidney dysfunction, (7) malignant tumor, connective tissue disease, coronary heart disease, stroke, and (8) take some drugs that affect the test (e.g., birth control pills, statins, etc.). All the selected persons were those of Chinese Han nationality who were not related to each other.

Main reagents

RPMI-1640 medium and fetal bovine serum were purchased from Gibco, and human lymphocyte separation solution 1.077 was purchased from Sigma. PE-labeled anti-human CD14 and FITC-labeled anti-human TLR4 antibodies were purchased from AbD Serotec, UK, and the ELISA test kit for MCP-1 was supplied by BioSource, USA. The adiponectin ELISA kit was supplied by Linco, USA.

Main methods

General data collection and grouping

(1) Collection of medical history: Collect the age of the subject, past illness and medication history, smoking status, etc.
(2) Body Mass Index (BMI) = weight/height^2 (kg/m^2) and height measurement: The subject is barefoot, standing in the "right position" on the bottom of the height gauge. The heel, the shin, and the two shoulders are placed against the height of the column, measuring the vertical distance from the top of the head to the heel, while recording the weight value.
(3) Determination of biochemical indicators: Venous blood was taken on a fasting stomach, serum was separated, and serum MCP-1 concentration was determined by ELISA. HbA1c determination: using a high-pressure liquid phase method. Blood routine application XE-2100 automatic analyzer detection. Determination of fasting blood glucose, blood lipids, renal function, and hsCRP: Venous blood was taken on an empty stomach and detected by Olympus automatic biochemical analyzer.
(4) Test group: Diagnostic criteria for diabetes according to the World Health Organization (WHO) Diabetes Diagnosis and Classification Standards in 1999; Divided according to the above diagnostic criteria: ①20 cases of normal glucose tolerance group (NGT) (male: female 12: 8),②50 cases of simple type 2 diabetes group (T2DM) (male: female 27:23), and ③50 cases of diabetic macroangiopathy group(male: female: 25:25). The HP-tip image color Doppler diagnostic device examines the lower extremity arteries (femoral artery, radial artery, anterior tibial artery, posterior tibial artery, and dorsal artery) and found arteriosclerotic plaques of the carotid artery and/or lower extremity arteries. Thrombosis or extensive irregular stenosis of the arteries; history of angina or myocardial infarction; diagnosis of coronary heart disease by dynamic electrocardiography and echocardiography or coronary angiography; history of cerebrovascular accident, ischemia of brain CT or MRI scan lesions; clinical manifestations of ischemic lesions of the lower extremities (intermittent breaks, ischemic pain).

A total of 20 healthy volunteers or normal controls who participated in the outpatient medical examination were selected. There were no significant differences in age, gender, blood routine, liver function, and renal function between the groups ($p > 0.05$). There were no significant differences in the incidence of hypertension, diabetic nephropathy, diabetic retinopathy, and smoking between the two groups ($p > 0.05$).

Collection of blood samples and isolation and culture of peripheral blood mononuclear cells (PBMC)

The subjects were collected for fasting venous blood in the morning, and the upper serum was taken and stored in a − 80 °C defreezer for use. At the same time, we sent the venous blood to the laboratory to test the blood routine, liver and kidney function, blood lipids, and other indicators. The remaining venous blood was placed in the heparin anticoagulation test tube for flow detection and PBMC separation. Peripheral blood PBMC was isolated by density gradient centrifugation of Ficoll lymphocyte separation solution, washed twice with serum-free medium, and added to complete RPM1–160 medium (containing 10% heat inactivated fetal bovine serum, penicillin, and streptomycin 1000 u /ml). To prepare a cell suspension, mix well and perform cell counting and trypan blue to identify cell viability (at least greater than 95%). The cell concentration was adjusted to 2×10^5 cells/ml and seeded into a 24-well plate, which was then placed in a 37 °C, 5% CO_2 incubator for subsequent experiments.

VEGF-A qRT-PCR detection

TRIzol extracts total cellular RNA, determines RNA purity and content, and reverse transcribes into cDNA. The PCR primer sequence is as follows: VEGF-A forward primer 5'-CGAAGTGGTGAAGTTCATGG-3', reverse primer 5'-GTACT CGATCTCATCAGGGT-3', GAPDH forward primer 5'-CAATGACCCCTTCAT TGACC-'', reverse primer 5 '-GACAAGCTTCCCGTTCTCAG-3'. The relative content of VEGF-A mRNA is expressed by $2^{-\Delta\Delta Ct}$. Use GAPDH as an internal reference.

Flow cytometry to detect CD14 + CD16 + monocyte mean fluorescence intensity (MFI)

150 μl of EDTA anticoagulated peripheral whole blood was added to the bottom of the flow tube, and 1 tube was reserved as a blank control tube. No fluorescent antibody was added, and the other tubes were respectively added with corresponding antibodies (10 μl each of CD14-PE and CD16-FITC) and oscillated. After mixing, protect it from light, incubate for 30 min at room temperature, add 1 ml of red blood cell lysate to the above liquid, and mix for 10 min; then add l ml PBS + 1% BSA solution to each tube and place in a centrifuge to 1600 rpm/min, centrifugation for 5 min; the supernatant was discarded, and the cells were washed twice; the polyformaldehyde was added to each tube for 10 min; the MFI of CD14 + CD16 + was detected by flow cytometry. The use of an isotype control at the same time makes it possible to effectively ensure the specificity of the antibody.

Cytokine detection

The concentrations of serum MCP-1 and adiponectin were measured by ELISA. According to the instructions of the ELISA kit (purchased from Biosource and Linco, USA), the absorbance of each well was measured at 450 nm [A(450)]. The MCP-1 content calculation result is expressed in pg/ml, and the adiponectin content calculation result is expressed in mg/L. The intra-assay and inter-assay coefficients of variation of the reagents were all < 10%, and 5 replicate wells were set for all samples.

Statistical processing

Statistical analysis was performed using SPSS 13.0 software, and the measurement data was expressed by ± S. The *t* test was used to compare the measurement data between the two groups, and the one-way ANOVA was used for the three or more measurement data. Correlation analysis was performed using Pearson correlation analysis. $p < 0.05$ was considered statistically significant.

Results

Patient's baseline characteristics

There were no significant differences in age, gender, DBP, liver function, renal function, peripheral blood leukocytes, and monocyte counts between the three groups ($p > 0.05$). The body mass index, HbA1c, fasting blood glucose, and SBP levels in the T2DM combined with macrovascular disease group were higher than those in the normal control group ($p < 0.05$; Table 1).

Flow analysis results of CD14 + CD16 + monocytes

Compared with NGT, the peripheral blood proinflammatory CD14 + CD16 + monocyte fluorescence intensity values were increased in the T2DM group and the T2DM combined with macrovascular disease group ($p < 0.05$), and the T2DM combined with macrovascular disease group was higher than the T2DM group, the difference was statistically significant ($p < 0.01$;Fig. 1).

mRNA detection of VEGF-A in PBMC

Compared with NGT, the expression of VEGF-A mRNA in peripheral blood PBMC of patients with T2DM was significantly higher, and the T2DM combined with macrovascular disease group was higher than the T2DM group alone, and the difference was significant($p < 0.05$;Fig. 2).

Table 1 Comparison of clinical parameters between 3 groups ($\bar{x} \pm s$)

Item	NGT	T2DM	T2DM combined with macrovascular disease
n (male/female)	12/8	27/23	25/25
Age(year)	47 ± 8	48 ± 7	50 ± 9
BMI(kg/m^2)	23.6 ± 3.8	23.9 ± 2.9	24.1 ± 6.6*#
HbA1c(%)	5.6 ± 0.3	5.8 ± 0.5	8.0 ± 1.6*#
Fasting plasma glucose (mmol/L)	5.8 ± 0.6	5.8 ± 0.4	8.8 ± 2.5*#
2 h postprandial plasma glucose (mmol/L)	7.2 ± 1.4	10.6 ± 5.2*	15.1 ± 3.6*#
SBP(mmHg)	128 ± 10	136 ± 18	138 ± 26*
DBP(mmHg)	78 ± 9	78 ± 10	82 ± 10

*# $p < 0.05$ vs NGR, $p < 0.05$ vs T2DM alone

Serum MCP-1 and adiponectin levels

Serum MCP-1 levels in T2DM combined with macrovascular disease group were higher than those in T2DM group and NGT group, the difference was statistically significant ($p < 0.05$). The T2DM group was higher than the NGT group, and the difference was statistically significant ($p < 0.05$;Table 2). Compared with NGT group, the serum adiponectin level in T2DM group and T2DM combined with macrovascular disease group was higher than that in the NGT group, the difference was statistically significant ($p < 0.05$). However, compared with the latter two groups, serum adiponectin concentration in the T2DM group was higher than that in the T2DM with macrovascular disease group, and the difference was not statistically significant ($p > 0.05$;Table 2).

Correlation analysis

The mean fluorescence intensity of CD14 + CD16 + monocytes was positively correlated with serum MCP-1 level ($r = 0.42$, $p < 0.05$) and was not associated with adiponectin ($p > 0.05$).

Discussion

Diabetes has become the third most serious chronic disease that threatens human health after cancer and cardiovascular disease. Diabetes with vascular disease is one of the main complications of diabetes, and it is the main cause of death and disability. As a basic disease of diabetic macroangiopathy, atherosclerosis is a chronic inflammatory disease induced by various factors. The exact mechanism of vascular endothelial dysfunction and promotion of atherosclerosis and development has been a hot spot of research. Cells can secrete a variety of inflammatory factors, promote inflammatory reactions, trigger or aggravate the occurrence, and deterioration of atherosclerosis.

CD14 + CD16 + monocytes are considered to be a subset of proinflammatory monocytes. Our previous study found that abnormal expression of CD14 + CD16 + monocytes may be associated with the microinflammation state of type 2 diabetic mellitus (T2DM), and cytokine-mediated chronic low-grade inflammatory response in patients with T2DM and diabetic kidney disease (DKD), non-specific immune system activation participates in the occurrence and development of T2DM

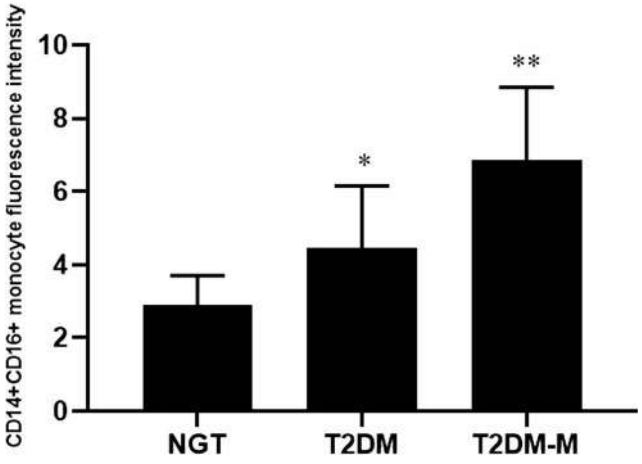

Fig. 1 CD14 + CD16 + monocyte fluorescence intensity values NGT Normal glucose tolerance, T2DM Type 2 diabetes mellitus alone, T2DM-M Type 2 diabetes mellitus with macroangiopathy. υs NGT, *:p<0.05; υs T2DM, **:p<0.01

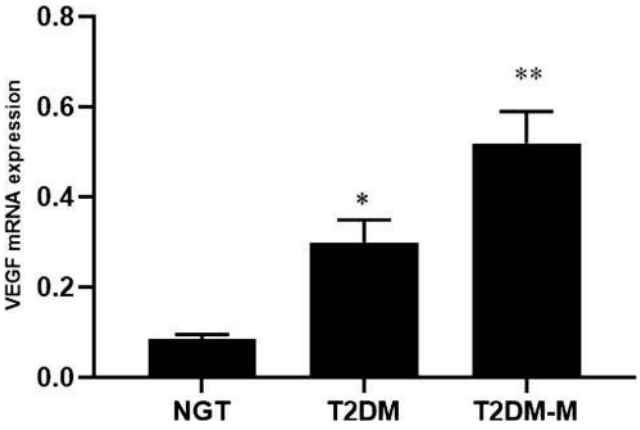

Fig. 2 VEGF-A mRNA expression in peripheral blood PBMC NGT Normal glucose tolerance, T2DM Type 2 diabetes mellitus alone, T2DM-M Type 2 diabetes mellitus with macroangiopathy. υs NGT, *:p<0.05; υs T2DM, **:p<0.05

Table 2 The serum MCP-1 and adiponectin levels from the groups (pg/ml$\bar{x} \pm s$)

Group	MCP-1	Adiponectin
Healthy subjects	18.9 ± 8.2	12.7 ± 3.3
T2DM group	32.4 ± 8.6**	6.3 ± 2.2**
T2DM-M group	39.1 ± 9.8**Δ	5.6 ± 2.4**Δ

*$p < 0.05$, **$p < 0.01$ ∿vs healthy subjects; Δ$p < 0.05$

and its complications [1, 2]. This study found that the peripheral blood proinflammatory CD14 + CD16 + monocyte fluorescence intensity values were significantly higher in patients with T2DM alone and T2DM with macrovascular disease ($p < 0.05$), and T2DM with macrovascular disease group was higher than T2DM group ($p < 0.01$). Serum MCP-1 levels in patients with T2DM were significantly higher than those in normal controls, and T2DM with macrovascular disease group was significantly higher than T2DM alone ($p < 0.05$). Compared with NGT group, the serum adiponectin level in T2DM group and T2DM combined with macrovascular disease group was higher than that in the NGT group, the difference was statistically significant ($p < 0.05$). However, compared with the latter two groups, serum adiponectin concentration in the T2DM group was higher than that in the T2DM with macrovascular disease group, and the difference was not statistically significant ($p > 0.05$).

Adiponectin is one of the most abundant protein products expressed in adipose tissue and is abundantly present in the blood circulation. The study found that it has anti-inflammatory and anti-atherosclerotic effects [3]. The serum adiponectin concentration in patients with T2DM in this study was significantly lower than that in normal subjects. This is similar to Scherer [4] and Li et al [5] and others. Our study also found that serum adiponectin levels were further elevated in T2DM with macrovascular disease patients. Although the correlation analysis indicated the mean fluorescence intensity of CD14 + CD16 + monocytes and serum adiponectin $p > 0.05$, there is a negative correlation trend from the trend, but the number of samples is small, which may affect the results.

Vascular endothelial growth factor (VEGF) is a cytokine that acts specifically on vascular endothelial cells, which promote monocyte infiltration and increases skin cell proliferation and vascular permeability [6, 7]. Gurbuz et al and Carbajo-Pescador et al. found that VEGF expression is involved in the activation of the JAK/STAT pathway. STAT3 is a direct transcriptional activator of the VEGF gene [8, 9]. In the past, our research showed that the expression of VEGF and vascular endothelial growth factor receptor (VEGF-R) genes in atherosclerosis was significantly higher than that in the control group and consistent with the changes in JAK2/STAT3 levels [10]. This is consistent with the findings of the Gurbuz et al and Carbajo-Pescador et al. The study also found that compared with NGT, the expression of VEGF mRNA in peripheral blood PBMC of T2DM patients was significantly increased, and T2DM combined with macrovascular disease group was higher than T2DM alone group ($p < 0.05$), suggesting that VEGF may promote T2DM with large blood vessels. Monocytes infiltrate the diseased patients and participate in the pathogenesis.

In conclusion, this study showed that patients with T2DM and T2DM with macrovascular disease had increased VEGF expression and increased MCP-1 concentration in peripheral blood mononuclear cells, which may increase the number of CD14 + CD16 + monocytes due to increased MCP-1 concentration. It is involved in the chronic inflammatory response in patients with T2DM and T2DM with macrovascular disease, which leads to the development of T2DM and its complications. This may be one of the important reasons for diabetic patients suffering from atherosclerotic diseases such as coronary heart disease and diabetic lower extremity macrovascular disease. However, because the control group is not easy to obtain, resulting in a small sample size, this is a deficiency in the course of this experiment, which will be further studied in subsequent experiments, the sample size will be expanded, and the verification will be further repeated.

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Compliance with ethical standards

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

Ethical approval The study was approved by the patient and by the Ethics Committee of Zunyi Medical University.

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ORIGINAL ARTICLE

Candidate gene association study of *UCP3* variant rs1800849 with T2D in Mizo population of Northeast India

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Abstract

Aim Uncoupling protein 3 (UCP3) has been identified as a type 2 diabetes (T2D) candidate gene and variant rs1800849 is of potential interest as it is present in the regulatory region of *UCP3*. The aim of the present candidate gene case-control association study was to evaluate the association of variant rs1800849 of *UCP3* with T2D in Mizo population from Northeast India.

Methodology The variation was genotyped using TaqMan allele discrimination assay in 767 individuals (425 cases and 342 healthy controls).

Results The variant rs1800849 of UCP3 was not found to be significantly associated with T2D (p-value = 0.733) in studied population group.

Conclusion Thus, it is concluded that the present study could not replicate the association of variant rs1800849 with genetic susceptibility to T2D in the Mizo population group. This absence of association highlights the genetic heterogeneity prevalent in Indian population groups and warrants screening of other T2D candidate genes in Mizo population group.

Keywords Type 2 diabetes · Mizo population · *UCP3*

Introduction

UCPs are the uncoupling proteins known to regulate mitochondrial proton leak and are further of 5 types, named UCP1 to UCP5. Among these, *UCP2* and *UCP3* are found to be significantly linked to T2D [1]. *UCP3* plays a prominent role in the downregulation of ROS in mitochondria [2, 3] and in free fatty acid (FFA) metabolism and transport which are potential mechanism for T2D pathogenesis [4]. The mRNA levels of *UCP3* were found to be decreased in skeletal muscles of patients suffering from T2D when compared with healthy control subjects [5] implying its protective role in the development of T2D. *UCP3* helps recover against cardiac IR, a cardiovascular complication in T2D which is known to contribute most to T2D mortality rate [6, 7]. UCP3 gene is also proposed to be a major PPAR-γ (subtype of PPAR) target known to be implicated in the modulation of insulin resistance [8]. Reduced activity of PPAR-γ is found to be associated with severe insulin resistance and diabetes in humans [9]. The variant rs1800849 of the UCP3 gene is of potential interest as it is present 6 bp upstream of TATA box and 4 bp downstream of a peroxisome peroliferator-activated receptor (PPAR) element and could therefore modify the PPAR responsiveness of the UCP3 gene [10, 11]. Until now, more than 120 loci for T2D have been identified [12, 13]. However, the genetics and heritability of diabetes is still ~ 80% unresolved [14].The prominent reason could be many of these loci remain un-replicated or many of the endogamous populations of the

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world are not yet screened. Very few genetic association studies have been focused on the Asian populations despite the fact that there is more diverse contributing to the target proportion of the global genetic pool.

With this background, we performed a case-control association study in Mizo population from Mizoram, a state in Northeast India. This population is one of the less explored populations but having diabetic prevalence of more than 9% [15]. Therefore, we evaluated the association of UCP3 gene variation rs1800849 with T2D in Mizo population.

Materials and methods

Sampling

Two millilitres of EDTA anti-coagulated venous blood samples were collected along with a written consent from Genesis Laboratory, Aizawl, Mizoram. T2D diagnosis was made in accordance with the World Health Organization criteria where fasting glucose level greater than 126 mg/dl and post prandial glucose greater than 200 mg/dl and healthy controls without the symptoms of diabetes mellitus, blood glucose levels within normal limits (below < 120 mg/dl) (follow-up report in diagnosis of diabetes) were taken [16, 17].The genomic DNA was extracted from the blood samples according to the protocol [18]. A total of 425 cases and 342 healthy controls were selected for analysis after quantitative and qualitative analysis of the isolated DNA samples by using spectrophotometry analysis with bio-spectrophotometer (Eppendorf, Hamburg, Germany) and agarose gel electrophoresis, respectively. DNA samples were diluted to a final concentration of 5 ng/μL. The study was approved by the Institutional Ethical Review Board (IERB) of Shri Mata Vaishno Devi University and Ethical committee of Mizoram University, Aizawl.

Genotyping

Genotyping of *UCP3* variant rs1800849 was performed with the help of TaqMan allele discrimination assay using Mx3005p Agilent real-time PCR (Strategene Agilent Technologies, Waldronn, Germany) following the protocol recommended by the manufacturer. UNG Master Mix (Applied Biosystems, Thermo Fischer Scientific, Foster City, CA, USA) and TaqMan assay (predesigned primers and probe labelled with FAM and VIC were supplied by Applied Biosystem, Thermo Fischer Scientific, Pleasanton, CA, USA) were used for genotyping. The PCR reactions were performed in a 96-well plate format with three non-template controls (NTCs) in each to check for foreign contamination of nucleic acids. The cycling conditions were adopted from our previous work, i.e. hold for 10 min at 95 °C, then 40 cycles of 95 °C for 15 s and 60 °C for 1 min [19]. The reaction volume was set at 10 μL for each well including 3 μL of DNA in each (except NTCs). Comparison of the post- and pre-scanned readings provided the allele call. More than 50 random samples were selected for re-genotyping in order to cross-validate the results and absolute concordance was observed, indicating the robustness of the assay.

Statistical analyses

Statistical analyses were done by using the Statistical Package for Social Sciences (SPSS) software (version 23; Chicago, IL). The clinical characteristics were compared in T2D cases and controls (supplementary table 1). Hardy-Weinberg equilibrium was estimated by performing chi-square (χ^2) analysis. Estimation of odds ratio (OR), its 95% CI and respective level of significance as *p* value was performed by logistic regression analyses in order to rule out the effect of plausible confounding factors like age, gender and BMI in different genetic models (additive, dominant and recessive). The power of the study was calculated statistically by PS software version 3.1.2 [20].

Results

The genotype frequencies of the variant rs1800849 of *UCP3* were found to be in agreement for commutatively cases and controls with HWE (*p* value = 0.707). The HWE observed for cases and controls separately was 0.416 and 0.980, respectively. The allele frequency observed for allele G in cases and controls was 0.88 and 0.874, respectively, whereas the observed frequency of allele A was 0.12 in cases and 0.126 in controls. The frequency of both the alleles was almost same in cases and controls (Table 1). The allelic OR observed for the variant rs1800849 of *UCP3* was 0.95 [0.70–1.29 at 95% CI], *p* value = 0.733. The variant rs1800849 of *UCP3* was also evaluated using different models of inheritance (the different models were selected with respect to the variant allele, i.e. 'allele A'), the OR observed in additive model was 0.9 [0.6–1.2 at 95% CI], *p* value 0.597, the OR observed in dominant model was 0.8 [0.60–1.20 at 95% CI], *p* value 0.479 and the OR observed in recessive model was 1.20 [0.30–3.80], *p* value 0.718, respectively. Nevertheless, the results did not vary after adjustment for age, gender and BMI (Table 1). Thus, it was observed that the variant rs1800849 of *UCP3* did not show any significant association with susceptibility to T2D in Mizo population group. The power of the study was 80.8% at a minor allele (A) frequency of 0.12 (from present study in controls) with an expected OR = 1.7 (as per the recently published association study in Chinese) [21], for this variation.

Table 1 Allelic frequency distribution and genetic association analyses of the variant rs1800849 of *UCP3* in Mizo population, India

Allelic Distribution			Association				
Allele	Cases n=425	Controls n=342	Models	OR [95% CI]	p value	OR* [95% CI]	p value*
G	0.880	0.874	Allelic	0.95 [0.7-1.29]	0.733	-	-
			Additive	0.9 [0.7-1.20]	0.737	0.9[0.6-1.20]	0.597
A	0.120	0.126	Dominant	0.9 [0.64-1.27]	0.553	0.8 [0.6-1.20]	0.479
			Recessive	1.46 [0.48-4.39]	0.500	1.2[0.3-3.80]	0.718

* Adjusted with age, gender and BMI

Discussion

The UCP3 gene had been implicated in protective role against insulin resistance [5] and as a mitochondrial anion carrier protein has protective effect against oxidative endothelial damage [22, 23]. It was observed that UCP3 mRNA expression increases during fasting in both lean and obese humans [24]. UCP3 was also found to influence insulin secretion in beta pancreatic cells [25]. The genetic variant rs1800849 located at 5′ UTR region was found to be associated with mRNA expression in skeletal muscle [26] and is speculated to influence UCP3 promoter activity that might lead to impaired beta cell functioning. The variant was first reported to be associated with T2D in a French cohort (OR = 0.50 with 95% CI of 0.26–0.96) [27]. The data was corroborated by two subsequent studies carried out in Brazilian obese women (OR = 0.30 with 95% CI of 0.11–0.82) and in Asian Indians (OR = 0.79 with 95% CI of 0.64–0.98) [28, 29]. Furthermore, strong association of the variant in Asians was suggested in two systemic meta-analysis with pooled OR of 1.22 (1.04–1.44) and 1.15 (1.03–1.28), respectively [30, 31]. Interestingly, the variant was found to be associated negatively with the development of T2D in Caucasian middle-aged men, Brazilian-Caucasian and Colombian population [31–33]. We performed this replication study in order to find out the association of UCP3 gene variant rs1800849 (G > A) in an unexplored endogamous population group from Mizoram [34]. However, in the present study, we found no significant association of the variant with T2D in the Mizo population in contrast to an earlier study in another population group of India (Vimaleswaran et al. [28]). We believe these observations are highlighting existing genetic heterogeneity in Indian population groups. However, these findings are in agreement with the genetic association studies in European and Danish population where no significant association of the variant rs1800849 of *UCP3* with T2D was observed [31, 35]. Such inconsistent results of *UCP3* variant rs1800849 in different ethnicities around the globe might substantiate the role of differences in genetic architecture, environmental factors and may be interactive effect of other candidate genes variants associated with T2D.

Conclusion

We conclude that the variant rs1800849 of *UCP3* is unlikely to play an independent role in the development of T2D in Mizo population of Northeast India. Furthermore, keeping genetic heterogeneity in mind, it is also emphasised that more variants of the T2D candidate genes are needed to be evaluated in Mizo population group to address the issue of missing heritability in Indian populations.

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Authors' contributions FL collected and processed the samples. SAS, VAS, SHS and TJP performed quantification and genotyping. SAS, VAS and IS performed the statistical analyses and prepared the manuscript. JZ primarily provided the samples. VIS and ER critically evaluated the manuscript. NSK and SWS primarily planned the study and critically evaluated the data and manuscript.

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Compliance with ethical standards

Ethical approval This study was approved by the ethical committee of Mizoram University, Aizawl, and Institutional Ethical Review Board (IERB) of Shri Mata Vaishno Devi University.

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

Statement of Human and Animal Rights The work has been approved by the ethical standards of the responsible committee on human experiments (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of informed consent Informed consent was obtained from all the patients and controls included in the study.

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ORIGINAL ARTICLE

Expression analysis of microRNA-155 in type 2 diabetes in Kashmiri population

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Abstract

Purpose The present study aimed to evaluate the expression of microRNA-155 (miR-155) in type 2 diabetes mellitus (T2DM) and assess its correlation with clinicopathological characteristics of this type.

Methods MiR-155 expression was detected in 20 T2DM blood samples and 23 blood samples of healthy controls using fluorescent reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The association between miR-155 expression and clinicopathological features was also analyzed.

Result We found higher values of quantitative parameters like blood pressure, blood sugar levels, and weight in cases compared to controls ($p < 0.05$). Among qualitative data we found that healthy patients were more physically active than T2DM patients ($p < 0.05$), while family history and smoking status were almost same in cases and controls. RT-qPCR results revealed that miR-155 expression levels were significantly decreased in T2DM cases (90%). However, miR-155 expression was not significantly correlated with various clinicopathological parameters ($p > 0.05$). We evaluated miR-155 expression between males and females; however, we found no gender-specific difference in the expression. Patients exhibiting high miR-155 expression levels were observed to have short duration of T2DM than that of patients with reduced expression of miR-155; however, this difference was not statistically significant ($p > 0.05$).

Conclusion The current results demonstrated that miR-155 may be involved in resistance against T2DM and may be negatively associated with clinical parameters of T2DM patients. The study was limited by its observational study design, so additional studies involving pathophysiology are required to clarify the role of miR-155 in T2DM.

Keywords MicroRNA-155 · Type 2 diabetes · Reverse transcription-quantitative polymerase chain reaction

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by high blood sugar, insulin resistance, and relative insulin deficiency. The chronic hyperglycemia of diabetes is associated with the risk of developing complications such as retinopathy, nephropathy, and neuropathy, influenced by many factors including duration of diabetes and genetic factors [1]. As a result of current treatments only a limited reduction in this risk has been achieved, and the management of these conditions remains a major problem for those with diabetes. New insights have come from human micro RNAs (miRNA) modulating several processes both physiological and pathological, by the posttranscriptional inhibition of gene expression. MicroRNAs (miRNAs) are small, single stranded, and typically 21–25 nucleotides long that normally bind to the 3′untranslated region of their target mRNA, leading to translational inhibition and/or mRNA degradation [2]. Several miRNAs have been identified as having a physiological role in diabetic complications.

In humans, MiR-155 is encoded by the *MIR155* host gene or *MIR155HG* (formerly called BIC (B cell Integration Cluster)) located in chromosome 21q21 [3]. It plays an

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important role in various physiological and pathological processes. Given that miR-155 has the potential to regulate multiple genes, dysregulation of the selected miR would be certain to have impact on many biological processes that are of direct relevance to T2DM.

Micro RNAs (miRNAs) are typically 21–25 nucleotides long. The active form of miRNA is generated after two-step nucleolytic processing from a longer RNA transcript called primary RNA (pri-RNA) that carries a hairpin-shaped secondary structure [2]. Two specialized RNA-cleaving enzymes are required to process the pri-miRNA transcript. The first enzyme is Drosha, a member of the RNase III family of enzymes [3]. This enzyme in nucleus, works together with an essential specificity subunit protein (called Pasha in some organisms and DGCR8 in others) [4], and together these two proteins form an active microprocessor complex. The pre-miRNA generated by Drosha is usually approximately 60–70 nucleotides long [5, 6]. The pre-miRNA is then exported to the cytoplasm where the second RNA cleavage occurs, carried out by Dicer [3, 7]. The active form generated by the action of Drosha and Dicer is the single-strand form called the "guide" RNA, which is then incorporated into a RISC protein complex [8]. Within this complex, the guide RNA strand recruits RNA-induced silencing complex (RISC) to a target RNA. A RISC contains, in addition to miRNA, various proteins including a member of Argonaute family (RNA-cleaving enzymes) responsible for the removal of miRNA passenger strands [9]. The base pairing between miRNA and mRNA is initiated by interactions of so-called "seed residues" typically the sequence between bases 2 and 8 of the miRNA [10–13]. The target RNAs are degraded, or their translation is inhibited depending upon the complementarities; if the sequences are complementary, the target is degraded; if the match is not good, there is inhibition of translation [14], both ultimately leading to silencing of gene expression.

Specific miRNAs such as miR-155 have been originally linked with the inflammatory response in which miRNA upregulation was found in multiple immune cell lineages by inflammatory cytokines, toll-like receptor ligands, and specific antigens [15–17]. However, the role of miR-155 in diabetes and its complications needs to be established. A recent study has shown that miR-155 expression is significantly decreased in peripheral blood mononuclear cells from T2DM patients [18]. One study carried out in diabetic mice found decreased expression of miR-155, and its over-expression prevented cardiac fibrosis in these mice [19]. Thus, it is hypothesized that there may be differential expression of miR-155 among T2DM subjects and may serve as useful biochemical parameter for disease progression. Evaluating the expression of miR-155 may also be useful in targeted therapy with clinical implications.

Materials and methods

Study design

This was a population-based study aimed at examining subjects from ethnic Kashmiri population. The study population comprised of 20 type 2 diabetes cases and 23 healthy controls. The clinical part of the study included prospective recruitment of T2DM patients from the Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura Srinagar J&K India. *Inclusion criteria*: (1) Patients of both sex and ≥ 18 years of age. (2) Patients willing to voluntarily participate in the study and belonging to the Kashmir valley were included. Recruitment was done randomly, and the data were analyzed by stratification of enrolled subjects as per population distribution. *Exclusion criteria*: (1) Unwilling patients and/or subjects not consenting to participate or comply with the study interventions. (2) Patients with type 1 diabetes, gestational diabetes, hypo/hyperthyroidism, autoimmune diseases, persistent infections, and other chronic diseases with similar manifestations.

Sample collection

A 1–2-mL whole blood sample of consenting subjects was obtained in EDTA vials for isolation of mononuclear cells for expression studies.

Peripheral blood mononuclear cell isolation

PBMNCs were isolated from whole blood by centrifugation in a density gradient medium (Lymphoprep™, Fresenius Kabi, Olso, Norway).

RNA isolation

Total RNA was first isolated from PBMNCs using TRIzol® reagent followed by DNase digestion to eliminate any genomic DNA. The integrity of RNA was checked on 1.5% agarose gel, and the quality and quantification of RNA were evaluated on a Nanodrop (Thermo Scientific) at 260 nm/280 nm absorbance.

Reverse transcription

RNA was reverse transcribed into cDNA using commercially available cDNA synthesis kit. MiR-155 was reverse-transcribed using stem loop primers and the microRNA reverse-transcription kit (Applied Biosystems, CA) according

to the manufacturer's protocol. The primer sequences for specific miR-155 and U6 reverse transcription are 5′-GTCG TATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACACCCCTAT-3′ and 5′-AACGCTTCACGAAT TTGCGT-3′, respectively. About 1.5 μL of RNA was reverse-transcribed in a 30-μL reaction volume for each assay.

Real-time PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was used for expression study. For qRT-PCR chemistry, SYBR Green dye was used. The Primer sequences for expression study are as follows:

MIR-155 F:5′-TGCGCTTAATGCTAATCGTG ATAGG-3′
MIR-155 R:5′-CCAGTGCAGGGTCCGAGGTATT-3′
U6 F:5′-GCTTCGGCAGCACATATACTAAAAT-3′
U6 R:5′-CGCTTCACGAATTTGCGTGTCAT-3′

Statistical analysis

Data were expressed as the mean ± SD, and statistical analysis was performed using SPSS software, version 20. The comparison of clinical variables with the miR-155 expression was done using Chi-square (χ^2) analysis/Fischer's exact test and independent samples *t* test. Real-time data of miR-155 were expressed as Cp/Ct values. Comparative analysis was performed using Livak Method (ΔΔCt), and results were expressed in terms of relative fold expression. The sample size was verified using G*Power (V3.1.9.4) software.

Results

A total of 43 subjects were enrolled: 20 T2DM patients and 23 healthy controls. Post-hoc power analysis was done by using G*Power (version 3.1.9.4) and power (1-β) turned out to be as high as 0.94 at the effect size of 1.11, $\alpha = 0.05$, and the given sample size [20].

Confirmation of successful miRNA extraction

Small fragments of RNA (< 200 bp) were acquired using the TRIzol® reagent. Electrophoresis indicated a bright 5S band, signifying successful extraction of miRNA (Fig. 1).

PCR results indicate miRNA expression We also performed simple PCR on cDNA obtained and successfully got two bands using agarose gel electrophoresis, corresponding to U6 (small nuclear ribonucleoprotein) and miR-155 gene (Fig. 2). MiR-155 expression was detected by means of SYBR Green fluorescence RT-qPCR. The amplification curves of the genes were smooth indicative of correct experiment conditions. The melting curve demonstrated sharp peaks, and the melting temperatures corresponded with the given gene products. The melting temperature for each gene product was similar in the various samples, confirming the high specificity of the amplified products.

PCR product analysis with agarose gel electrophoresis There were two clear bands: 89 and 65 bp, which were consistent for the reference gene U6 and target gene miR-155 respectively. No other impurity bands were observed, representative of the correct experimental conditions and primer designing (Fig. 3).

Association between clinicopathological parameters of T2DM cases and controls We found a significant difference of parameters like blood sugar fasting, systolic and diastolic pressure, weight, and physical activity in cases and controls ($p < 0.05$; Table 1).

MiR-155 is downregulated in type 2 diabetic clinical specimens We evaluated the basal expression of miR-155 in PBMNCs of T2D patients, and control PCR analysis revealed

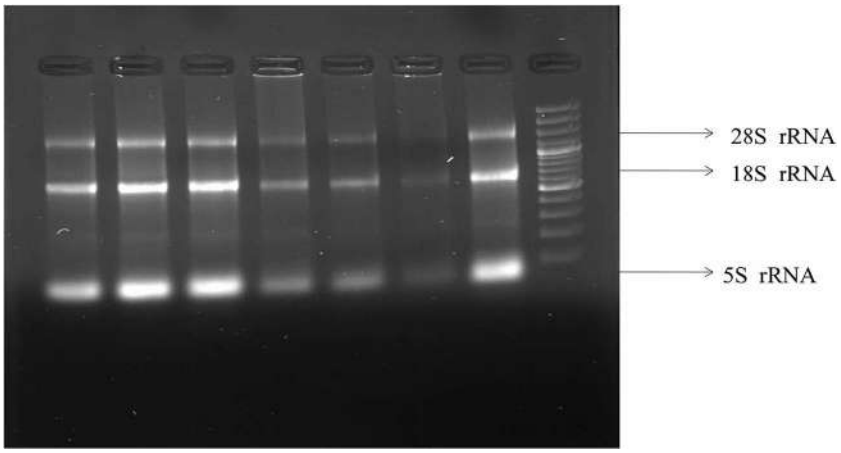

Fig. 1 Representative gel picture showing RNA run on 1.5% agarose gel

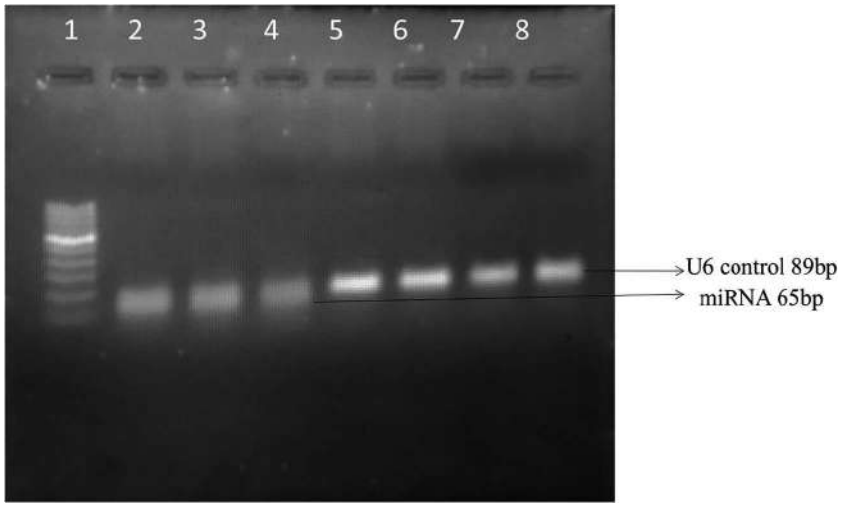

Fig. 2 Representative gel picture showing conventional PCR products (1.5%). Lane 1; 50 bp DNA ladder. Lane 2, 3, and 4; 65 bp miR-155. Lane 5, 6, 7, and 8; 89 bp U6

a significantly lower expression of miR-155 in PBMNCs from T2DM patients compared with healthy controls. MiR-155 expression was downregulated in 90% (18/20) of samples, compared with that of control samples. The mean level of downregulation (fold change expression) was 0.554 ± 0.813 (Fig. 4).

Association between miR-155 expression levels and clinicopathological parameters of T2DM No association was observed between miR-155 expression and the various parameters ($p > 0.05$; Table 2) as there were no significant differences in relative expression values between categories of different variables. We also tried to evaluate miR-155 expression between males and females; however, we found no gender-specific difference in the expression.

Discussion

T2DM is a chronic process, which involves a number of steps, including abnormal regulation of various cell signaling pathways. At present, a key area of diabetes research is elucidation of the association between miR expression and T2DM. This is the first study carried out in our population that has analyzed the miR-155 expression in T2DM subjects with those of healthy control subjects. Selection of participants was limited to Kashmiri population. The present study revealed that miR-155 expression was downregulated in T2DM consistent with previous findings by Corral-Fernandez et al [18]. Several studies have demonstrated that the microRNAs have a relationship with type 2 diabetes mellitus. Majority of them showed the similar results

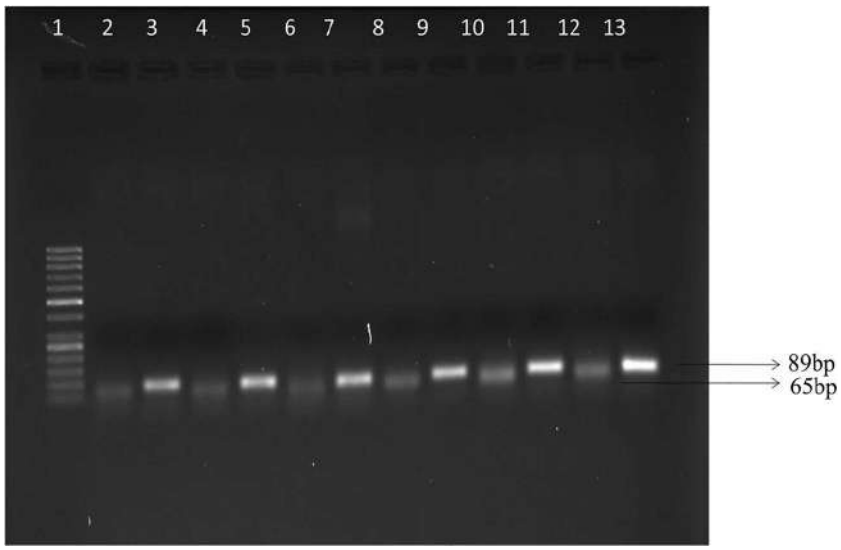

Fig. 3 Representative gel picture of PCR products (1.5%) carried out to confirm the results of real time. PCR. Lane 1; 50 bp DNA ladder. Lane 2, 4, 6, 8, 10, and 12; 65 bp miR-155. Lane 3, 5, 7, 9, 11, and 13; 89 bp U6

Table 1 Clinical characteristics of T2DM and controls among the study population

(a)

Parameters	Cases ($n = 20$) Mean ± SD	Controls ($n = 23$) Mean ± SD	p value
Age	45.3 ± 11.921	43.08696 ± 12.273	0.55
Blood sugar fasting (mg/dl)	156.85 ± 49.01479	95.47826 ± 8.360	*< 0.0001*
Systolic blood pressure (mmHg)	129.3 ± 14.98455	120.6522 ± 8.020478	*0.020*
Diastolic blood pressure (mmHg)	83.75 ± 9.301245	78.47826 ± 5.922757	*0.030*
Height (cm)	160.8995 ± 6.590389	158.9439 ± 5.614	0.30
Weight (kg)	68.3 ± 9.701329	63.04348 ± 6.52602	*0.04*
BMI (kg/m^2)	26.275 ± 2.469365	24.841 ± 2.308	0.056

(b)

Parameters	Cases ($n = 20$)	Controls ($n = 23$)	OR (CI)	p value
Gender				
Male	9	11	0.8926	1.000
Female	11	12	(0.2682–2.971)	
Family history				
Yes	9	6	2.318	0.2193
No	11	17	(0.6434–8.353)	
Smoking status				
Ever	8	3	4.444	0.0781
Never	12	20	(0.9838–20.078)	
Physical activity				
Yes	10	19	0.2105	*0.0483*
No	10	4	(0.05244–0.8451)	

In (a), p value of < 0.05 at 95% confidence interval was considered to be significant.

In (b), Fisher's exact test and odds ratio analysis were used, where $p < 0.05$ at 95% confidence interval was considered to be significant

regarding the expression of miR-155 like those reported in populations from Mexico [18], Egypt [21], Iran [22], Germany [23], and China [24], in which the miR-155 levels were found to be downregulated in T2DM subjects compared to healthy controls. In another study, MiR-155 level was downregulated in serum from T2D patients and has been confirmed to be involved in blood glucose control and diabetes. Thus, miR-155 was found to positively regulate insulin sensitivity with potential applications for diagnosis and treatment of diabetes [24]. Recently, Hubal et al. found that miR-155-5p improved insulin resistance and also enhanced insulin sensitivity in obese patients [25]. Another study revealed that miR-155 was significantly reduced in diabetic patients with coronary artery disease (CAD) in comparison to healthy controls [26]. MiR-155 is a multifunctional microRNA playing an important role in inflammation. MiR-155 has been identified as an important factor involved in macrophage response to different types of inflammatory mediators. Inflammatory mediators including IFN-β, polyriboinosinic–polyribocytidylic acid (poly IC), or tumor necrosis factor-a (TNF-α) can induce miR-155 expression in monocytes and macrophages [15]. Furthermore, miR-155 has been implicated with atherosclerosis [27], thus might be involved in vascular diseases involved in T2DM. In addition, antagomiR-155 inhibiting miR-155 can decrease lipid-loading in macrophages and thus reducing atherosclerotic plaques [28]. The expression of miR-155 is not elevated in our diabetic patients, although it plays an important role in inflammation, it may be a possible target to reduce the inflammation in diabetic complications.

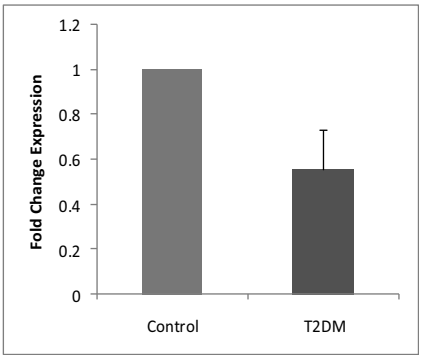

Fig. 4 Relative expression of miR-155 in study subjects

The present study revealed no association between miR-155 expression levels and clinicopathological parameters like blood sugar fasting and post prandial, blood pressure, and

Table 2 Analysis of miR-155 expression and the clinicopathological parameters of T2DM patients

Parameters	*N*	Relative fold expression Mean ± SD	*p* value
Gender			
Male	9	0.5994 ± 1.11536	0.827
Female	11	0.5164 ± 0.50716	
Age			
≥ 50	8	0.3603 ± 0.52480	0.399
< 50	12	0.6827 ± 0.95972	
Family history			
Yes	9	0.2278 ± 0.16370	0.106
No	11	0.8204 ± 1.02983	
Smoking status			
Ever	8	0.7256 ± 1.17044	0.455
Never	12	0.4392 ± 0.48387	
Physical activity			
Yes	10	0.4874 ± 0.49972	726
No	10	0.6201 ± 1.06578	
HbA1c (%)			
≥ 6.5	15	0.6760 ± 0.91190	255
< 6.5	5	0.1869 ± 0.07040	
BSF (mg/dl)			
< 126	4	0.3304 ± 0.42942	
127–180	10	0.6499 ± 1.04851	
181–250	5	0.6306 ± 0.62943	
> 250	1	0.1013 ± 0.00	0.872
BSPP (mg/dl)			
< 140	2	0.1192 ± 0.08094	
141–180	6	1.0230 ± 1.28723	0.497
181–250	8	0.3189 ± 0.2614	
251–300	3	0.6820 ± 0.83513	
> 300	1	0.1013 ± 0.00	
Systolic blood pressure (mmHg)			
≥ 120	17	0.5315 ± 0.83298	
< 120	3	0.6796 ± 0.83694	0.780
Diastolic blood pressure (mmHg)			
≥ 80	18	0.5162 ± 0.81074	0.550
< 80	2	0.8921 ± 1.06308	
BMI (kg/m^2)			
≥ 30	1	0.2619 ± 0.00	0.723
< 30	19	0.5691 ± 0.83230	

$p < 0.05$ at 95% confidence interval was considered to be significant

HbA1c in contrast to other studies. These contrasting findings might be due to different ethnic background, patient's profile, and procedural differences in the quantification of miR-155, including the RNA isolation, qPCR, and estimation of gene expression [29].

This study was focused on the subjects who developed diabetes that are already increasing at an alarming rate and posing a threat to balanced healthcare. The altered expression of miRNA has been correlated with diabetes and may be used to identify high-risk individuals prone to getting diabetes. There is a need to confirm the present findings in larger replication considering the number of participants involved in our study. Further studies are needed for establishing association of miR-155 and T2DM in Kashmiri population. The results may be important in the ongoing effort to diagnose T2DM subjects paving way to timely prevention and reversibility of risk factors. This study has the potential to aid in earlier identification of high risk individuals and better management of the early disease. However, the study was limited by its study design, a relatively low number of subjects, methodology followed, and multiple statistical testing.

Our finding suggests that miR-155 may have some contribution to the protection against the development of T2DM in Kashmiri population because it was found to be downregulated in the diabetics compared to healthy control group. However, further replication study is required before a firm conclusion can be reached.

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Compliance with ethical standards

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

Ethics approval The study has been approved by Institutional Ethics Committee, Sher-i-Kashmir Institute of Medical Sciences, Srinagar under IEC/SKIMS Protocol #104/2017. This study involved human participants, and the procedures performed were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration.

This article does not contain any studies with animals performed by any of the authors.

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

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ORIGINAL ARTICLE

Human APRIL and FGF-21 and adhesion molecules in relation to cognitive function in elderly diabetic patients

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Abstract

Aim A diverse combination of etiologies such as vascular and inflammatory factors and social and physical inactivity may take place in the etiology of cognitive dysfunction (CD). Diabetes mellitus (DM) may contribute to CD over insulin resistance, inflammation, and vascular risk factors. However, mechanisms included in the process are not very clear. We aimed to investigate serum levels of selected biomarkers as a proliferation-inducing ligand (APRIL), FGF-21, P-selectin, soluble vascular cell, and intercellular adhesion molecules-1 (sVCAM-1 and sICAM-1) in elderly patients with DM in relation to cognitive function.

Methods A group of 80 elderly type 2 diabetic patients from the outpatient clinic, consisting of 40 patients with CD (mini-mental state examination (MMSE) score < 24) and 40 individuals without CD were enrolled in the study. Anthropometric, sociodemographic, and functional-glycemic evaluations were determined. Biomarker levels were determined by enzyme-linked immunosorbent assay.

Results Median sICAM-1 and FGF-21 levels were higher, and P-selectin level, activities of daily living (ADL), instrumental ADL, MNA, and MMSE scores were lower in the CD group ($p = 0.002$, $p = 0.010$, $p = 0.001$, $p = 0.001$, $p < 0.001$, $p = 0.005$, $p < 0.001$, respectively). There was no significant difference between the groups regarding age, gender, living status, education, cigarette and alcohol consumption, antidiabetic therapy as well as comorbidities such as hypertension and other diseases, depression, body composition, sVCAM-1, APRIL levels, and related biochemical values.

Conclusion Median sICAM-1 and FGF-21 levels were higher and P-selectin level was lower in older diabetic patients with CD than in patients with normal cognitive status. Understanding the mechanisms may lead to the prevention or delay of CD in those patients.

Keywords Type 2 diabetes mellitus · Cognitive function · Adhesion molecules · FGF-21 · APRIL

Introduction

The population is getting older worldwide, and non-communicable diseases are becoming challenging as their prevalences increase by aging like dementia syndromes and diabetes mellitus. Predictions indicate a higher burden, composing nearly three-quarters of all deaths by the year 2020 [1, 2]. The number of diabetic patients was 425 million in 2017 and is estimated to rise to 629 million by 2045 [1]. Besides, about 35.6 million people suffered dementia globally in 2010, and the number of affected people is estimated to double almost every 20 years rising to 115.4 million in 2050, reflecting the severity of the situation [3].

Though there are numerous mechanisms suggested to be associated with cognitive dysfunction (CD) and dementia syndromes, the mechanisms and concrete solutions have failed to be addressed up to date [4]. Besides the well-established risk factors such as family history, age, sex, and genetic factors which are non-modifiable; lifestyle factors such as education, body mass index (BMI), smoking, and social network; and physiological risk factors or cardiovascular-cerebrovascular risk factors like type 2 diabetes mellitus (DMII) and

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hypertension may lead to cognitive decline [5, 6]. An increased risk of dementia in diabetic or prediabetic patients was not proven in some prospective studies; however, many population-based studies report such relationships [7, 8]. In selected recent studies, relations among diabetes and microvascular lesions (e.g., white matter hyperintensities likely due to demyelination and loss of axons) or Alzheimer's disease–associated neurodegenerative markers (e.g., cortical gray matter and hippocampal atrophy) were reported [7, 9–11]. Recently, in a population-based cohort study ($n = 2746$, age ≥ 60 years) with a 9-year follow-up, Marseglia et al. reported that diabetes and prediabetes might predict microvascular lesions among older adults without CD and accelerate cognitive decline in diabetes [7]. Ultimately, regarding the relationship among DMII and CD; chronic complications of diabetes may be involved over several mechanisms including increased cardiovascular risk factors and atherosclerosis and may involve insulin resistance and inflammatory mechanisms in addition to vascular pathology [6]. At the beginning and progression of atherosclerosis, the interaction of monocytes with the endothelium occurs with adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) [12]. P-selectin, a glycoprotein adhesion molecule located on platelets and endothelial cells, is involved in the adhesion of leukocytes to inflamed endothelium [13]. On the other hand, it has been shown in animal studies that cell adhesion molecules of the selectin family are required in the deployment of inflammation to the brain from the periphery in the context of liver inflammation [14]. Besides, a proliferation-inducing ligand (APRIL), a tumor necrosis factor (TNF) superfamily (TNFSF) member expressed by monocytes, macrophages, dendritic cells, and T cells, is upregulated by a variety of immune cells in response to proinflammatory cytokines [15]. The members of TNFSF also play an important role in the activation, proliferation, differentiation, and migration of immune cells into the central nervous system (CNS), and some of them are known to control alloimmunity, autoimmunity, and immunity [16]. Lastly, fibroblast growth factor 21 (FGF-21) is reported to play an important role in the metabolic regulation as an endocrine hormone by increasing glucose tolerance and insulin sensitivity and is regarded as the missing link between peripheral metabolic tissues and the brain [17, 18].

Though the relationship between diabetes and dementia is documented, the exact mechanisms underlying cognitive decline in diabetes are not clear. Besides, it is suggested that peripheral inflammation may cause activation of immune responses in the CNS [13]. So, given the probable association of DMII and inflammation with cognitive decline, and the assumption of activation of immune responses in the CNS caused by peripheral inflammation [13], we hypothesized a relationship among cognitive function and adhesion molecules, APRIL, and FGF-21 in elderly patients with DMII. To the best of our knowledge, there is no study in the literature investigating cognitive function in diabetic older patients related to biomarkers such as APRIL, FGF21, and P-selectin. Accordingly, we performed this study to examine the role of these factors in elderly diabetic patients in relation to CD.

Materials and methods

Patients

Diabetic patients over 60 years of age admitted to the Department of Geriatrics and Neurology were consecutively enrolled in the study. Cognitive function was assessed by mini-mental state examination (MMSE) test where points ≤ 23 were evaluated as CD and points ≥ 24 were evaluated as normal cognitive status. Out of 180 patients admitted, individuals who did not want to participate in the study and did not give informed consent ($n = 27$); patients who were not evaluated with MMSE ($n = 8$); and patients with acute ($n = 34$) and chronic inflammatory disorders ($n = 17$), malignities ($n = 5$), severe heart failure ($n = 4$), acute cardiovascular ($n = 3$) and cerebrovascular disease ($n = 2$) were excluded from the study. Finally, two groups consisting of both 40 diabetic patients with CD and normal cognitive status were formed.

Anthropometric and socio-demographic data of the patients were recorded. Activities of daily living (ADL) and instrumental ADL (IADL) were assessed by Katz index and Lawton-Brody IADL, respectively, for which higher scores were suggestive of independency. Nutritional status was assessed by Mini-Nutritional Assessment (MNA), and Yesavage geriatric depression scale-short form (GDS-SF) was performed for depression assessment. Comorbidities were classified as hypertension and other diseases (other disease group comprised of conditions like thyroid disorders, goiter, chronic obstructive pulmonary disease, Parkinson's disease). Glycemic status regarding HbA1c levels, fasting, and postprandial glucose levels were evaluated retrospectively from hospital records that were recorded at the examination period of the study for the routine control process.

Biochemical analyses

Peripheral venous blood (8 ml) samples were taken from the patients. Venous blood samples were centrifuged for approximately 20 min (2000–3000 rpm) and serum phases were separated. The samples were stored at − 80 °C until they were analyzed.

Regarding serum soluble ICAM-1 and VCAM1 (sICAM-1 and sVCAM-1), P-selectin, FGF-21, and APRIL levels, human sICAM-1 (YH Biosearch Laboratory, Shanghai, China), human sVCAM-1 (YH Biosearch Laboratory, Shanghai, China), P -Selectin (YH Biosearch Laboratory, Shanghai,

China), FGF-21 (YH Biosearch Laboratory, Shangai, China), and human APRIL (SunRed Biotechnology Campany, Shanghai, China) were measured using enzyme-linked immunosorbent assay. For absorbance measurements, a Multiskan™ FC microplate photometer (Thermo Scientific, USA) was used.

Statistical analysis

For descriptive statistics, the mean ± standard deviations of the continuous variables and the frequency and the percentage of the categorical variables were determined. Normality was assessed by the Shapiro-Wilk test, Student's *t* test was used for those with parametric comparisons, and the Mann-Whitney *U* test was applied for those with nonparametric comparisons in the continuous variable group**.** Variables were expressed as means and standard deviations or medians with interquartile ranges (IQR) where available. The comparison of discrete groups was determined with χ^2, Fisher's exact test, odds ratio (OR), and 95% confidence interval (95% CI). A value of $p < 0.05$ was considered statistically significant. The version of Windows (25.0, SPSS Inc., USA) of Statistical Package for Social Sciences was used for the statistical tests.

Results

No statistically significant differences in age or sex were observed among patients with CD (age; median 73.5 (IQR) (12) years; 26 females, 14 males) and the group with normal cognitive status (age; median 71 (9) years; 33 females, 7 males) ($p = 0.130$ and $p = 0.126$, respectively). There was no significant difference between the CD group and the normal cognitive status group in terms of living status, education level, cigarette and alcohol consumption, and oral antidiabetic or insulin therapy, as well as comorbidities such as hypertension and other diseases, BMI, glycemic status, serum low-density lipoprotein, creatinine levels, and GDS-SF as shown in Table 1. There was a significant decrease in the DMII + CD group when the two groups were compared in terms of ADL, IADL, MNA, and MMSE scores ($p = 0.001$, $p < 0.001$, $p = 0.005$, and $p < 0.001$, respectively). Diabetic patients with mild cognitive impairment and moderate to severe dysfunction in the cognitive dysfunction group consisted of a nearly similar number of diabetic older patients with MMSE scores $18 \leq \text{MMSE} < 24$ ($n = 18$) and MMSE < 18 ($n = 22$), respectively. The characteristics of the CD group and the normal cognitive status group are shown in Table 1.

Median serum levels of sVCAM-1, sICAM-1, FGF-21, and human APRIL were higher in diabetic patients with CD than in diabetic patients with normal cognitive status, but lower for P-selectin. Among them, the levels of sICAM-1 and FGF-21 were significantly higher, and P-selectin level was significantly lower in the DMII + CD group than in the group with normal cognitive status ($p = 0.002$, $p = 0.010$, and $p = 0.001$, respectively). There was no significant difference between the two groups in terms of median sVCAM-1 and human APRIL levels as shown in Table 2 ($p = 0.100$ and $p = 0.256$, respectively).

Discussion

In the present study, we showed that serum sICAM-1, P-selectin, and FGF-21 levels were significantly different among the patients with CD and DMII than in the patients with normal cognitive status and DMII in a sample of older patients.

It is well known that cardiovascular risk factors like hypertension and hyperlipidemia, age, sex, education-social network, body composition, and smoking may lead to cognitive decline [5, 6]. Depression is common in older diabetic patients, and depression has been shown to be a predictor of CD among elderly patients with type 2 DM [19, 20]. However, the groups with and without CD in our study were similar regarding the aforementioned factors. This may be because of the small sample size and the cross-sectional design of the study and exposure to similar factors arising from DMII presence and older age in both the groups. Cognitive dysfunction and physical dysfunction are common in older adults with DMII [21]. Besides, dementia is associated with functional decline and this condition presents an increased burden for the patients, carers, and nations all around the world. As expected, the CD group of diabetic patients had significantly lower ADL and IADL scores in this study. Patients with diabetes and CD may have a spectrum ranging from mild cognitive impairment to severe dysfunction [21]. The cognitive dysfunction group consisted of a nearly similar number of diabetic older patients with $18 \leq \text{MMSE} < 24$ ($n = 18$) and MMSE < 18 ($n = 22$) in our study. Additionally, glycemic status and antidiabetic therapy regimen may affect the cognitive state. In a recent study, no significant relationship has been reported among CD and treatment adherence [22]**.** In a study from Turkey, poor glycemic control has been shown to be negatively associated with cognitive performance in diabetic patients, but antidiabetic therapy regimen was not related to the cognitive status [23]. The findings from studies on the impact of glycemic control on CD are inconsistent possibly owing to the lack of long follow-ups in studies with negative results [7]. Likewise, fasting blood glucose and HbA1c levels were similar in the CD group and in the group with normal cognitive status in our study design. Besides, antidiabetic regimen also did not differ among both the groups. Poor nutritional status is reported to be associated with cognitive decline [17]. Consistent with the literature, the patients with

Table 1 Characteristics of the diabetic older patients in relation to cognitive function

	Normal cognitive status ($n = 40$)	Cognitive dysfunction ($n = 40$)	OR (%95 CI)	p
Age (year)	71 (9)	73.5 (12)		0.130
Sex (male/ female), *n*	7/33	14/26	2.538 (0.88–6.54)	0.126
Living status, *n* (%)				
With spouse	28 (70)	27 (67.5)	1.000	–
At nursing home	8 (20)	10 (25)	1.296 (0.45–3.78)	0.634
Alone	4 (10)	3 (7.5)	0.778 (0.16–3.81)	0.756
Education level, *n* (%)				
Uneducated	0 (0)	3 (7.5)	–	–
Primary education	33 (82.5)	33 (82.5)	1.000	–
High school and above	7 (17.5)	4 (10)	0.571 (0.15–2.14)	0.406
Cigarette use (no/yes), *n*	7/33	9/31	0.731 (0.26–2.24)	0.781
Alcohol use (no/yes), *n*	10/30	15/25	0.556 (0.23–1.42)	0.335
Insulin use (no/yes), *n*	15/25	17/23	0.812 (0.35–2.08)	0.819
Oral antidiabetic use (no/yes), *n*	28/12	21/19	2.111 (0.81–5.35)	0.168
Hypertension (no/yes), *n*	14/26	14/26	1.000 (0.41–2.44)	0.999
Other Diseases[a] (no/yes), *n*	8/32	14/26	0.464 (0.16–1.28)	0.210
BMI (kg/m^2)	29.8 ± 5.5	28.3 ± 3.7		0.323
Fasting blood sugar (mg/dL)	143.5 (49)	127 (118)		0.746
HbA1c (%)	7.4 (2)	7.1 (1.6)		0.119
LDL cholesterol (mg/dL)	112.7 ± 34.6	122.8 ± 33.3		0.277
Creatinine (mg/dL)	0.95 (0.4)	0.91 (0.7)		0.994
Mini-mental state test score	28 (3)	17 (7)		< 0.001
Geriatric depression scale-short form score	3 (6)	1 (3)		0.070
Katz score	6 (1)	5 (4)		0.001
Lawton-Brody IADL score	14.5 (6)	5 (12)		< 0.001
MNA score	25.5 (5.8)	21.5 (7)		0.005

BMI, body mass index; *LDL cholesterol*, low-density lipoprotein cholesterol; *IADL*, instrumental activities of daily living; *MNA*, mini-nutritional assessment. [a] Goiter, thyroid disorders, chronic obstructive pulmonary disease, and Parkinson's disease

CD were at risk of malnutrition, though the patients in both the groups were similar in terms of body composition, and not underweight.

Beta-cell autoimmunity may lead to the activation of acute-phase response by hypersecretion of interleukins, C-reactive protein, and TNF-α in older adults with diabetes. Dysregulation in signaling by TNFRSF members can promote B cell survival and proliferation leading to autoimmunity and neoplasia [24]. The serum level of APRIL was reported to be lower in DMII and type I diabetic patients with respect to controls, and similar among patients with and without gestational diabetes mellitus [25, 26]. The serum level of APRIL was similar in both groups of older diabetics in our study regarding cognitive function. As we were not able to locate studies on cognition of older diabetic patients related to APRIL in the literature, we suggest that more studies should be executed in this regard.

Age, obesity, metabolic syndrome, and diabetes are found to be associated with increased FGF21 levels [17, 27, 28]. It is suggested that FGF-21 may act in a pleiotropic fashion in different tissues or organs, and it may possess beneficial effects on glucose and lipid metabolism and insulin sensitivity in animal-based studies [28]. Serum FGF-21 levels were shown to be increased in diabetic patients whether recently diagnosed or with a long duration of DMII compared with those in controls, in a previous study [28]. The authors suggested that the paradoxically elevated levels of serum FGF-21 might be related to defensive response or DMII may have caused resistance to FGF-21 activity and a compensatory up-regulation [28]. There is growing evidence on the beneficial effect of FGF21 on the brain, and mainly experimental studies indicate that FGF21 may improve cognition due to decreased oxidative stress and inflammation, and also increased antioxidants and anti-inflammatory molecules possibly via FGF receptor–independent mechanisms [29]. In a recent study on high-fat diet–induced obese diabetic mice, it was shown that rFGF21 has modulated diabetes-related CD [30]. In the study by Phrommintikul et al., cognitive impairment was associated

Table 2 Comparison of sICAM-1, sVCAM-1, P-selectin, FGF-21, and APRIL levels in relation to cognitive function

	Normal cognitive status ($n = 40$)	Cognitive dysfunction ($n = 40$)	p
sVCAM-1 (ng/mL)	687.9 (124.6)	718.5 (214.5)	0,100
sICAM-1 (ng/mL)	362.7 (88.7)	410.8 (156.8)	0.002
P-selectin (ng/mL)	96.8 (15.7)	67.9 (42.7)	0.001
FGF-21 (pg/mL)	309.7 (55)	334.7 (63.3)	0.010
APRIL (ng/mL)	13.8 (2.4)	14 (5.2)	0.256

sVCAM-1, soluble vascular cell adhesion molecule 1; *sICAM-1*, soluble intercellular adhesion molecule 1; *APRIL*, a proliferation-inducing ligand; *FGF-21*, fibroblast growth factor

with FGF21 level in non-elderly patients, but not in elderly patients with metabolic syndrome [17]. In our study, FGF-21 levels were higher in diabetic patients with CD. Research of FGF21 on cognition and DMII in humans is scarce, so there is a need for studies in humans.

Endothelial dysfunction and vascular markers are linked to white matter disease [31]. Cell adhesion molecules ICAM-1 and VCAM-1 are substantial for firm adhesion and transendothelial migration for which shed, soluble isoforms appear after induction of those on the cell surface by inflammatory mediators [32]. Increased sVCAM-1 levels have been reported in several diseases such as coronary and peripheral atherosclerosis, hypertension, diabetes mellitus, cognitive impairment, and Alzheimer's disease [33, 34]. In a study by Yoon et al. studying sICAM-1, VCAM-1, P-selectin, E-selectin, and fractalkine in 2017, they showed that higher circulating ICAM-1 at average ages 32 and 40 was associated with lower cognitive skills at an average age 50 [35]. However, in a population-based prospective cohort study, sICAM-1 and sVCAM-1 were not associated with dementia [36]. Elderly diabetic patients with mild cognitive impairment were shown to have elevated sVCAM-1 levels [37]. Increased sICAM-1 levels were associated with silent brain infarctions and periventricular white matter lesion progression predicting impairment in psychomotor function in DMII [38]. Patients with DMII and higher baseline sICAM and sVCAM levels were associated with a significant decrease in cerebral vasoreactivity, vasodilation, and decline in executive functions after 2 years of follow-up in a prospective study [39]. Serum sICAM-1 levels were significantly higher in the CD group in our study where median sVCAM-1 level was also higher in the CD group, but it was statistically nonsignificant. This situation might have occurred because of the size of our sample. Further prospective studies with a larger study population are needed. Among adhesion molecules, P-selectin belongs to the selectin superfamily and functions in leukocyte rolling, cell activation, and adhesion to the platelets and endothelial cells [31]. As the selectins take place in the initial contact of circulating immune cells with the vascular endothelium and this process may eventually lead to the transfer of immune cells across the blood-brain barrier into the CNS, increased P-selectin level may induce atherosclerosis and lead to poorer neurological outcomes and increased white matter lesions [31, 40, 41]. Higher P-selectin levels have been reported in DMII patients compared with healthy controls, and in a recent study, higher CD62P levels were shown to be associated with white matter lesions accompanied by CD [42, 43]. In a study with a small sample carried out in coronary artery disease patients, it was shown that patients with cognitive impairment presented significantly higher platelet activation compared with the patients without cognitive impairment [44]. In our study, serum P-selectin level was lower in elderly diabetic patients with CD. To the best of our knowledge, no other study has researched cognitive function of diabetic elderly patients in association with P-selectin. Older diabetic patients with or without CD share strong potent factors related to insulin resistance and aging, but several other confounding factors such as medications might complicate the results. So, studies on P-selectin examining the medications broadly might better show the relationships.

The strength of our study is that several biomarkers are analyzed in the same diabetic elderly population and some of them such as FGF21, APRIL, and P-selectin are novel for CD research in diabetic humans. The limitations of this study include the small sample size and the diverse composition of CD. However, a remarkable number of studies investigating the biomarkers we have researched have enrolled a similar number of patients. We did not compose a pure vascular or Alzheimer's disease group; we enrolled simply diabetic patients with scores over and below 24 in MMSE. A mixed pathology in elderly patients with CD might be expected, indeed. So, composing such a group was not intended to be reached. A detailed list of medications other than antidiabetic therapy was not available. Medications such as antihypertensive, anticoagulating, and lipid-lowering agents might have an impact on the levels of the biomarkers. Besides, the duration of diabetes was not also considered. Lastly, the cross-sectional design of the study makes it hard to reflect the results of a

temporal change. So, the results of our study should be interpreted considering those issues.

Conclusion

Diverse mechanisms in different patient groups need to be investigated regarding the complex mechanisms that may take place in the process and progress of cognitive decline. Diabetes mellitus is a disease which accompanies several chronic complications; CD is also noteworthy among them leading to all kinds of burden. However, the overlapping factors and the mechanisms included are not very clear. We showed that serum sICAM-1, P-selectin, and FGF-21 levels were significantly different in elderly diabetic patients with CD and in patients with normal cognitive status. Understanding the mechanisms and the relationships in the process of cognitive decline in diabetic elderly patients may lead to the prevention or delay of CD in those patients. Further longitudinal studies investigating the systemic process with biomarkers in a larger sample size with diverse glycemic status, antidiabetic treatment, and body composition are needed to explore future CD in humans.

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Compliance with ethical standards

Ethical approval Written informed consent was obtained from all participants or the guardians of the patients, and the study was approved by the Ege University Ethical Committee (decision no: 14-9.1/2).

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

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ORIGINAL ARTICLE

Relationship between serum cystatin C level and pregnancy complications and abnormal glucose tolerance at 6-week postpartum in patients with gestational diabetes mellitus

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Abstract

Objective This study aimed to investigate the relationship between serum cystatin C level and pregnancy complications and abnormal glucose tolerance at 6-week postpartum in patients with gestational diabetes mellitus (GDM).

Methods Clinical data of 298 cases of GDM delivered in Taizhou People's Hospital from January 2017 to June 2018 were retrospectively analyzed. According to the level of cystatin C before delivery, they were divided into normal group (≤ 1.1 mg/L) and elevated group (> 1.1 mg/L). The general situation, complications of pregnancy, and the incidence of abnormal glucose tolerance at 6 weeks after delivery were compared between the two groups. According to concomitant gestational hypertension in late pregnancy, the patients were divided into group A (GDM with hypertensive disorder complicating pregnancy (HDCP)) and group B (GDM without HDCP). The differences of age, parity, body mass index (BMI) and mean arterial pressure (MAP) in the first trimester of pregnancy, levels of cystatin C, creatinine, urinary microalbumin, and glycosylated hemoglobin in 24-week gestation and before delivering were compared between the two groups.

Results There was no significant difference in age and parity between the normal group and the elevated group ($p > 0.05$). The levels of BMI (25.06 ± 3.46 vs 26.34 ± 3.65 kg/m2), creatinine (38.76 ± 16.52 vs 59.75 ± 17.82 mmol/L), and urinary microalbumin (37.11 ± 49.20 vs 61.25 ± 43.52 mg/L) and the incidence of premature delivery (9.35% vs 17.8%), premature rupture of membranes (11.22% vs 20.94%), and abnormal glucose tolerance at 6-week postpartum (18.69% vs 42.93%) in the normal group of cystatin C were significantly lower than that in the elevated group of cystatin C ($p < 0.05$). There was no significant difference in the incidence of hypertension (8.41% vs 7.85%) and oligohydramnios (33.64% vs 24.61%) between the two groups ($p > 0.05$). The clinical characteristics of group A (GDM with HDCP) and group B (GDM without HDCP) were compared. The results showed that the levels of age, BMI, and mean arterial pressure (MAP) were significantly different ($p < 0.001$). The results of binary logistic regression analysis showed that the significance value of MAP was 0.005 after controlling age factors, which indicated that MAP was an independent risk factor of GDM with HDCP, and the OR value of MAP was 1.420 (1.109–1.817).

Conclusion The increase of cystatin C in patients with GDM does not increase the risk of HDCP, but it does increase the risks of premature delivery, PROM, and abnormal glucose tolerance at 6-week postpartum. Cystatin C is a biological index that may be valuable in predicting the occurrence of these diseases in GDM patients. The independent risk factor of GDM complicating HDCP is MAP.

Keywords Cystatin C · Gestational diabetes mellitus · Pregnancy complications · Abnormal glucose tolerance at 6-week postpartum

Introduction

In recent years, the incidence of gestational diabetes mellitus (GDM) has increased significantly. Diabetes mellitus can cause microangiopathy, kidney damage, and other organ damage, which are harmful to pregnant women and fetuses. The most common complications of GDM are fetal macrosomia,

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preeclampsia, and premature delivery [1]. However, the occurrence of these complications in patients with GDM is not only related to quality of blood glucose control.

Cystatin C is a cysteine protease inhibitor. Its main physiological function is to regulate the activity of cysteine protease. When the activity of cysteine protease increases, the remodeling of vascular wall will become more obvious. At the same time, the body increases the secretion of cystatin C through its own regulation mechanism. High level of cystatin C can balance the activity of cysteine protease and directly regulate the remodeling of vascular wall. Physiological hemodynamic changes of pregnant women in order to meet the needs of pregnancy are crucial to good pregnancy outcomes [2]; otherwise, they often lead to adverse pregnancy outcomes [3]. These physiological changes include reduced vascular resistance, increased cardiac output, and increased vascular content through sodium and water retention [4]. Cystatin C is undoubtedly playing an important role as a regulator of vascular wall remodeling in this process.

Cystatin C was considered as a biological index of early renal damage in the past, but now many studies have found that cystatin C also has many non-renal biological characteristics. Some studies suggest that cystatin c is associated with the onset of preeclampsia [5–9], but whether it has similar predictive value in patients with gestational diabetes mellitus is unknown. At the same time, some studies have suggested that cystatin c is associated with premature delivery and PROM [10]. Whether this biomarker is a predictor of adverse obstetric outcomes has not been determined yet [6, 10, 11].

Insulin resistance in pregnant women disappears quickly after delivery, and abnormal postpartum glucose tolerance suggests that abnormal glucose tolerance may exist before pregnancy without being detected. Impaired glucose tolerance is a type of glucose intolerance, i.e., pre-diabetes, which is a risk marker of diabetes and cardiovascular disease. Therefore, this part of the population is very worthy of attention. It is believed that cystatin C is closely related to the onset of diabetes mellitus [12–15], so whether cystatin C is related to postpartum impaired glucose tolerance in GDM patients is a question worth discussing, because this group of people has a high risk of developing diabetes in the future.

We designed this study to explore the relationship between cystatin C and pregnancy complications and impaired glucose tolerance at 6-week postpartum of GDM patients.

Materials and methods

Materials

We collected the data of 298 pregnant women diagnosed as GDM who had prenatal examination and delivery at Taizhou People's Hospital from January 2017 to July 2018. We used two grouping methods. The first grouping method was to divide patients into normal group (≤ 1.1 mg/L) and elevated group (> 1.1 mg/L) based on the level of cystatin C detected before delivery. The second grouping method was based on whether these patients complicated gestational hypertension in late pregnancy, and patients were divided into two groups: group A (GDM with hypertensive disorder complicating pregnancy (HDCP)) and group B (GDM without HDCP). The inclusion criteria included the following indicators: regular antenatal examination in our hospital, 75 g oral glucose tolerance test (OGTT) diagnosed as "GDM" in 24–28 weeks of pregnancy, received the standard treatment of GDM in our hospital (including diet therapy and drug treatment), and delivered in our hospital. The exclusion criteria included the following indicators: pregnant women known to have previous diabetes mellitus, chronic hypertension, kidney disease, or other systemic diseases.

In our hospital, GDM screening is routinely conducted at 24–28 weeks of gestation. All participants were instructed regarding the preparation for the OGTT. We use 75-g oral glucose tolerance test (OGTT) for the identification of GDM. The OGTT was performed in the morning, after fasting for at least 8 h. A GDM diagnosis was based on the following criteria established by the Ninth Edition of Gynecology and Obstetrics Published by People's Health Publishing House [6]: The three blood sugar values should be lower than 5.1, 10.0, and 8.5 mmol/L before and 1 and 2 h after taking sugar. GDM is diagnosed if any of the blood sugar values meet or exceed the above criteria. The same method and criteria were used to determine abnormal glucose tolerance at 6-week postpartum.

Methods

Demographic information and anthropometric measures were all collected in the hospital, when these patients accepted their first antenatal examination in the first trimester, including information of age, parity, pregnant time, adverse maternal history, smoking behavior, alcohol consumption, history of diabetes mellitus, chronic hypertension, kidney disease, and history of other systemic diseases. The height, weight, and blood pressure will be regulately measured in the first antenatal examination, and the weight and blood pressure will be measured in each antenatal examination. Other laboratory parameters included level of cystatin C, creatinine (Cr), glycosylated hemoglobin (HbA1c), and microalbuminuria (mALB) at 24–28 weeks of gestation and before delivery. In our hospital, blood routine examination, urine routine examination, and liver and kidney function examination are carried out in the first prenatal examination, 24–28 weeks, 34–36 weeks, and before delivery, while cystatin C, creatinine, and urinary microalbumin are included in these examinations. Patients diagnosed with gestational diabetes will be routinely tested for glycosylated hemoglobin at 24–28 weeks, 34–36 weeks,

and before delivery. We retrieved this part of data of those patients diagnosed as GDM. We still collected data of neonatal weight and Apgar score, and incidence of HDCP, premature delivery, premature rupture of membranes (PROM), oligohydramnios, and abnormal glucose tolerance at 6-week postpartum of these patients.

The age, parity, and body mass index (BMI) in the first trimester; the levels of Cr, mALB, and HbA1c before delivery; neonatal weight; neonatal Apgar score, and the incidence of HDCP, premature delivery, PROM, oligohydramnios, and abnormal glucose tolerance at 6-week postpartum were compared between cystatin C normal group and elevated group. The age, parity, and BMI in the first trimester; the mean arterial pressure (MAP) in the first trimester; and the levels of cystatin C, Cr, mALB, and HbA1c at 24–28 weeks of gestation and before delivery were compared between group A (GDM with HDCP) and group B (GDM without HDCP).

BMI was calculated as body weight (kg) divided by the square of the height (m). MAP was calculated as the sum of systolic pressure plus twice diastolic pressure divided by three. Glucose was determined by glucose oxidase method, serum cystatin C, and urine microprotein by immunoturbidimetry; creatinine by enzyme method; and glycosylated hemoglobin by ion exchange chromatography; reagent was provided by Roche kit; Hitachi Roche Modular P800 automatic biochemical analyzer was used.

Statistical analysis All the statistical analyses were performed using the SPSS 16.0 (SPSS, Inc.). The variables were presented as mean ± standard deviation. Differences among the grouping variables were compared by the Student's *t* test. Categorical variables were presented as frequency percentage, and intergroup comparisons were conducted using the Chi square test. $p < 0.05$ was considered statistically significant. Binary logistic regression analysis was used to find the independent risk factor of GDM with HDCP.

Results

Comparison of the clinical characteristics of cystatin C normal group (≤ 1.1 mg/L) and elevated group (> 1.1 mg/L).

The clinical characteristics of patients in cystatin C normal group (≤ 1.1 mg/L) and elevated group (> 1.1 mg/L) are presented in Table 1. A total of 298 pregnant women were enrolled in this study. There were 107 patients in cystatin C normal group and 191 patients in cystatin C elevated group. The average age and the average parity between these two groups had no significant difference ($p > 0.05$). The levels of BMI, Cr, and mALB in the normal cystatin C group were significantly lower than those in the elevated cystatin C group ($p < 0.05$). The incidence of premature delivery, PROM, and abnormal glucose tolerance at 6-week postpartum was significantly increased in the elevated cystatin C group ($p < 0.05$). And the level of HbA1c, neonatal weight, neonatal Apgar score, and the incidence of HDCP and oligohydramnios between these two groups had no significant difference ($p > 0.05$). The incidence of abnormal glucose tolerance at 6-week postpartum in all the 298 patients with GDM was 34.23%.

Comparison of the clinical characteristics of group A (GDM with HDCP) and group B (GDM without HDCP).

The clinical characteristics of patients in group A (GDM with HDCP) and group B (GDM without HDCP) are presented in Table 2. A total of 298 pregnant women were enrolled in this study, and the incidence of HDCP in these GDM patients after 28 weeks of pregnancy is 8.05%. There were 24 in group A and 274 in group B. There were significant differences between group A and group B, including age, BMI, and MAP (all $p < 0.001$). There was no difference in the cystatin C, Cr, mALB, and HbA1c levels between the two groups (all $p > 0.05$), as well as parity ($p > 0.05$). The results of binary logistic regression analysis are presented in Table 3, with GDM combined with HDCP as the dependent variable, age as the control variable, and BMI and MAP as the independent variables. The results showed that the significance value of MAP was 0.005 after controlling age factors, which indicated that MAP was an independent risk factor of GDM with HDCP, and the OR value of MAP was 1.420 (1.109–1.817).

Discussion

In general, this study preliminarily explored the relationship between cystatin C level and pregnancy outcomes of GDM patients in Chinese pregnant women. In the first part of the study, we found the incidence of PROM, premature delivery, and abnormal glucose tolerance at 6-week postpartum significantly increased in GDM patients with elevated cystatin C level. However, there was no significant difference in the incidence of HDCP between cystatin C normal group and elevated group. The second part of the study further confirmed this point. In the second part, we found that the occurrence of HDCP in GDM patients was related to age, BMI, and MAP, and MAP was an independent risk factor.

Cystatin C was known as a marker of inflammation [16], and inflammation was associated with premature delivery and PROM. A previous study emphasized that the levels of cystatin C in early pregnancy in patients with premature delivery and PROM were significantly higher than those without such diseases (0.58 ± 0.07 vs 0.55 ± 0.07, $p = 0.041$; 0.58 ± 0.07 vs 0.55 ± 0.07, $p = 0.036$) [10]. It had a similar conclusion to our study. In addition, cystatin C was reported to be

Table 1 Comparison of the clinical characteristics of cystatin C normal group(≤ 1.1 mg/L) and elevated group (> 1.1 mg/L)

Characteristics	Cystatin C normal group	elevated group	*p*
Age (years)	30.56 ± 3.25	29.75 ± 5.60	> 0.05
Parity	1.67 ± 0.71	1.63 ± 0.72	> 0.05
BMI (kg/m^2)	25.06 ± 3.46	26.34 ± 3.65	< 0.05
Cr (mmol/L)	38.76 ± 16.52	59.75 ± 17.82	< 0.001
mALB (mg/L)	37.11 ± 49.20	61.25 ± 43.52	< 0.001
HbA1c (%)	5.49 ± 0.36	5.4 ± 1.48	> 0.05
HDCP *n* (%)	9(8.41)	15(7.85)	> 0.05
Premature delivery *n* (%)	10(9.35)	34(17.8)	< 0.05
PROM *n* (%)	12(11.22)	40(20.94)	< 0.05
Oligohydramnios *n* (%)	36(33.64)	47(24.61)	> 0.05
Neonatal weight (kg)	3640 ± 361.95	3498.13 ± 560.87	> 0.05
Neonatal Apgar score 1 min	8.56 ± 0.53	7.88 ± 1.20	> 0.05
Neonatal Apgar score 5 min	9.56 ± 0.53	9.13 ± 0.62	> 0.05
OGTT abnormal at Six-week postpartum *n* (%)	20(18.69)	82(42.93)	< 0.05

related to preterm delivery of preeclampsia patients, and the incidence of preterm delivery in patients with elevated cystatin C was significantly increased [17]. In addition, we also found that the levels of mALB and Cr significantly increased in the elevated cystatin C group. These three indicators were all biomarkers for evaluating the early renal damage in diabetes.

Interestingly, a recent study suggested that cystatin C levels were positively correlated with birth weight of newborns [18]. This can be explained by the potential associations between serum cystatin C levels and obesity and high BMI. However, according to the findings of our study, there was no significant correlation between cystatin C level and neonatal weight in GDM patients. The metabolic characteristics of GDM women lead to the increase of cystatin C, which may have something to do with it. It can be seen that the predictive value of Cystatin C is significantly different between GDM women and normal pregnant women.

From the past studies, we can see that the detection rate of abnormal OGTT in GDM patients at 6–8-week postpartum is very high. A 2003 study of Wu et al. [19] from China reported that the abnormal rate of OGTT in GDM pregnant women at 2-month postpartum was 45.6%. Another 2007 study of Yinchuan et al. [20] reported that the detection rate of abnormal OGTT in 6–8-week postpartum was 43.27%, also from China. Incidence of postpartum abnormal OGTT in GDM patients in this study was 34.23%, which was lower than that in previous studies. This may be related to the stricter diagnosis of GDM after the International Diabetes and Pregnancy Research Group (IADPSG) issued new diagnostic criteria for GDM in 2010.

The data showed that the incidence of postpartum abnormal OGTT was significantly increased in women with elevated cystatin C levels in the third trimester of pregnancy. Over the years, we have found that cystatin C is closely related to

Table 2 Comparison of the clinical characteristics of group A (GDM with HDCP) and group B (GDM without HDCP)

Characteristics	Group A	Group B	*p*
Age (years)	35.46 ± 3.40	29.33 ± 4.05	< 0.001
parity	1.64 ± 0.75	1.61 ± 0.72	> 0.05
BMI (kg/m^2)	30.45 ± 1.23	26.62 ± 3.53	< 0.001
MAP (mmHg)	95.89 ± 3.91	86.84 ± 6.74	< 0.001
Cystatin C in 24–28 weeks(mg/L)	0.95 ± 0.12	0.98 ± 0.18	> 0.05
Cr in 24–28 weeks (mmol/l)	46.46 ± 7.16	45.89 ± 8.23	> 0.05
mALB in 24–28 weeks(mg/L)	46.96 ± 35.18	45.22 ± 32.29	> 0.05
HbA1c in 24–28 weeks (%)	5.65 ± 0.31	5.63 ± 0.36	> 0.05
Cystatin C before delivering(mg/L)	1.19 ± 0.17	1.2 ± 0.25	> 0.05
Cr before delivering (mmol/l)	47.27 ± 6.88	46.93 ± 8.62	> 0.05
mALB before delivering(mg/L)	58.86 ± 43.11	56.52 ± 75.60	> 0.05
HbA1c before delivering (%)	5.72 ± 0.46	5.67 ± 0.38	> 0.05

Table 3 Risk factors for GDM with HDCP by binary logistic regression analysis

Step	Characteristics	B	S.E.	Wald	Sig.	OR	95% C.I. for OR	
							Lower	Upper
1	Age	0.418	0.103	16.412	0.000	1.519	1.241	1.859
2	Age	0.522	0.179	8.537	0.003	1.686	1.188	2.394
	BMI (km/m^2)	0.239	0.181	1.738	0.187	1.270	0.890	1.812
	MAP (mmHg)	0.350	0.126	7.740	0.005	1.420	1.109	1.817

insulin resistance [12–15]. Insulin resistance may still exist after delivery in GDM patients with elevated cystatin C and lead to abnormal postpartum glucose tolerance, although the underlying causal mechanism between them is currently unknown. For these patients, they still need close monitoring and dietary guidance after termination of pregnancy, because of a higher risk of postpartum abnormal glucose tolerance.

Cystatin C as high risk factor for preeclampsia in pregnant women has been emerging in recent years [5–9]. But in our study, we found that there was no significant correlation between the levels of cystatin C and the incidence of HDCP in GDM patients, but the increase of maternal age, BMI, and MAP were the risk factors.

Yogev et al. [21] found that higher maternal age ($p = 0.03$) and pre-pregnancy body mass index ($p = 0.03$) were both risk factors for preeclampsia in patients with GDM. The results of this study were consistent with those of ours. But after the binary logistic regression analysis of age control, we found that MAP was an independent risk factor of GDM with HDCP. It reminds us that in the perinatal care of patients with GDM, we should pay more attention to their MAP in early pregnancy. For patients with high MAP in early pregnancy, we may take some preventive measures to prevent the occurrence of HDCP.

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Compliance with ethical standards

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

Ethics statement This study was approved by the Ethics Committee of Taizhou People's Hospital in Jiangsu Province. It was designed in accordance with the principle of the Helsinki Declaration. Written informed consent was obtained from all of participants.

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ORIGINAL ARTICLE

The risk factors for tuberculosis patients with diabetes mellitus living in Western China: a retrospective study conducted from 2014 to 2018

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Abstract

Objective To study risk factors for tuberculosis (TB) patients with diabetes mellitus (DM) living in Western China and analyze the baseline characteristics and clinical data of those patients for developing an effective screening strategy.

Methods We enrolled 3548 TB patients who were admitted to our hospital from 2014 to 2018. The baseline characteristics and clinical data of TB patients with and without DM were compared. Besides, risk factors were presented, and their effects on TB patients with and without DM were analyzed.

Results The prevalence of DM among TB patients was 7.7%, which increased with elevation of the patients' age, and 63.1% of TB patients with DM had hemoglobin A1c (HbA1c) ≥ 7.0%. The prevalence of DM in the Han patients with TB was the highest (8.8%), which was roughly three times higher than that in the Tibetan patients with TB (3.0%). In the multivariate logistic regression analysis, elevated values of the patients' age (odds ratio (OR), 1.047 (1.033–1.062, $p < 0.01$), blood pressure (OR, 1.735 (1.101–2.734), $p = 0.04$), proportion of cavity in pulmonary TB (PTB) (OR, 2.167 (1.272–3.656), $p = 0.004$), fasting blood glucose (OR, 2.248 (1.997–2.555), $p < 0.001$), erythrocyte sedimentation rate (ESR) (OR, 1.007 (1.001–1.012), $p = 0.027$), and proportion of patients with PTB (OR, 2.426 (1.425–4.104), $p < 0.001$) were significantly associated with increased prevalence of DM in TB patients. For evaluation of the model, the receiver operating characteristic (ROC) curve was plotted, in which the area under the curve (AUC) value of 0.924 was obtained for an optimal cutoff value of 0.052. The re-sampling method was utilized to verify the regression model, and the mean squared error (MSE) was 0.00026.

Conclusions The prevalence of DM in TB patients is high and is associated with severe clinical symptoms. Therefore, early screening of DM for TB patients is highly recommended.

Keywords Tuberculosis · Diabetes mellitus · Risk factors · Western China

Introduction

Both tuberculosis (TB) and diabetes mellitus (DM) are common disorders worldwide. The global prevalence of DM increased from 4.7 to 8.5% since the 1980s, and it is estimated that the rate will rise to 552 million in 2030 [1]. In contrast, TB incidence has annually declined by 1.5% on an average; however, 10.4 million new (incident) TB cases and 1.4 million TB deaths have been reported in 2019 [2, 3]. Recently, a strong evidence showed that TB and DM have a vital link that can negatively influence TB treatment [4, 5]. According to a systematic review of 13 observational studies, DM could noticeably increase the risk of active TB [6]. A prospective study demonstrated that DM is an independent risk factor for TB patients after adjusting for confounding factors [7]. Using dynamic TB transmission models, Pan et al. pointed out that reducing the prevalence of DM by an absolute level of 6.6% to 13.8% may accelerate the decline of TB incidence by an absolute level of 11.5% to 2.2% and TB mortality by 8.7% to 19.4% in 13 high-burden countries [8].

China ranked the third for TB incidence among 30 high-burden countries, according to the latest report released in 2016 by the World Health Organization (WHO) [1], and stood

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in the first place in the world's number of DM cases, accounting for 110 million people in 2015, according to the International Diabetes Federation's statistics [9]. To date, 156 thousand adult diabetes patients with TB were reported in China, ranking the second worldwide, and 17% of adult TB cases were estimated to be attributable to DM [10]. A recently conducted national TB survey in China showed that the prevalence of active TB in individuals old aged ≥ 15 years is 459 per 100,000 and the prevalence of smear-negative TB is 66 per 100,000. As reported previously, the prevalence of active and smear-positive TB in the Western China is 695/100,000 and 105/100,000, respectively, which is markedly higher than that in the Eastern and Central regions [11]. On the other hand, the prevalence of DM in the Western China is lower than that in the Eastern and Central regions [12].

A number of scholars reported racial and ethnic differences in the prevalence of DM: the prevalence of DM for minority population is lower than that for the Han population [13]. To date, there were no data on the prevalence of DM for TB patients in the Western China. In the present study, we aimed to assess the prevalence of DM for TB patients living in Western China and analyze the baseline characteristics and clinical data of those patients to develop an effective screening strategy.

Study subjects and methods

Study subjects

This retrospective study conducted from January 2014 to December 2018 at the West China Hospital, Sichuan University, Chengdu, China. Equipped with a large-scale specialized tuberculosis care unit and the endocrine medical unit, this hospital can recruit a large number of patients with TB with and without DM. A total of 3548 patients (2073 men and 1475 women) were enrolled in the present study. Eligible patients were independently diagnosed by two respiratory physicians based on typical symptoms (e.g., low fever, fatigue, loss of appetite, cough, hemoptysis, and positive result of the Mantoux test), bacteriological evidence (smear microscopy, culture, and TB-DNA), radiological evidence of active TB, and appropriate response to antituberculosis chemotherapy. Among them, TB-DNA-positive was found in 100 patients, mycobacterium TB was detected in 29 patients, positive culture of mycobacterium tuberculosis was found in 2 patients, and the presence of cavity was observed in 32 patients' chest X-rays.

Experiment research

All the patients' baseline and clinical data were acquired from the hospital information system that included final diagnosis, age, gender, ethnic group, status of residence, body mass index (BMI), blood pressure, alcohol intake, smoking history, and clinical symptoms (e.g., cough, weight loss, fever, night sweats, and anorexia). The present study provided clinical and laboratory data of TB patients with and without DM as follows: (1) TB patients with DM may be accompanied by hepatic and renal dysfunction, due to their diseases and/or the usage of antituberculosis drugs. DM patients often present with dyslipidemia. Therefore, we employed a biochemical analysis of hepatic function, renal function, and lipid profiles. (2) Infectious diseases, such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV), are definite risk factors for TB patients [14–17]. Hence, we added viral serum indicators.

All laboratory data were completed in the Department of Laboratory Medicine of West China Hospital, and all results were comparable and traceable. Besides, fasting blood sugar level was detected by the hexokinase method, while standard methods identified lipid profiles, liver function, and renal function: all the parameters were measured by Cobas 8000 Modular Analyzer (Roche, Basel, Switzerland). Hemoglobin A1c (HbA1c) was measured by the high-performance liquid chromatography (HPLC; G8; Tosoh Bioscience, Griesheim, Germany), and erythrocyte sedimentation rate (ESR) was quantified by TEST1 (ALIFAX, Polverara, Italy). Additionally, hepatitis C virus (HCV), hepatitis B surface antigen (HBsAg), and hepatitis B core antibody (HBcAb) were detected by electrochemiluminescence (Cobas 601; Roche, Basel, Switzerland), HIV was confirmed by the Western blotting, and TB-DNA was assessed by quantitative polymerase chain reaction (qPCR; LightCycler® 480 System; Roche, Basel, Switzerland). TB-DNA has become a routine diagnostic testing method for TB patients in several countries, including China, with higher diagnostic accuracy than acid-fast bacilli (AFB) smear and culture [18]. The results of AFB smear and culture were interpreted by two qualified pulmonologists. Qualified pulmonologists interpreted X-ray or computed tomography (CT) findings of chest for diagnosing cavity. The cavity in pulmonary TB (PTB) is relatively thin walled, and there are often satellite foci around it. TB was diagnosed according to the WHO's guideline (2010), and diagnosis of DM was carried out according to WHO's guidelines (1999). Three qualified physicians verified the final diagnosis of TB and DM. HbA1c was used to assess DM with glycemic control, reasonable glycemic control was defined as HbA1C < 7.0%, and poor glycemic control was specified as HbA1C ≥ 7.0%.

Statistical analysis

Normally distributed data were expressed as mean ± standard deviation (SD), data with skewed distribution were presented as median (interquartile range [IQR]), and categorical variables were expressed as number (percentage). Continuous

variables (with normal distribution) were evaluated with the Student's t test, while differences in variations with skewed distribution were assessed using the Mann-Whitney U test. For categorical variables, differences between two groups and among three groups were compared by using the Fisher's exact and chi-squared tests, respectively. Moreover, the collinearity diagnosis was performed to avoid some confounding correlations. Multicollinearity was measured by variance inflation factor (VIF) and tolerance; multicollinearity was defined as $5 < \text{VIF} < 10$. Furthermore, variables that were associated at $p = 0.1$ level in the univariate analysis were included in the multivariate logistic regression analysis. BMI, smear, TB-DNA, and HBcAb were removed due to incomplete data. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to evaluate the risk of the mentioned factors in TB patients with DM. $p < 0.05$ was considered statistically significant. Statistical analyses were conducted by SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). R programming language (ver. 3.5.2) was used to plot the receiver operating characteristic (ROC) curve and nomogram.

Results

Baseline and clinical characteristics of TB patients with and without DM

The baseline characteristics of TB patients with and without DM are presented in Table 1. There were statistically significant differences in the data based on patients' age, gender, BMI, alcohol intake, high blood pressure, the number of Han patients, and presence of cavity in PTB between the group of TB patients with DM and the group of TB patients without DM (all $p < 0.001$). Additionally, for the Tibetan patients, significant differences were noted in the data based on patients' status of residence (i.e., the number of patients living in rural areas), smoking history, and presence of extrapulmonary TB (EPTB) between the group of TB patients with DM and the group of TB patients without DM (all $p < 0.001$).

Regarding clinical symptoms, cough (45.8%) was the most common prevalent symptom among all TB patients, and the number of patients presenting with cough was noticeably higher in the group of TB patients with DM than those without DM (58.6% vs. 44.7%, $p < 0.001$). The number of patients presenting with fever, night sweats, anorexia, and body weight loss was similar in both the groups (Table 1).

Regarding ethnic groups, the majority of patients were the Han patients (80.7%), the Tibetans (12.1%), and the Yi patients (5.2%). Regarding the prevalence of TB with DM, the Han patients had the highest portion (8.8%), which was about triple higher than the Tibetan patients (3.0%). According to the occupational analysis, we found that the majority of patients were retired (56.4%).

Significant differences in smoking history were found in the three ethnic groups ($p < 0.001$): 31.0% in the Han patients, 16.5% in the Tibetan patients, and 27% in the Yi patients. Alcohol intake was similar in the three ethnic groups. For clinical symptoms of TB, the number of Yi patients with cough and fever symptoms was remarkably lower than the Han patients and the Tibetan patients (both $p < 0.001$), and the number of Yi patients with night sweats was markedly higher than that of other two ethnic groups ($p = 0.006$). Tibetan patients had significantly higher weight loss than Han patients and Yi patients ($p = 0.008$). The number of patients with symptoms of anorexia was similar among the three ethnic groups. Regarding nutritional status (BMI and albumin), according to the ethnicity, it was unveiled that there were no significant differences in BMI among the three ethnic groups, and although significant differences were noted in albumin level among the three ethnic groups ($p < 0.001$), all the albumin levels were in the reference intervals. The details are presented in Table 2.

We further analyzed the laboratory parameters separately in PTB group and EPTB group. We found that there were significant differences between the two groups, including albumin level, fasting blood sugar level, ESR, smear-positive, culture-positive, TB-DNA, HCV, HIV, and HBcAb (Supplementary Table S2).

Effect of glycemic control on TB patients with DM

There were 249 patients in TB group with DM, and 63.1% (157/249) of patients had poor glycemic control (HbA1C $\geq$ 7.0%**)**. The proportion of patients with PTB (64.3% vs. 50.0%) and the proportion of patients presenting with night sweats (32.7% vs. 20.0%, $p = 0.0321$) were elevated among patients with HbA1C $\geq$ 7.0% compared with those with HbA1c < 7.0%. Additionally, the proportion of patients with positive-smear (38.1% vs. 28.6%, $p = 0.227$), smoking history (52.9% vs. 45.6%, $p = 0.269$), alcohol intake (45.9% vs. 35.6%, $p = 0.114$), presence of cavity in PTB (32.5% vs. 23.9% $p = 0.152$), and cough (61.1% vs. 53.3 $p = 0.223$) were elevated in group of HbA1C $\geq$ 7.0% compared with group of HbA1c < 7.0%. Moreover, in order to illustrate the figures visually more appealing, we illustrated the main findings using stacked column charts (Fig. S1a–g).

Risk factors for TB patients with DM

The clinical data of TB patients with and without DM are summarized in Table 3. The levels of fasting blood glucose, creatinine, triglyceride, ESR, proportion of positive-smear (35.1% vs. 15.1%, $p < 0.001$), proportion of TB-DNA-positive patients (52.5% vs. 30.7%, $p < 0.001$), and proportion

Table 1 Baseline characteristics and clinical data of the study subjects.

Characteristics	TB patients without DM (n = 3275)	TB patients with DM (n = 273)	p
Male (%)	1869/3275 (50.1)	204/273 (74.7)	< 0.001
Age (years old)	38.47 ± 17.78	56.61 ± 12.95	< 0.001
Body mass index (kg/m^2)	20.65 ± 3.75	22.31 ± 3.60	< 0.001
High blood pressure	452/3211 (14.1)	84/271 (31.0)	< 0.001
Ethnic groups			< 0.001
Han	2628/3275 (80.2)	254/273 (93.0)	
Tibetan	421/3275 (12.9)	13/273 (4.8)	
Yi	183/3275 (5.6)	4/273 (1.5)	
Qiang	16/3275 (0.5)	0/273 (0)	
Other	27/3275 (0.8)	2/273 (0.7)	
Occupation			< 0.001
Farmer	9/508 (1.8)	0/43 (0)	
Office clerk	30/508 (5.9)	0/43 (0)	
Worker	14/508 (2.8)	0/43 (0)	
Student	171/508 (33.7)	0/43 (0)	
Teacher	12/508 (2.4)	0/43 (0)	
Retired	268/508 (52.8)	43/43 (100)	
Unemployed	4/508 (0.8)	0/43 (0)	
Rural (%)	1531/3256 (47.0)	103/273 (37.7)	0.003
Smoking (%)	886/1121 (79.0)	136/271 (50.2)	< 0.001
Alcohol intake (%)	712/2550 (21.9)	111/271 (41.0)	< 0.001
Clinical symptoms			
Cough (%)	1459/3261 (44.7)	160/273 (58.6)	< 0.001
Fever (%)	1263/3260 (38.7)	99/273 (36.3)	0.419
Weight loss (%)	1104/3259 (33.9)	102/273 (37.4)	0.243
Night sweats (%)	851/3258 (26.1)	76/273 (27.8)	0.535
Anorexia (%)	1026/3261 (31.5)	93/273 (34.1)	0.374
Cavity (%)	337/3258 (10.3)	76/273 (27.8)	< 0.001
Type of TB			< 0.001
PTB only (%)	1055/3275 (32.2)	159/273 (58.2)	
EPTB only (%)	1758/3275 (53.7)	71/273 (26.0)	
PTB combined with EPTB (%)	462/3275 (14.1)	43/273 (15.8)	

Data were presented as mean ± standard deviation (SD) or absolute number (percentage)

TB tuberculosis, *PTB* pulmonary TB, *EPTB* extrapulmonary TB

of HBcAb-positive patients (78.2% vs. 57.9%, $p < 0.001$) were significantly higher in TB patients with DM compared with TB patients without DM (all $p < 0.05$). Albumin level in TB patients with DM was lower than that in TB patients without DM (all $p < 0.05$). Other laboratory parameters (i.e., alanine aminotransferase, low-density lipoprotein cholesterol, positive-culture, positive HIV, positive HCV, and positive HBsAg) were similar in both groups of patients.

In the final multivariate logistic regression model, elevated values of parameters, such as age (OR, 1.047 (1.033–1.062, $p < 0.01$), blood pressure (OR, 1.735 (1.101–2.734), $p = 0.04$), presence of cavity in PTB (OR, 2.167 (1.272–3.656), $p = 0.004$), fasting blood glucose (OR, 2.248 (1.997–2.555), $p < 0.001$), ESR (OR, 1.007 (1.001–1.012), $p = 0.027$), and proportion of patients with PTB (compared with EPTB) (OR, 2.426 (1.425–4.104), $p < 0.001$), were significantly associated with increased prevalence of DM in TB patients. Similar associations were not observed for sex, alcohol intake, ethnic groups, or other laboratory parameters (Table 4).

Besides, the receiver operating characteristic (ROC) curve analysis yielded an area under the curve (AUC) of 0.924 for an optimal cutoff value of 0.052 (95% CI, 0.817–0.878) (Fig. 1). In order to verify the regression model, we used the re-sampling method, and the mean squared error (MSE) was 0.00026 (Fig. 2).

Table 2 Lifestyle, nutritional status, and TB-related clinical symptoms of the Han, Tibetan, and Yi patients

Characteristics	Han patients (n = 2882)	Tibetan patients (n = 434)	Yi patients (n = 187)	p
Lifestyle				
Smoking history (%)	886/2862 (31.0)	71/430 (16.5)	50/185 (27.0)	< 0.001
Alcohol intake (%)	686/2861 (24.0)	87/431 (20.2)	42/185 (22.7)	0.315
Rural areas (%)	1239/2868 (43.2)	262/430 (60.8)	107/186 (57.5)	< 0.001
Clinical symptoms				
Cough (%)	1334/2872 (46.4)	205/431 (47.5)	67/186 (36.0)	< 0.001
Fever (%)	1138/2871 (39.6)	164/431 (38.0)	47/186 (25.3)	< 0.001
Weight loss (%)	963/2870 (33.6)	175/431 (40.5)	56/186 (30.1)	0.008
Night sweats (%)	726/2869 (25.3)	124/431 (28.7)	65/186 (34.9)	0.006
Anorexia (%)	914/2872 (31.8)	140/431 (32.4)	55/186 (29.6)	0.779
Nutritional status				
BMI	20.46 ± 4.42	20.52 ± 5.61	19.31 ± 4.58	0.062
Albumin (g/L)	39.21 ± 6.51	37.23 ± 6.07	37.12 ± 6.97	< 0.001

Data were presented as absolute number (percentage). *BMI* body mass index

Discussion

According to the global TB report released in 2019 by the WHO, China is still one of the countries with the heaviest burden of TB. Thus, we conducted the first sizeable hospital-based study to explore the association between DM and TB in Western China. The current study showed that the prevalence of DM in TB patients was remarkable and increased with the elevation of age.

In the present research, we found a high proportion (7.7%) of TB patients who suffered from DM, which was higher than that in the general population (4.6%). Being male, living in urban areas, and alcohol intake were the main characteristics of patients who were more susceptible to DM. Wu et al. carried out a retrospective analysis from 2007 to 2008 in a downtown area in Shanghai (China) and found that 19.9% of TB patients were complicated by DM [19]. Wang et al. reported a prevalence of 6.3% from 2010 to 2012 in rural areas of

Table 3 Laboratory data of study subjects

Parameters	TB patients without DM (n = 3275)	TB patients with DM (n = 273)	p value
Albumin (g/L)	38.54 ± 6.5	36.54 ± 6.97	< 0.001
ALT (IU/L)	17 (11, 29)	18 (11, 32)	0.483
Creatinine (umol/L)	61.3 (49.75, 74.5)	68 (54, 83)	< 0.001
Fating glucose (mmol/L)	5.04 (4.64, 5.49)	7.77 (6.42, 10.69)	< 0.001
Triglyceride (mmol/L)	0.99 (0.76,1.33)	1.29 (0.94, 1.79)	< 0.001
Total cholesterol (mmol/L)	4.00 ± 1.14	4.11 ± 1.18	0.129
LDL cholesterol (mmol/L)	2.27 ± 0.85	2.32 ± 0.88	0.408
Erythrocyte sedimentation rate (mm/h)	32 (6.95, 64)	56 (28, 91)	< 0.001
Smear-positive (%)	268/1776 (15.1)	59/168 (35.1)	< 0.001
Culture-positive (%)	8/408 (2)	0/22 (0)	0.187
TB-DNA-positive (%)	324/1056 (30.7)	42/80 (52.5)	< 0.001
HCV-positive (%)	23/2299 (1.0)	2/173 (1.2)	0.844
HIV-positive (%)	24/2296 (1.0)	2/173 (1.2)	0.891
HBsAg-positive (%)	230/2123 (10.8)	16/146 (11.0)	0.962
HBcAb-positive (%)	1129/2124 (57.9)	115/147 (78.2)	< 0.001

Data were presented as mean ± standard deviation (SD) or absolute number (percentage) or median (interquartile range [IQR]). *TB* tuberculosis, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *ALT* alanine aminotransferase, *HBsAg* hepatitis B surface antigen, *HBcAb* hepatitis B core antibody, *HIV* human immunodeficiency virus, *HCV* hepatitis C virus

Table 4 Logistics regression analyses for the association of DM with clinical characteristics and laboratory parameters (diabetics vs. non-diabetics)

	Univariate regression analysis		Multivariable regression analysis*		
Parameters	OR (95% CI)	*p*	Coefficient	OR (95% CI)	*p*
Age	1.058 (1.050–1.066)	< 0.001	0.046	1.047 (1.033–1.062)	< 0.001
Sex (female/male)	0.449 (0.339–0.596)	< 0.001			
Body mass index	1.103 (1.054–1.115)	< 0.001			
High blood pressure	2.742 (2.081–3.613)	< 0.001	0.673	1.735 (1.101–2.734)	0.004
Ethnic groups					
Tibetan	0.319 (0.181–0.562)	< 0.001			
Yi	0.226 (0.083–0.614)	0.004			
Status of residence	1.465 (1.136–1.889)	0.003			
Smoking history	0.372 (0.290–0.478)	< 0.001			
Alcohol intake	2.473 (1.914–3.194)	< 0.001			
Cough	1.749 (1.361–2.247)	< 0.001			
Cavity	3.344 (2.508–4.458)	< 0.001	0.773	2.167 (1.272–3.656)	0.004
Fasting blood glucose (mmol/L)	2.332 (2.131–2.553)	< 0.001	0.810	2.248 (1.997–2.555)	< 0.001
Albumin (g/L)	0.958 (0.941–0.975)	< 0.001			
ALT (IU/L)	1.001 (1.000–1.002)	0.098			
Creatinine (umol/L)	1.003 (1.000–1.008)	0.008			
Triglyceride (mmol/L)	1.642 (1.416–1.903)	< 0.001			
Total cholesterol (mmol/L)	1.087 (0.976–1.211)	0.129			
LDL cholesterol (mmol/L)	1.067 (0.920–1.238)	0.391			
ESR (mm/h)	1.008 (1.004–1.021)	< 0.001	0.007	1.007 (1.001–1.012)	0.027
Smear-positive (%)	3.046 (2.163–4.289)	< 0.001			
Tuberculosis-DNA-positive	1.283 (0.783–2.104)	0.332			
HBcAb-positive	2.615 (1.751–3.905)	< 0.001			
PTB (compared with EPTB)	3.7427 (2.792–4.987)	< 0.001	0.886	2.426 (1.425–4.104)	< 0.001

PTB pulmonary tuberculosis, *EPTB* extrapulmonary TB, *ESR* erythrocyte sedimentation rate, *ALT* alanine aminotransferase, *LDL* low-density lipoprotein, *CI* confidence interval. *Adjusted for sex, age, smoking history, alcohol intake, status of residence, blood pressure, ethnic groups, cough, cavities, fasting blood glucose, albumin, ALT, creatinine, triglyceride, ESR, type of TB

Shandong province in north China [20]. Li et al. demonstrated that prevalence of DM was significantly higher in patients living in urban areas (14.0%) than those in rural areas (10.6%), as well as being higher in hospitalized patients (13.5%) than those who attended the TB clinics (8.5%) [21]. The reason for the high rate of DM in TB patients has not been well understood yet, while it may be related to symptoms of TB, including fever, loss of appetite, and weight loss, directly influencing metabolism. Furthermore, studies showed that DM might exhibit delayed innate immunity and stronger adaptive immune responses to mycobacterium TB [22, 23].

In the current research, we, for the first time, indicated the proportion of TB patients with DM for different ethnic groups in Western China. Tian et al. reported that DM is a risk factor for the Han patients with TB, while the Tibetan patients were not involved [24]. In the current large sample study, the Han group's TB patients were more likely to suffer from DM, while the proportion of the Tibetan group's TB patients with DM was lower, and the proportion of the Yi and Qiang groups' TB patients with DM was similar to the Han patients. The smoking history, urbanization level, and clinical manifestation of TB patients were remarkably different among the groups in our study, which may be, in part, related to the fact that the proportion of TB patients with DM was different among the groups. Studies found that single-nucleotide polymorphisms (SNPs) were different between the Tibetan and Han populations [25, 26]. However, further research needs to be carried out to indicate whether SNPs among different groups are associated with the prevalence of DM in TB patients.

Compared with EPTB, PTB was found as an independent risk for DM in TB patients in the present research, which is consistent with results of a previous study [2]. TB is an infectious disease caused by the bacillus *M. tuberculosis*, typically influencing the lung. Radiographic manifestations of sputum smear-positive PTB patients with DM were reported earlier; however, the results are not consistent because of difference in

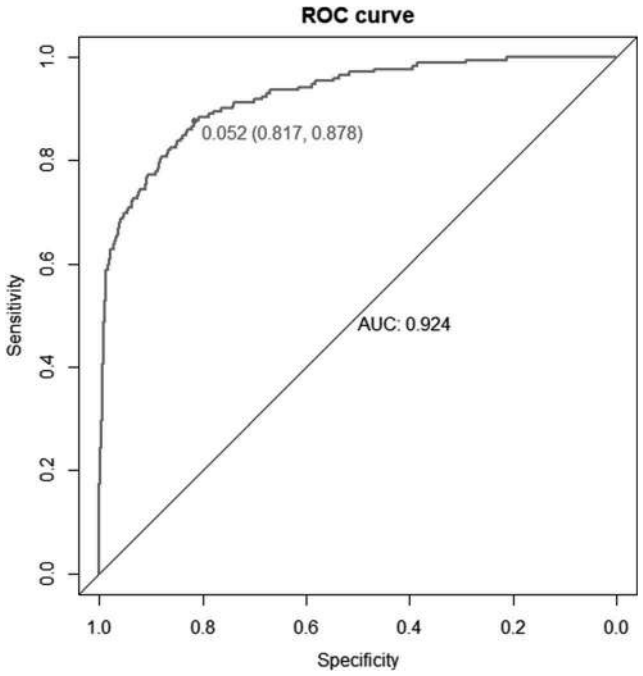

Fig. 1 ROC curve to predict the performance of the logistics regression model. The area under the curve (AUC) of 0.924 was achieved for an optimal cutoff value of 0.052 (95% CI, 0.817–0.878)

the number and baseline characteristics of patients [27, 28]. Magee et al. demonstrated that patients with DM had more cavities (OR 2.26) and higher prevalence of smear-positive TB (OR 2.37) in newly diagnosed TB patients [29]. The results of the current study indicated that the proportions of cavity and smear-positive TB were noticeably increased in TB patients with DM, reflecting that high-risk transmission and more severe lung performance may appear in TB patients with DM compared with those without DM.

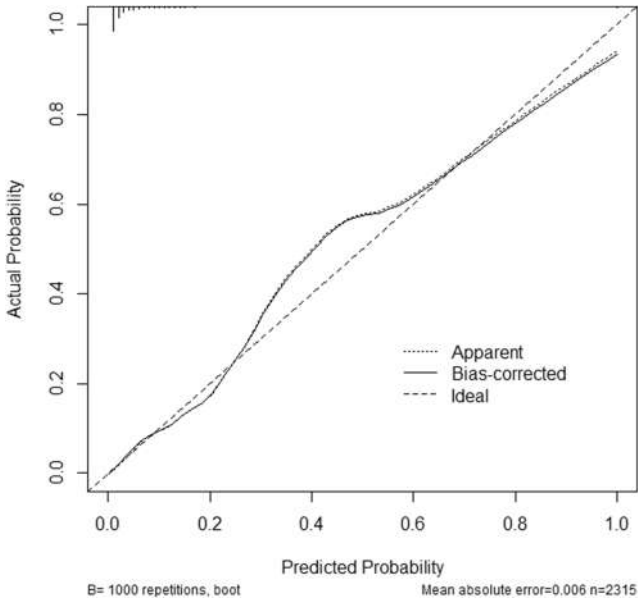

Fig. 2 Internal verification of the model by re-sampling. The mean squared error (MSE) was 0.00026

Previous researches primarily concentrated on the association of social-demographic characteristics and clinical features in TB patients with DM [30, 31]. However, insufficient data were collected with respect to laboratory parameters. In the current framework, it is highly essential to point out the association between TB patients with DM and numerous laboratory parameters. TB patients with DM had higher fasting blood glucose level, with a sensitivity that varied from 66 to 85%. A number of scholars reported the difference in the performance of lipid profile [20, 32]. The present research unveiled that although albumin level was not taken as a risk factor into account for TB patients with DM in multivariate logistic regression analysis, that level was lower in TB patients with DM compared with those without DM, demonstrating that patients with DM are undernourished and are unable to overcome the disease. Demir et al. reported that the occult HBV infection rate is higher in diabetics than that in healthy controls [14]. HBV infection is common in China; therefore, in the current research, we investigated the prevalence of HBV in our patients. In the proportion of HBsAg-positive patients, there were no significant differences between TB patients with and without DM, while the proportion of HBcAb-positive patients was higher in the group of TB patients with DM, suggesting a higher rate of HBV infection in those patients. Our study showed that the proportion of HIV-positive was similar in both groups.

Only 36.9% of the patients had adequately controlled diabetes (HbA1c < 7.0%) in the current study. Leung et al. assessed the effects of DM and diabetic control on tuberculosis risk with adjustment for sociodemographic and other background variables. They demonstrated that among diabetics, higher risks of active, culture-confirmed, and pulmonary TB were observed with baseline HbA1c ≥ 7% [27]. Sputum smear-positive PTB patients with type 1 diabetes had higher HbA1c level than those with sputum smear-negative PTB [33]. We also found that uncontrolled DM was associated with higher proportion of patients with PTB. An inverse correlation was observed between only EPTB and only PTB patients with uncontrolled DM. Further analysis demonstrated that it might result from high proportion of men, elevated age, and increased fasting blood glucose level (Supplementary Table. S2).

The outcomes of the current research showed that the proportion of smear-positive PTB cases was higher in subjects with HbA1c ≥ 7.0% compared with those with HbA1c ≤ 7.0%. The associations between HbA1c and mycobacterium TB may be related to impaired T cell-mediated immune responses and the decreased counts and proportions in peripheral blood mononuclear cells [34–36]. It is suggested that further attention should be given to control blood glucose levels in smear-positive PTB patients, in addition to the necessity of paying attention with high priority to DM patients with night sweats for glycemic control.

The main strength points of this study are that it presented a relatively complete clinical and laboratory data, highly assisting physicians, and visualization of the model which was more conducive for clinicians. In terms of limitations, firstly, since the study subjects were from a single tertiary hospital, this may restrict the generalizability of our results to the general population and other institutions or countries. Secondly, there is a certain degree of admission rate bias, for example, inpatients who tended to have more severe symptoms than outpatients; hence, we obtained the study conclusion only from the inpatients, and a multicenter study needs to be conducted to confirm our results. Thirdly, the detection of resistant genes was not included in this study because only a limited number of study subjects chose the test for economic reasons. However, given our study findings, it may be an effective method of screening specific groups of TB patients, including the elderly, presence of cavity in PTB cases, and patients with increased fasting blood glucose level.

Conclusion

In summary, according to the clinical manifestations and laboratory indicators, the status of TB patients with DM is more sensitive than those without DM. To effectively control these two diseases, early screening of DM for TB patients is essential, actively controlling the blood glucose level in TB patients as well as decreasing the spread of mycobacterium TB. To achieve more reliable data and elaborate the shortcomings of this study, a multicenter study is required.

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Compliance with ethical standards The study received approval from the Ethics Committee of West China Hospital, Sichuan University (Approval No. 198 (2014)).

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

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ORIGINAL ARTICLE

Sensorimotor impairments, postural instability, and risk of falling in older adults with diabetic peripheral neuropathy

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Abstract

Background Older adults with type 2 diabetes mellitus (DM) have high incidence of falls. The aim of this study was to compare sensorimotor functions, balance, mobility, fear of falling, and fall history in older people with DM (with and without neuropathy) and non-diabetic healthy controls.

Methods We enrolled 153 participants aged 50–70 years: 51 people with diabetic peripheral neuropathy (D-PN), 52 with diabetes without neuropathy (D-noPN), and 50 healthy controls (HC). Participants completed a fear of falling assessment and detailed test battery comprising sensorimotor functions, lower limb strength, contrast vision, reaction time, balance, and mobility from which a composite physiological fall risk score (PFRS) was derived. In addition, a fall history of the past 3 months was recorded.

Results Post hoc comparisons of ANOVA test revealed the D-PN had significant deficits than the other two groups in tests of lower limb sensation, knee extension strength, reaction time, postural sway, one leg standing, sit-to-stand and the timed up and go test. The D-PN had the highest fear of falling (30.18 ± 6.75) and the highest PFRS (1.68 ± 1.13). PFRS for the D-noPN (0.74 ± 0.80) was intermediate between HC (0.49 ± 0.96) and DP-N groups. Thirty-four D-PN participants (66.7%), 19 D-noPN participants (36.5%), and 7 HC (14.0%) reported one or more falls in the past 3 months (Chi^2 test for trend = 28.1, df = 2, $p < 0.001$).

Conclusions Older people with diabetic neuropathy have impaired sensorimotor function, balance, mobility, and associated increased fear of falling and fall rates. This population may benefit from fall risk assessments involving the above measures, and subsequent interventions targeted to deficits amenable to correction.

Keywords Postural control · Sensorimotor functions · Diabetic neuropathy · Fall risk

Introduction

Diabetes mellitus (DM) affects approximately 8.5% of people aged over 18 years [1] and more than 16% of people aged over 65 years [2]. With changing demography and higher rates of diagnosis, the prevalence of DM is expected to increase from 366 million in 2011 to 553 million in 2030 [3]. DM was previously considered as a disease of the developed world, but has now become a global epidemic, with two-thirds of the world diabetic population living in developing countries [4]. Sri Lanka is no exception, with prevalence studies undertaken over the past 30 years showing a relentless upward surge of DM in both urban and rural districts. In consequence, DM is having major impacts on sufferers and the health care system. A study carried out in 2005 in adults aged between 35 and 65 years in four provinces in Sri Lanka reported the prevalence of DM as 14.2% in men and 13.5% in women [5]. According to Sri Lanka Diabetes and Cardiovascular Study

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(SLDCS) which was carried out in the same year (i.e., 2005), the national prevalence of diabetes mellitus in Sri Lanka was 10.3% [6]. The SLDCS also reported that one in five adults in Sri Lanka has either diabetes or pre-diabetes and one-third of those with diabetes were undiagnosed [6].

Diabetic peripheral neuropathy (D-PN) is a common and costly chronic complication and a major factor that reduces mobility and quality of life of DM patients [7]. D-PN affects up to 50% of people with DM and usually starts with damage to the peripheral sensory nerves which progresses later to motor and autonomic nerves [8, 9]. National prevalence of D-PN in 2012, according to diabetic neuropathy symptom score among all patients, in patients with already established diabetes and newly diagnosed patients were 48.1%, 59.1%, and 28.8% respectively [10]. Diabetic neuropathy causes progressive loss of proprioception, reflexes, and lower limb strength, which lead to increased postural instability [11]. Studies have reported a significant association between D-PN severity and fall risk [12, 13] with one study reporting that people with DPN are 15 times more likely to experience a fall compared with healthy controls [14].

Risk factors in DM, and particularly for those with neuropathy, extend well beyond sensory loss. Previous studies have found that in addition to sensory loss and advanced disease status [15], muscle weakness [16], increased postural sway [17], gait and mobility impairments [18, 19], and fear of falling [15] increase fall risk in people with DM. However, previous studies have used only a limited range of tests to assess potential risk factors for falls, and many have used expensive high-tech equipment that is unavailable in most clinical settings throughout the world.

Therefore, the aim of this case control study was to compare a broad set of sensorimotor, balance, and functional mobility measures in community-living people with DM (with and without diagnosed peripheral neuropathy) and people without DM. We hypothesized that those with DM would have multiple physiological and functional impairments and significantly increased composite physiological fall risk scores, and this would be particularly the case for those with diabetic peripheral neuropathy. The study findings may elucidate explanatory and modifiable risk factors for falls in people with DM.

Materials and methods

Participants

One hundred fifty-three participants ($n = 153$; females = 68) comprised the study sample: 51 with diabetic peripheral neuropathy (D-PN group), 52 with non-neuropathic diabetes (D-noPN group), and 50 without diabetes (healthy control (HC) group). An endocrinologist examined all participants with diabetes. Peripheral neuropathy was confirmed using (a) validated symptom scores, (b) examination scores for D-PN, (c) nerve conduction time tests, and (d) vibration perception thresholds (see neuropathy assessment below in 2.3.1). If three out of four tests were positive, participants were classified as having D-PN. HbA1c tests were used to assess glycemic control in the D-PN and D-noPN groups and exclude undiagnosed diabetes in the HC group.

Inclusion criteria for the diabetes groups included having diabetes for more than 5 years, aged 50–70 years, living in the community, and being able to understand instructions and able to ambulate household distances without an assistive device. Participants in all groups with significant central nervous system dysfunctions, musculoskeletal deformity, or lower limb pathologies that affect balance were excluded.

Informed verbal and written consent was obtained from all the participants prior to study participation. The study was designed and conducted in compliance with the Declaration of Helsinki.

Anthropometric, demographic, and medical measures

Anthropometric, demographic, and medical information were obtained by means of an interviewer-administered questionnaire and clinical examination. Fear of falling was assessed with the Icon-FES [20]. The shortened version of Icon-FES scale is an interview-based questionnaire using a combination of pictures and matching short phrases. Icon-FES provides information on level of concern about falls for a range of activities of daily living. The shortened Icon-FES version contains 10 items scored on a 4-point scale (1 = not at all concerned to 4 = very concerned).

Physical assessments

Neuropathy and lower limb sensation Diabetic Neuropathy Symptom (DNS) [21] and Diabetic Neuropathy Examination (DNE) [21] scores were used for diagnosing diabetic polyneuropathy. The DNS score comprises four items: symptoms of unsteadiness in walking, neuropathic pain, paresthesia, and numbness. Each symptom is scored as 1 point with a score of 1 sufficient to indicate D-PN. The DNE score contains two items concerning muscle strength, one concerning reflexes, and five concerning sensation. Each item is scored from 0 (normal) to 2 (2 severely disturbed). The maximum score is 16 points, and a score > 3 points is indicative of D-PN.

Nerve conduction studies of tibial and sural nerve velocity, amplitude, and latency values were assessed by a neurophysiologist using a Natus Xltek nerve conduction device (Koll Center Parkway Suite, Pleasanton, CA, USA) [21]. Vibration perception thresholds of the big toe were measured using a Biothesiometer (Bio-Medical Instrument Co., OH, USA) [21].

Tactile sensitivity was assessed with a Semmes-Weinstein pressure aesthesiometer comprising 20 nylon filaments of equal length with varying diameter [21]. The filaments were applied to the center of the lateral malleolus, and pressure measurements were expressed as logarithms of the bending force in milligrams. Lower limb proprioception was measured using a lower limb matching task with participants sitting and eyes closed. Errors in matching the great toes were recorded using a protractor inscribed on a vertical clear acrylic sheet (60 × 60 × 1 cm) placed between the legs [22].

Muscle strength Maximal isometric quadriceps strength was measured in both legs while participants were seated on a high chair (so that feet did not touch the floor) with the hips and knees flexed to 90° [22]. A strain gauge was fixed horizontally with straps on the lower shin, after which the participant was given three attempts with the dominant leg to push against the strap as forcefully as possible. In addition, the five times sit to stand test was administered [23]. Participants were given a test trial and then the second trial was taken as the test result.

Balance and gait Postural sway was assessed using a sway meter that measured displacements of the body at the level of the waist. Testing was performed with participants standing on the floor and on a foam rubber mat (40 × 40 × 15 cm thick) with eyes open and closed [22]. Sway path (number of mm squares traversed by the sway meter pen) for each 30 s test was recorded. Standing balance was also assessed by timing how long participants could stand on one leg maximum of 30 s [24]. Functional mobility was assessed with the Timed Up and Go (TUG) test [25]. Participants were asked to rise from a chair, walk forward 3 m at their usual walking pace, turn 180°, walk back to the chair, and sit down. The TUG time was obtained by timing how long a participant took to complete the whole process.

Contrast vision and reaction time Visual contrast sensitivity was assessed using the Melbourne Edge Test, which presents 20 circular patches containing edges with reducing contrast [22]. Correct identification of the orientation of the edge on the patches provides a measure of contrast sensitivity in decibel units, where 1 dB = 10 log10 contrast. Simple reaction time in milliseconds was assessed using a light as a stimulus and a button press as the response [22].

Physiological fall risk Physiological fall risk was estimated using the Physiological Profile Assessment (PPA) [22] which comprises five of the above physical tests evaluating key functions of the human balance system: lower limb proprioception, visual contrast sensitivity, knee extension strength, simple reaction time, and postural sway when standing on a compliant surface [21, 22]. The five PPA components were weighted to compute a composite PPA fall risk score expressed in standard (z-score) units, with high scores indicating poorer physical performance. In multivariate models, weighted contributions from these five variables provide a fall risk score that can predict community-dwelling older Caucasian people at risk of multiple falling over a 12-month period with 75% accuracy [26].

Falls

Falls were defined as unexpected events which resulted in the participant unintentionally coming to the ground, floor, or other lower level [27]. Participants who reported one or more falls in the past 3 months were defined as fallers.

Statistical analysis

Variables with right skewed distributions (tibial nerve velocity, tactile sensitivity, five time sit to stand time, sway, one leg stand tests, and TUG time) were normalized using log transformations. One-way ANOVAs with Tukey post hoc test between group comparisons were used in all analyses. Falls status was assessed with the Chi^2 test for trend. A 5% limit of significance was applied. Analyses were conducted using SPSS for windows (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) and Epi Info (Centers for Disease Control and Prevention in Atlanta, GA).

Results

Anthropometric, demographic, and medical measures

The D-PN, D-noPN, and HC groups were well matched for age, height, and weight. The D-PN group had longer disease duration than the D-noPN group, and both diabetes groups were taking significantly more medications than the HC group (Table 1). Twenty-six D-PN participants (51%) and five D-noPN participants (9.6%) were insulin dependent.

Lower limb sensation

There were significant differences among the three groups for all four lower limb sensation tests: tibial nerve conduction velocity ($p < 0.001$), vibration perceptual thresholds ($p < 0.001$), tactile sensitivity ($p < 0.001$), and proprioception ($p < 0.001$). Post hoc comparisons indicated both diabetes groups had significantly poorer scores in all tests compared with the HC group, and that the D-PN group had poorer scores than the D-noPN group in the vibration perceptual threshold and tactile sensitivity tests (Table 1). D-noPN group also has significantly poorer scores than HC in tibial nerve velocity, vibration perception threshold, proprioception, and tactile sensitivity (Table 1).

Table 1 Anthropometric, demographic, and medical measures for the D-PN, D-noPN, and HC groups

Risk factor	D-PN ($n = 51$)	D-noPN ($n = 52$)	HC ($n = 50$)	p value
Demographic/medical				
Age (years)	61.6 ± 6.2	61.5 ± 5.9	59.9 ± 7.3	0.347
Height (cm)	156 ± 8	157 ± 7	159 ± 8	0.161
Weight (kg)	60.9 ± 10.3	62.6 ± 8.4	62.4 ± 11.4	0.633
DM duration	17.5 ± 7.6	11.8 ± .7.0	0	< 0.001**
Number of medications	7.4 ± 2.5	6.3 ± 2.67	1.9 ± 2.9	< 0.001**
Neuropathy status				
Symptom score	2.33 ± 1.12	.44 ± .85	0.14 ± 0.35	< 0.001**
Examination score	5.33 ± 2.96	1.04 ± 1.33	0.20 ± 0.49	< 0.001**

**$p < 0.001$, *$p < 0.05$

Diabetes mellitus duration, number of medications, symptom, and examination score were significantly different among groups

Muscle strength, balance, gait, and reaction time

There were significant differences among the three groups for the simple reaction time ($p = 0.001$), knee extension strength ($p < 0.001$), five times sit to stand ($p < 0.001$) (Table 2), one leg stand ($p < 0.001$), timed up and go ($p < 0.001$), and sway on foam eyes open and eyes closed tests (Table 3). Post hoc comparisons indicated both diabetes groups had significantly slower five times sit to stand times compared with the HC group, and that the D-PN group had poorer scores than both the D-noPN and HC groups in the simple reaction time, knee extension strength, one leg stand, timed up and go, and sway on foam eyes open and eyes closed tests (Tables 2 and 3).

Falls, physiological fall risk, and fear of falling

Thirty-four D-PN participants (66.7%), 19 D-noPN participants (36.5%), and 7 HC (14.0%) reported one or more falls in the past 3 months (Chi^2 test for trend = 28.1, df = 2, $p < 0.001$).

Compared with the HC group, the D-noPN and D-PN groups had markedly increased odds of being a faller: 3.54 and 12.3 increased odds respectively. Mean PPA and Icon-FES scores differed significantly among the groups in the ANOVA, and post hoc analyses revealed that the D-PN group had higher PPA scores than both the HC and D-noPN groups and that Icon-FES scores differed significantly between each group. The PPA fall risk profiles for the D-PN and D-noPN groups are presented in Fig. 1. These profiles indicate that the D-noPN group had a similar profile to the reference group of people without diabetes aged 65 years and over, whereas the D-PN group had below average scores in the tests of proprioception, knee extension strength, reaction time, and postural sway.

Discussion

In this study, we found that sensorimotor, balance, and functional mobility measures were impaired in older people with

Table 2 Sensorimotor test performances for the D-PN, D-noPN, and HC groups

Sensorimotor function	HC	D-noPN	D-PN	F statistic	HC vs. D-noPN p	HC vs. D-PN p	D-noPN vs. D-PN p
Tibial nerve velocity# (m/s)	44.38 ± 6.77	40.89 ± 4.43	39.69 ± 6.95	8.49	0.013	< 0.001	0.392
Vibration perception threshold (v)	11.45 ± 3.95	16.34 ± 7.11	39.93 ± 9.43	226.98	0.002	< 0.001	< 0.001
Proprioception (degrees)	1.54 ± 1.35	2.25 ± 1.21	2.67 ± 1.45	10.24	0.015	< 0.001	0.212
Tactile sensitivity (bending force)	3.51 ± .58	3.80 ± .49	4.45 ± .93	23.83	0.039	< 0.001	< 0.001
Contrast sensitivity (dB)	20.84 ± 1.97	20.62 ± 2.12	19.86 ± 3.0	2.28	0.885	0.107	0.256
Knee extension strength (kg)	28.02 ± 8.76	24.94 ± 6.70	20.46 ± 7.71	12.14	0.115	< 0.001	0.011
Simple reaction time (ms)	241.59 ± 45.34	249.32 ± 43.12	275.76 ± 50.91	7.52	0.679	0.001	0.013
Five times sit to stand test (s)	11.14 ± 2.21	12.45 ± 2.58	14.23 ± 3.60	15.25	0.036	< 0.001	0.007

#n; D-PN = 39, HC = 52, DC = 49 (tibial nerve velocity was not detected in the remainder of participants)

High scores in the vibration sense, proprioception, tactile sensitivity, and reaction time, and low scores in the tibial nerve, contrast sensitivity, and knee extension strength assessments in D-PN group were reported compared with D-noPN and HC groups

Table 3 Balance, mobility, fear of falling, and risk of falling among the D-PN, D-noPN, and HC groups

Sensorimotor function	HC	D-noPN	D-PN	F statistic	HC vs. D-noPN *p*	HC vs. D-PN *p*	D-noPN vs. D-PN *p*
Sway floor, eyes open-path (mm)	64.02 ± 28.55	69.54 ± 31.70	74.24 ± 33.41	1.22	0.619	0.269	0.804
Sway floor, eyes closed-path (mm)	85.66 ± 40.54	84.56 ± 34.64	106.49 ± 54.01	3.21	0.969	0.059	0.098
Sway foam, eyes opened-path (mm)	159.86 ± 99.95	179.27 ± 84.41	223.35 ± 96.42	7.88	0.246	< 0.001	0.05
Sway foam, eyes closed-path (mm)	382.84 ± 138.19	418.54 ± 164.56	491.94 ± 188.08	5.75	1.000	< 0.001	0.003
One leg stand test (s)	28.60 ± 3.93	26.77 ± 5.71	15.93 ± 10.11	47.46	0.398	< 0.001	< 0.001
Timed up and go test (s)	6.51 ± 0.96	7.01 ± 1.1	8.51 ± 2.11	25.09	0.209	< 0.001	< 0.001
PPA fall risk score	.49 ± 0.96	.74 ± .80	1.68 ± 1.13	21.98	0.381	< 0.001	< 0.001
Icon-FES score	20.72 ± 7.75	24.65 ± 7.2	30.18 ± 6.75	21.78	0.019	< 0.001	< 0.001

High scores in the sway tests, timed up and go test, PPA, and Icon-FES assessments significantly higher in D-PN compared with D-noPN and HC groups

DM, and this is more so the case in people with diabetic neuropathy. Specifically, we found that those with diabetic neuropathy had significantly poorer knee extension strength, reaction time, standing balance, and mobility in addition to lower limb sensory loss. Such deficits across a diverse range of neuromuscular systems provide insight into why older people with DM are at increased risk of falls.

As expected, the D-PN group exhibited significant loss in all lower limb tests important for sensory integrity: tibial nerve conduction velocity, vibration perceptual thresholds, tactile sensitivity, and proprioception. However, while the D-noPN group did not have manifested neuropathy, they also had significantly impaired sensory loss in all four sensory measures, compared with the healthy controls. This indicates that there is

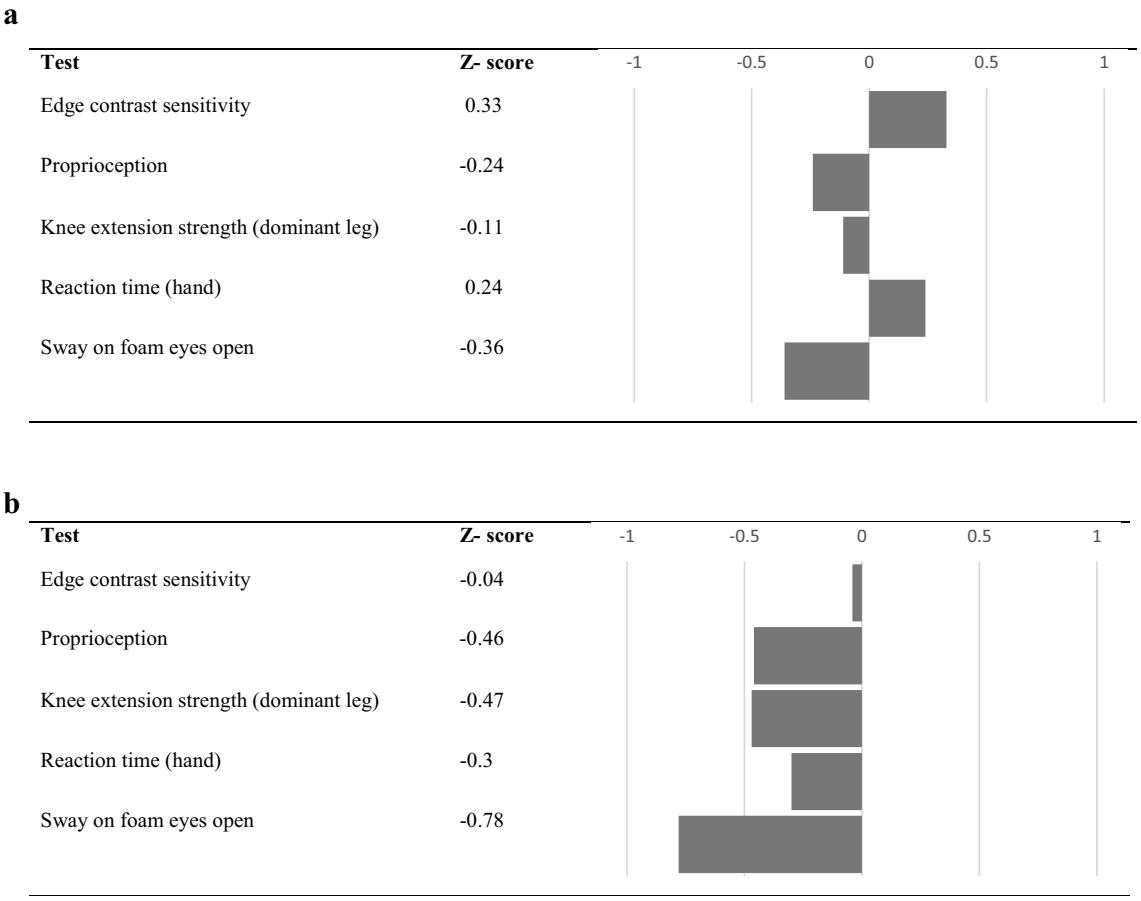

Fig. 1 Physiological falls risk profiles for the D-noPN (panel **a**) and D-PN (panel **b**) groups. Each bar represents a mean *Z*-score for the two groups based on normative values in community-dwelling people ≥ 65 [22]. Larger positive or negative *Z*-scores represent deviation from the general older population, with negative scores representing poorer performance

significant sub-clinical sensory loss in people with diabetes without manifested neuropathy that may warrant further assessment and management. These findings align with previous work that has reported impairments in proprioception and lack of sensory feedback cueing contribute to reduced postural control [28, 29] and impair balance recovery when people are exposed to unexpected perturbations [30]. Further deleterious effects are evident in people with D-PN adopting a rigid postural control strategy once they perceive increased sway. This stiffening strategy is associated with level of neuropathy and history of DM [28].

In addition to sensory loss, previous studies have documented that people with type 2 DM have reduced muscle mass [31–33], poor functional strength, balance, and gait impairments [34]. It has been shown that postural sway is increased in people with D-PN [35–37] and especially in eye closure test conditions [28, 38]. Some studies have reported the postural sway with eyes open in older people with D-PN is comparable with postural sway with eyes closed of healthy adults [30, 36]. Furthermore, D-PN patients tend to use a hip strategy rather than an ankle strategy for balance tests when they are deprived of visual inputs [39]. We also found that older people with DM have significant impairments in the physiological domains of strength, reaction time, and balance. It is likely that this impaired mobility as assessed with the TUG test results from impairments to multiple sensorimotor and balance domains, as it has been shown that these underpin this functional mobility measure [40].

DPN group has increased risk of falls compared with other groups. This elevated risk aligns with previous studies [12, 13, 41] that have been conducted both retrospectively [13] and prospectively [41]. Increased fall risk was associated with concomitant increases in concern about falling and physiological fall risk. The PPA profiles show the D-noPN group had similar test scores to a significantly older (i.e., 65+ years) reference group without diabetes and the D-PN group had scores below these reference levels indicating impaired functioning (Fig. 1). Furthermore, the average composite PPA score for the D-PN group of 1.68 indicates a marked risk of falls based on population norms [42].

Strengths of this study include the setting of the study in a developing country in which DM is a serious health issue, and the inclusion of a broad range of sensorimotor and balance measures and the recruitment of large samples of older community-living people with DM with and without peripheral neuropathy along with a well-matched group of healthy controls. We also acknowledge certain limitations. First, we did not include measures of executive functioning such as cognition or mood as part of the test battery, and factors implicated with fall risk in older people and clinical groups with balance impairment [43]. Second, falls were ascertained retrospectively. Further studies with large samples and prospective follow-up for falls are required to develop definitive fall risk profiles for people with DM.

Our findings reveal that older people with DM, with and without neuropathy, have multiple sensorimotor, balance, and mobility impairments, many of which are amenable to intervention. Most of the assessments used here comprise simple "low-tech" tests with scope for widespread use in clinical settings. The identification of specific impairments would aid greatly in diabetic management, through education, medication prescription, and lifestyle modification comprising healthy diets and regular exercise containing balance training.

In conclusion, the study findings indicate that older people with DM have impaired sensorimotor function, balance, and mobility, and associated increased fear of falling and fall rates. Older people with DM, and especially those with neuropathy, would benefit from fall risk assessments involving the above measures, and subsequent interventions targeted to deficits amenable to correction.

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Compliance with ethical standards This study was approved by the Ethical Review Committee of the Faculty of Medicine, University of Colombo (EC-15-166) and the National Hospital of Sri Lanka, Research Ethics Committee (ETH/COM/2016). All participants signed informed consent prior to their participation.

Conflict of interest The PPA (NeuRA FallScreen) is commercially available through Neuroscience Research Australia.

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ORIGINAL ARTICLE

Association between hemoglobin A1c and acute ischemic stroke among patients with type-2 diabetes: a case-control study

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Abstract

Background At present, stroke is a major health burden and the leading cause of death and long-term disability in the elderly. Ischemic stroke is the most common stroke type in the Thai population. Causes of ischemic stroke are likely to be multifactorial.

Objective The purpose of this study was to determine the association between hemoglobin A1c (HbA1c) and acute ischemic stroke (AIS) among Thai patients with type-2 diabetes (PTDs).

Methods A hospital-based case-control study was conducted among PTDs attending the Bhuddasothorn hospital with 100 cases and 300 controls from 2013 to 2016. Cases were defined as PTDs who had an AIS and diagnosed by neurologists and computed tomography (CT) scan and controls were PTDs who did not have AIS. Cases and controls were matched by gender, age (± 5 years), residential area, and attending duration. Data were collected using a questionnaire comprising 2 parts: demographic characteristics and medical data.

Results Conditional logistic regression was applied to estimate the effect of HbA1c on acute ischemic stroke among PTDs. Univariable conditional logistic regression showed risk factors for AIS among the PTDs comprised history of dyslipidemia, history of atrial fibrillation, diastolic blood pressure, systolic blood pressure, HbA1c, history of CVD, FPG, creatinine, and microvascular complications ($p < 0.05$). For multivariable conditional logistic regression, after controlling for potential confounders, it revealed a HbA1c of 8–8.9% and higher increased the risk of AIS by a factor of 7.9 and 10.9 times, respectively (OR = 7.9, 95%CI = 3.0–20.9; OR = 10.9, 95%CI = 4.3–27.9).

Conclusions Ongoing surveillance of HbA1c among PTDs should be conducted alongside knowledge sharing of glycemic control and stroke prevention as an essential measure to prevent developing AIS risk.

Keywords HbA1c · Acute ischemic stroke · Patients with type-2 diabetes

Introduction

Cerebrovascular diseases (CVD) mainly include ischemic stroke and hemorrhagic stroke. At present, it has been well established that stroke is the second leading cause of death worldwide according to ischemic heart disease [1, 2]. Its social burden increased from 38 to 61 million stroke cases between 1990 and 2020 [3]. The Global Burden of Disease, Injuries,

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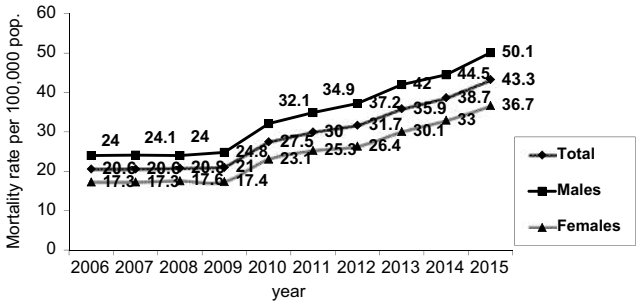

Fig. 1 Mortality rate of stroke. Thailand, 2006–2015

and Risk Factors Study (GBD) reported approximately 16.9 million new stroke cases in 2010 [4]. Most comprised ischemic stroke (11.6 million cases) ending in 2.8 million deaths [5–7]. The GBD has expected 12 million deaths and 200 million disability-adjusted life years (DALYS) lost from stroke in 2030 [4]. In Thailand, stroke is a major health burden and the leading cause of death and long-term disability in the elderly [8]. According to the data from the Thai's Ministry of Public Health, over 50,000 stroke deaths occur annually, presenting an increasing trend of stroke death rate during the past 10 years, as shown in Fig. 1 [9, 10].

Similar to other parts of the world, ischemic stroke is the most common stroke type in the Thai population. Causes of ischemic stroke are likely to be multifactorial. Effect of hyperglycemia showed higher risk of ischemic stroke, mainly in western countries [11–14]. In Thailand, quite a few studies have reported this association among PTDs. Therefore, the purpose of the study was carried out to determine the association between HbA1c and AIS occurrence among PTDs.

Variable definitions

Acute ischemic stroke is caused by a critical reduction of regional cerebral blood flow when the critical blood flow reduction lasts beyond a critical duration. One of the most widely used stroke schemes is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, which divides ischemic stroke into five categories: large artery atherosclerosis, cardiac embolism, small artery/lacunar occlusion, stroke of other determined etiology, and stroke of undetermined etiology [15, 16]. Diagnosis was determined based on the code of I63, the International Classification of Diseases, 10th revision (ICD-10). Acute ischemic stroke in the present study referred to PTDs had diagnosed with the neurologists and CT scan.

HbA1c is a form of hemoglobin that is measured primarily to identify the 3-month average plasma glucose concentration. It reflects an average plasma glucose over the previous 2–3 months. With the property of without fasting, it is a preferred test for assessing glycemic control among people with diabetes [17].

Methods

Study design, sample size, and sampling technique

A hospital-based matched case-control study (1:3) was conducted at the Bhuddasothorn hospital, Chachoengsao from 2013 to 2016 to identify the effect of HbA1c and AIS risk among PTDs. A total of 100 AIS cases and 300 controls were included in the study. The cases comprised PTDs newly diagnosed with AIS by neurologists and CT scan during the study period and controls were PTDs who did not have AIS. Of the 400 participants, cases and controls were matched by age (± 5 years), residential area, and duration of attending. Both cases and controls used medical records comprising demographic factors, laboratory and medical data, and AIS status. The sample size was calculated by the formula of Dupont [18], where P_o (0.04) and P_1 (0.138) were the proportions of exposure in controls and cases [19]; $Z_{\alpha/2} = 1.96$ at $\alpha = 0.05$; $Z_{\beta} = 0.84$ at $\beta = 0.20$. The calculated sample size was at least 91 among cases and 273 among controls. Subjects totaled 100 cases and 300 controls for the present study. The authors used the average medical data during the past 3 years for exposed factors.

Data analysis

Data were tabulated by means of descriptive statistics, univariable analyses, and multivariable analyses. Categorical variables were given as a frequency and percentage, crude odds ratio, 95% confidence interval (CI) of OR, and *p* value. Statistical descriptions were comprised of the mean, minimum and maximum, standard deviation (SD), frequencies, and

Table 1 General characteristics of cases and controls

Characteristics	Cases ($n = 100$)		Controls ($n = 300$)		*p* value[a]
	n	%	*n*	%	
Age gr. (years)					1.000
< 50	7	7.0	21	7.0	
50–59	21	21.0	62	20.7	
60–69	29	29.0	87	29.0	
≥ 70	43	43.0	130	43.3	
Mean (SD)	66.9 (11.1)		66.9 (10.9)		
Min-max	42–90		43–89		
Gender					1.000
Male	32	32.0	96	32.0	
Female	68	68.0	204	68.0	
Duration of DM (years)					0.584
< 5	20	20.0	75	25.0	
5–10	68	68.0	191	63.7	
> 10	12	12.0	34	11.3	

[a] Chi-square test

Table 2 Univariable conditional logistic regression analysis of characteristics associated with AIS patients

Characteristics	Cases (*n* %)		Controls (*n* %)		OR_c	95%CI	*p* value[a]
History of DLP							
No	79	79.0	296	98.7	1		
Yes	21	21.0	4	1.3	29.6	6.9–126.4	< 0.001*
History of AF							
No	94	94.0	299	99.7	1		
Yes	6	6.0	1	0.3	17.9	2.2–149.5	0.001*
Diastolic BP (mmHg)							
< 90	87	87.0	293	97.7	1		
≥ 90	13	13.0	7	2.3	7.1	2.5–20.1	< 0.001*
Systolic BP (mmHg)							
< 140	62	62.0	231	77.0	1		
≥ 140	38	38.0	69	23.0	2.2	1.3–3.8	0.003*
HbA1c (%)							
< 7	15	15.0	139	46.3	1		
7–7.9	15	15.0	65	21.7	2.2	1.0–4.9	0.07*
9–8.9	26	26.0	48	16.0	5.6	2.6–12.0	< 0.001*
≥ 9	44	44.0	48	16.0	9.5	4.6–19.8	< 0.001*
History of CVD							
No	93	93.0	294	98.0	1		
Yes	7	7.0	137	2.0	3.5	1.2–10.4	0.024*
FPG (mg/dl)							
< 126	22	22.0	127	42.3	1		
≥ 126	78	78.0	173	57.7	3.4	1.9–6.3	< 0.001*
Total cholesterol (mg/dl)							
< 200	70	70.0	233	77.7	1		
≥ 200	30	30.0	67	22.3	1.5	0.9–2.6	0.113
Blood creatinine (mg/dl)							
≤ 1	48	48.0	177	59.0	1		
> 1	52	52.0	123	41.0	1.7	1.0–2.8	0.037*
Active smoking							
No	86	86.0	273	91.0	1		
Yes	14	14.0	27	9.0	1.9	0.9–4.5	0.108
Alcohol consumption							
No	96	96.0	297	99.0	1		
Yes	4	4.0	3	1.0	4.0	0.9–17.9	0.070
Microvascular complications							
No	64	64.0	212	70.7	1		
1	22	22.0	68	22.7	1.1	0.6–1.9	0.741
> 1	14	14.0	20	6.6	2.4	1.1–5.2	0.024*
Body mass index (kg/m^2)							
18.5–22.9	34	34.0	88	29.3	1		
23.0–24.9	23	23.0	55	18.3	1.0	0.6–1.9	0.903
25.0–29.9	29	29.0	107	35.7	0.7	0.4–1.2	0.198
≥ 30.0	12	12.0	44	14.7	0.7	0.3–1.5	0.316
< 18.5	2	2.0	6	2.0	0.9	0.2–4.6	0.892

[a] Univariable conditional logistic regression analysis performed on 100 matched pairs

AIS, acute ischemic stroke; *DLP*, dyslipidemia; *AF*, atrial fibrillation; *BP*, blood pressure; *HbA1c*, hemoglobin A1c; *CVD*, cardiovascular diseases; *FPG*, fasting plasma glucose; OR_c, crude odds ratio; *CI*, confidence interval

*Significant at *p* value < 0.05

percentages. Univariable conditional logistic regression analysis was performed to differentiate proportional exposures between AIS patients and controls for categorical variables. Adjusted odds ratio and the 95%CI of OR were calculated from multivariable conditional logistic regression to examine associations between HbA1c and AIS occurrence, adjusted for potential confounding factors. All analyses were performed using the statistical software STATA (Release 12, StataCorp LP, College Station, TX, USA, serial number: 4012044037). A *p* value of < 0.05 was considered statistically significant in the analyses.

Results

Characteristics of sample

A total of 400 PTDs participated in the case-control study. The average age of subjects was 67 years. Table 1 outlined their demographic characteristics. To summarize, the majority were aged ≥ 70 years (43.0%, 43.3%), female (68%), and duration of DM 5–10 years (68.0%, 63.7%). As shown in Table 1, no

Table 3 Multivariable conditional logistic regression analysis of HbA1c associated with AIS patients

Variables	OR_c	95%CI	*p* value	OR_{adj}	95%CI	*p* value
HbA1c (%)						
< 7	1			1		
7.0–7.9	2.2	1.0–4.9	0.07	2.2	0.8–5.8	0.117
8.0–8.9	5.6	2.6–12.0	< 0.001	7.9	3.0–20.9	< 0.001
≥ 9	9.5	4.6–19.8	< 0.001	10.9	4.3–27.9	< 0.001

significant difference was observed regarding demographics at baseline among PTDs ($p > 0.05$).

AIS and risk factors

Using a univariable conditional logistic regression analysis, we found possible risk factors of AIS among PTDs included history of DLP, history of AF, diastolic BP, systolic BP, HbA1c, history of CVD, FPG, blood creatinine, and microvascular complications ($p < 0.05$), as shown in Table 2. Concerning multivariable conditional logistic regression analysis, HbA1c showed the association with AIS occurrence after controlling for possible confounding factors (family history of DLP, history of AF, diastolic BP, systolic BP, HbA1c, history of CVD, FPG, blood creatinine, and microvascular complications) and a HbA1c level of ≥ 8% was significantly associated with AIS. The higher the HbA1c level, the greater the odds of having an AIS (for HbA1c 8–8.9%: $OR_{adj} = 7.9$, 95%CI = 3.0–20.9; for HbA1c ≥ 9%: $OR_{adj} = 10.9$, 95%CI = 4.3–27.9), as shown in Table 3.

Characteristics of AIS patients

Cases were the newly PTDs with AIS diagnosed by neurologists and CT scan confirmation during 2013–2016 (incidence cases). Majority of them were with weight 65–74 kg (31%) and height 150–159 cm (51%). Considering on body size, 64% showed overweight and obesity.

OR_c, crude odds ratio; *AIS*, acute ischemic stroke; *CI*, confidence interval; *LRT*, likelihood ratio test *OR_{adj}*, adjusted odds ratio for history of DLP, history of AF, diastolic BP, systolic BP, history of CVD, FPG, blood creatinine, and microvascular complications

Discussion

Study participants comprised PTDs attending the Bhuddasothorn hospital, Chachoengsao Province. Finding from the present study, most comprised females (68%) and aged ≥ 70 years (43%). Results of multivariable analyses showed an association between HbA1c and AIS among PTDs when controlling potential factors which were consistent with related studies [20–22]. In addition, some studies found diabetic control was able to prevent from stroke [23, 24] while some studies found no association [25–28]). The present study showed 85% of HbA1c ≥ 7 mg/dl among PTDs with AIS.

Hyperglycemia was able to cause stroke because continual high blood glucose would activate the mechanism of glycation among glucose and amino groups. Then the advanced glycation end products (AGEs) were made and they would deviate the protein's function, such as low-density lipoprotein (LDL) at apo B, and then the abnormal LDL was caught and captured by macrophage; this mechanism would accumulate fatty plague at the endothelial wall that then made the endothelial malfunction, lack of elasticity, and atherosclerosis [29]. In summary, the excess blood glucose level had a direct effect on the endothelial wall of thickening and less elasticity. Accumulated plague would cause endothelial stenosis and occlusion, and reduce cerebral blood flow (CBF). When CBF is insufficient for the brain tissue, the brain cells would die and cause the ischemic stroke.

Primary prevention involves appropriate dietary control, for example, using the dietary approach to stop hypertension [30, 31], e.g., maintaining a cereal, vegetables, fruit, and low-fat diet. PTDs were a risk group of stroke [14, 32]. Therefore, glycemic control to normal level should be practiced continually to help reduce AIS risk. Currently, the American Heart Association and the American Stroke Association recommend that the ideal glucose level after AIS is between 140 and 180 mg/dl [33]. In addition, PTDs mostly present hypertension [34]. Therefore, hypertension control constitutes a crucial factor in reducing stroke risk [35–37]. The recommended blood pressure was < 130/80 mmHg [38]. At present, many tools are available to evaluate stroke, namely the Stroke Risk Quiz of the American Heart Association/American Stroke Association [39] and the National Institute of Health Stroke Scale (NIHSS) Neurologic Examination [40, 41]. For Thais aged 35–70 years, the Thai CV risk score [42] was made to evaluate stroke. There are some advantages of this case-control study. First, Bhuddasothorn hospital is the tertiary care center for the eastern part of Thailand. Second, they are easily identified, and provide sufficient numbers. Finally, cases were reduced classification bias. Some limitations of this study should be considered in interpreting the results. First, the study was a hospital-based matched case-control study; therefore, the representative of target population could not be mentioned. Second, selecting suitable controls was difficult. However, we matched cases and controls by age, residence, and duration of attending.

Conclusion

In summary, a surveillance system of HbA1c among risk groups should be conducted along with providing knowledge of stroke and self-care prevention, implementing health-promoting campaigns including proper regular exercise, healthy diet, weight control, risk avoidance such as smoking and alcohol consumption, and basic techniques for maintaining a healthy lifestyle among risk groups, as they will help extensively reduce risk of developing stroke [43, 44].

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Authors' contribution W.C. carried out the data collection and drafted the manuscript. W.B. undertook the literature searches. All authors conceived the study idea, participated in its design, and performed the statistical analyses. All authors have read and approved the final version to be published.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest on finance and non-finance.

Ethical approval The study protocol was reviewed and approved by the Ethics Committee for Research in Human Subjects of the Faculty of Public Health, Mahidol University (171/2557), the Ethics Committee for Research in Human Subjects of the Chachoengsao Public Health Office (PH_CCO_REC 004/58), and the Ethics Committee for Research of the Bhuddasothorn hospital (BSH-IRB 005/2558). The information was collected using medical records. Confidentiality was well maintained using an anonymous technique throughout the study to ensure privacy and the results were analyzed as a whole group.

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

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ORIGINAL ARTICLE

Inflammatory response and timeline of chronic complications in patients with type 1 and 2 diabetes mellitus

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Abstract

Background and aim The chronic complications of diabetes mellitus (DM) are accompanied by inflammatory manifestations. Our study aimed to assess the association between inflammatory status, reflected by C-reactive protein (CRP) values and the evolution of type 1 and 2 DM patients, evaluated by glycated hemoglobin (HbA1c) levels, the length of disease duration, the average time until the onset of microvascular complications and their risk of occurrence.

Methods We conducted a retrospective observational study, involving 192 patients, randomly selected from the medical records of the Centre for Diabetes Mellitus, Nutrition and Metabolic Diseases, Cluj-Napoca, Romania.

Results We noted significant differences between the two patient groups concerning HbA1c levels in patients with stage I nephropathy and CRP values in those with retinopathy. A significant positive correlation between the levels of studied biomarkers and disease duration was noted for type 1, but not for type 2 patients. We found a higher risk of chronic complications in patients with type 2, compared to those with type 1 DM: the relative risk was higher by 1.87 (1.59–1.97) times for nephropathy, 2.57 (1.56–4.18) times for retinopathy and 3.66 (3.00–3.82) times for neuropathy.

Conclusion Our study indicates a direct link between systemic inflammation and the timely progression of type 1 DM. In patients with type 2 DM, no statistical significance was found between the levels of studied biomarkers and the occurrence of microvascular complications. Nephropathy appeared sooner in type 1 DM patients, while retinopathy and neuropathy had a similar pattern of occurrence in both types of patients.

Keywords Diabetes mellitus · C-reactive protein · Glycated Hemoglobin · Inflammation · Chronic complications

Introduction

Diabetes is regarded as a major healthcare problem, currently affecting 463 million people worldwide. By 2045, it is estimated that this number will reach 700 million people. In Europe, the latest estimate showed that 59.3 million people were affected by diabetes, with a continuously rising prevalence. One of the most affected areas is Romania, with a prevalence of over 8.8% and a proportion of undiagnosed diabetes of 20.7% [1].

Diabetes mellitus (DM) is a chronic and complex disease, requiring continuous medical care, with multi-factorial risk-reduction strategies, that extend beyond glycemic control [2], in order to prevent or delay the onset of chronic vascular complications, represented by microvascular (neuropathy, retinopathy and nephropathy) and macrovascular lesions (coronary artery disease, peripheral arteriopathy and cerebrovascular disease) [3].

Vascular endothelial dysfunction is an important factor in the pathogenesis of micro- and macroangiopathy, both in type 1 and type 2 diabetes [4]. Endothelial lesions are the cornerstone for both the initiation and the progression of vascular complications [5]. The term endothelial dysfunction refers to endothelial damage by loss of its physiological properties and the occurrence of pathological attributes, such as prothrombotic, proinflammatory and vasoconstrictive properties [6].

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Besides chronic hyperglycemia, many other factors are involved in the pathogenesis of diabetes-related endothelial dysfunction, including the high sensitivity C-reactive protein (hsCRP). The C-reactive protein (CRP) is widely used in current clinical practice to monitor chronic and acute inflammatory conditions. Its elevated levels are positively associated with metabolic syndrome and have been recognized as an independent risk factor for coronary artery disease [7]. Some studies support the hypothesis that CRP, even within normal limits, may be an important precursor of metabolic syndrome and type 2 diabetes [8–10].

CRP belongs to the pentraxin family of calcium binding proteins. In humans, the molecule is composed of five identical, non-glycosylated polypeptide subunits, each containing 206 amino acid residues [11]. In clinical practice, CRP is a non-specific biochemical marker, useful for the screening of organic inflammatory disorders, for the monitoring of inflammatory response and treatment efficacy in infectious diseases, and for the detection of intercurrent infection in immunocompromised patients or in disorders characterized by modest or even absent acute disease response [12].

Glycated hemoglobin (HbA1c) is the result of non-enzymatic glycosylation of hemoglobin and is widely used as an indicator of glycemic control during the past 2–3 months. Besides its correlation with mean glycemic levels over the previous months, HbA1c is an important indicator of the risk of chronic complications. Its clinical usefulness has been demonstrated by the tight correlation with cardiovascular morbidity and mortality, indicating that an increase of only 1% in HbA1c may cause an increase of up to 10% in the mortality by cardiovascular events [2, 13].

The aim of this study was to assess the association between inflammatory status (reflected by CRP values) and the control (evaluated by HbA1c levels) and timely evolution of both type 1 and 2 DM (indicated by the length of disease duration, the average time span until the onset of chronic microvascular complications and their risk of occurrence).

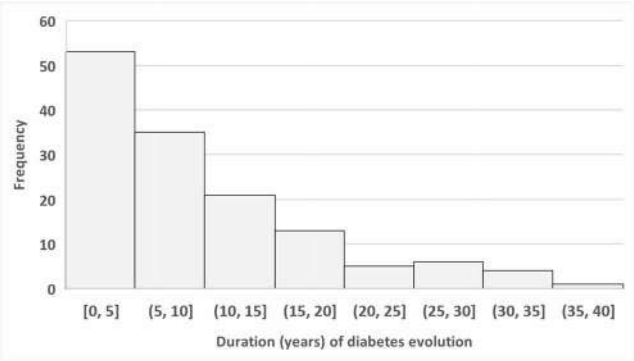

Fig. 1 Disease duration (in years) in patients with type 1 diabetes mellitus

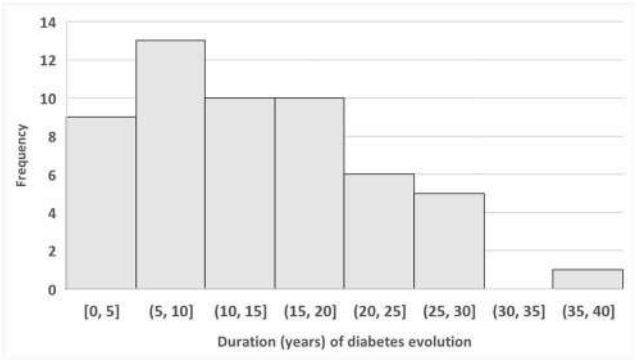

Fig. 2 Disease duration (in years) in patients with type 2 diabetes mellitus

Material and methods

Patients

We conducted a retrospective observational study, involving 192 patients with DM, diagnosed and followed up at the Centre for Diabetes Mellitus, Nutrition and Metabolic Diseases, Cluj-Napoca, Romania, between January 2018 and February 2019. Among them, 138 had type 1 and 54 had type 2 DM. Patients with confirmed diagnosis and complete medical data were enrolled. The diagnosis of DM and of its chronic complications were established according to the criteria of the American Diabetes Association [2]. Patients diagnosed with hematological disorders (e.g. anemia, thalassemia), cancer, nephrotic syndrome, liver cirrhosis, hypo or hyperthyroidism were excluded, as were those with an estimated glomerular filtration rate < 30 mL /min /1.73 m^2, patients undergoing anti-inflammatory treatment, pregnant or breast-feeding women or subjects currently affected by other inflammatory conditions, susceptible of interfering with CRP and/or HbA1c levels [14]. Type 1 DM patients were treated with personalized doses of long and short acting insulin, while type 2 DM patients were treated with hypoglycemia agents and/or long and short acting insulin.

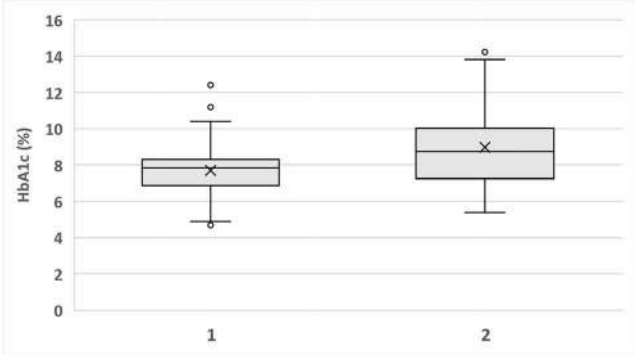

Fig. 3 Median values of glycated hemoglobin (HbA1c) in type 1 (left) and type 2 (right) diabetic patients

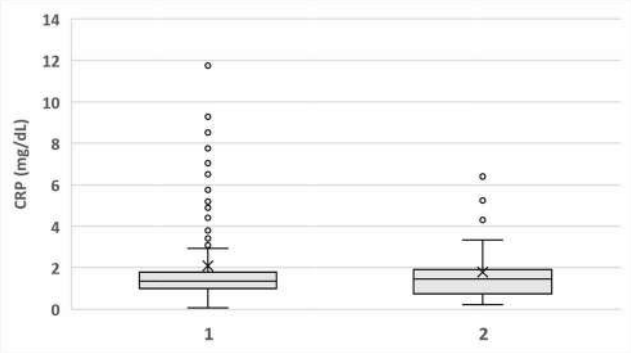

Fig. 4 Median values of C-reactive protein (CRP) in type 1 (left) and type 2 (right) diabetic patients

Study protocol

Data collection was performed using patient medical records and the following items were registered for each patient: the type of diabetes, the age and duration of disease evolution, the presence of arterial hypertension and of chronic microvascular complications (neuropathy, retinopathy and nephropathy), the serum levels of cholesterol, triglycerides, HbA1c and CRP. The diagnosis of diabetic neuropathy was based on clinical criteria [15]. For the diagnosis and staging of diabetic retinopathy we used the ETDRS (Early Treatment Diabetic Retinopathy Study) classification [16]. Diabetic nephropathy was divided into three stages, on the basis of urinary albumin to creatinine ratio (under 30 mg/g = stage I, 30–300 mg/g = stage II, over 300 mg/g = stage III) according to the KDIGO (Kidney Disease: Improving Global Outcomes) classification [17]. Hypercholesterolemia was defined by serum cholesterol levels above 200 mg/dL and hypertriglyceridemia as serum triglyceride concentrations superior to 150 mg/dL.

Statistical analysis

Statistical analysis was performed with the IBM SPSS V25 program. The normality of distribution of quantitative variables was tested using the Kolmogorov-Smirnov test. Non-parametric (Mann-Whitney U or Kruskal-Wallis) tests were subsequently used for data analysis, assuming significant differences for $p < 0.05$. Data linearity was checked with the Spearman correlation coefficient. Quantitative variables were presented as means and standard deviation or as medians and quartiles. For the description of qualitative variables, we used frequency tables, and contingency tables were employed to analyse their association.

Results

Description of clinical and biological parameters

Of the 192 patients included in our study, 138 (71.90%) had type 1 and 54 (28.10%) had type 2 DM. Their average age at the time of enrolment was 22.41 (± 9.75) years for those with type 1 DM and 64.22 (± 8.92) years for those with type 2 DM. The mean duration of disease progression was 9.95 (± 8.52) years in patients with type 1 DM (Fig. 1) and 14.37 (± 8.35) years in patients with type 2 DM (Fig. 2).

The median value of HbA1c was 7.85% in type 1 and 8.74% in type 2 DM. Median CRP values were 1.34 mg/dL (Fig. 3) and 1.44 mg/dL (Fig. 4) in patients with type 1 and 2 DM, respectively (Table 1).

Retinopathy was present in 24 of the 138 type 1 DM patients (17.39%) and in another 24 of the 54 patients with type 2 DM (44.44%). Neuropathy was diagnosed in 37 patients with type 1 (26.81%) and in 53 patients with type 2 DM (98.15%). Stage III nephropathy was present in 30 patients with type 1 (21.74%) and in 5 patients with type 2 DM (9.26%). Hypercholesterolemia was detected in 34 patients with type 1 (24.64%) and in 45 patients with type 2 DM (83.33%). A total of 27 patients with type 1 (19.57%) and 28 patients with type 2 DM (51.85%) had hypertriglyceridemia. Arterial hypertension was absent in 118 patients with type 1 (85.51%) and in 5 patients with type 2 DM (9.26%), while stage III hypertension was present in 29 subjects with type 2 DM (53.70%).

We noticed significant differences between the two groups regarding the age of patients, the duration of disease and the percentage of HbA1c ($p < 0.01$, Mann-Whitney U test). There

Table 1 Clinical and biological parameters in type 1 and 2 diabetic patients

	Age (years)		Disease duration (years)		HbA1c (%)		CRP mg/dL	
	Median (Q1-Q3)	p value	Median (Q1-Q3)	p value	Median (Q1-Q3)	p value	Median (Q1-Q3)	p value
Type 1 DM	22 (16–28)	**0.000***	8 (4–14)	**0.000***	7.85 (6.90–8.30)	**0.000***	1.34 (0.99–1.78)	0.752*
Type 2 DM	66 (58–70)		14 (8–20)		8.74 (7.24–10.02)		1.44 (0.73–1.90)	

DM: diabetes mellitus; HbA1c: glycated hemoglobin; CRP: C-reactive protein; Q1: quartile 1; Q3: quartile 3.

*Mann-Whitney U test, significance level at 0.05

Table 2 Distribution of glycated hemoglobin (HbA1c) and C-reactive protein (CRP) values in type 1 and 2 diabetic patients according to complications

	***Type 1* vs *type 2* DM**	***Type 1* vs *type 2* DM**	***Type 1 DM***				***Type 2 DM***			
	Hb A1c (%) p value	*CRP mg/dL p value*	*Hb A1c (%) Median (Q1-Q3)*	*p value*	*CRP mg/dL Median (Q1-Q3)*	*p value*	*HbA1c (%) Median (Q1-Q3)*	*p value*	*CRP mg/dL Median (Q1-Q3)*	*p value*
Nephropathy										
Absent	0.692	0.276	7.20 (6.50–7.90)	**<0.010***	1.31 (0.95–1.63)	**0.003***	7.71 (6.06–9.36)	0.825*	1.76 (1.45–2.07)	0.065*
Present, stage I	**0.030**	0.526	7.80 (6.85–8.00)		1.14 (0.85–1.34)		8.91 (7.13–10.03)		1.34 (0.61–1.87)	
Present, stage II	0.374	**0.047**	8.10 (7.80–8.90)		1.57 (1.31–3.56)		8.30 (7.24–9.97)		1.40 (0.54–1.78)	
Present, stage III	0.802	0.141	8.50 (8.10–9.30)		1.49 (1.02–4.98)		8.73 (7.31–10.36)		4.30 (2.08–5.25)	
Retinopathy										
Present	0.765	**0.043**	8.70 (8.15–9.20)	**<0.010****	2.35 (1.24–5.09)	**0.001****	8.74 (7.26–10.02)	0.821**	1.49 (0.62–2.07)	0.657**
Absent	**<0.001**	0.772	7.60 (6.70–8.10)		1.30 (0.99–1.63)		8.62 (7.24–10.02)		1.44 (0.76–1.84)	
Neuropathy										
Present	0.251	0.238	8.20 (7.90–8.90)	**<0.010****	1.49 (0.99–3.80)	0.194**	8.74 (7.25–10.02)	0.444**	1.43 (0.73–1.84)	0.407**
Absent	0.843	0.333	7.60 (6.80–8.20)		1.31 (1.01–1.67)		7.21 (7.21–7.21)		2.07 (2.07–2.07)	
Hyper-cholesterolemia										
Present	0.917	**0.022**	8.35 (7.90–9.30)	**<0.010****	1.72 (1.06–5.33)	**0.004****	8.50 (7.21–9.97)	0.198**	1.43 (0.73–1.80)	0.378**
Absent	**<0.001**	0.340	7.45 (6.70–8.20)		1.31 (0.97–1.64)		8.89 (8.45–10.36)		1.90 (1.15–2.68)	
Hyper-triglyceridemia										
Present	0.661	0.143	8.70 (7.90–9.30)	**<0.010****	1.72 (1.07–4.98)	**0.023****	8.74 (7.31–10.05)	0.723**	1.47 (0.77–1.82)	0.945**
Absent	**0.003**	0.854	7.60 (6.70–8.20)		1.31 (0.97–1.66)		8.60 (7.21–10.04)		1.43 (0.73–1.90)	
Arterial hypertension										
Absent	**0.001**	0.433	7.60 (7.30–7.90)	**<0.010***	1.31 (1.20–1.40)	**0.047***	10.40 (8.89–11.50)	0.123*	1.15 (0.54–1.38)	0.838*
Present, stage I	0.267	1.000	8.70 (8.20–10.40)		1.29 (1.07–5.90)		9.90 (8.45–13.82)		1.30 (0.43–2.68)	
Present, stage II	0.708	0.115	9.30(8.30–10.4)		1.87 (1.12–5.33)		9.07 (7.24–10.36)		1.51 (0.84–2.08)	
Present, stage III	–	–	–		–		8.30 (7.07–9.48)		1.50 (0.76–1.83)	

DM: diabetes mellitus; HbA1c: glycated hemoglobin; CRP: C-reactive protein; Q1: quartile 1; Q3: quartile 3.

* Kruskal-Wallis test, significance level at 0.05

** Mann-Whitney U test, significance level at 0.05

were no statistically significant differences between serum CRP values among the two groups.

Complication-induced differences in biological parameters among the two groups

In type 1 DM patients included in our study, we found statistically significant differences between HbA1c values and the presence of nephropathy, retinopathy and neuropathy (Table 2, Fig. 5). Regarding serum CRP levels in patients with type 1 DM, statistically significant differences were observed for the association with nephropathy, retinopathy and arterial hypertension, but not with neuropathy.

In the group of patients with type 2 DM, the differences between HbA1c and CRP values associated with either microvascular complications, or arterial hypertension, did not reach statistical significance (Fig. 6).

Statistically significant differences were noted between the two groups of diabetic patients concerning HbA1c levels in patients with stage I diabetic nephropathy, and CRP values in patients with retinopathy. On the other hand, the occurrence of diabetic neuropathy did not induce any statistically significant differences in either HbA1c, or CRP levels, between the two patient groups.

Evaluation of the risk of chronic complications according to the type of diabetes

Our results indicate a higher relative risk of chronic complications in patients with type 2, compared to those with type 1 DM. The relative risk of occurrence of nephropathy was 1.87 (1.58–1.98) times higher in type 2, than in type 1 DM. For retinopathy, the relative risk of occurrence was 2.38 (1.42–3.93) times higher in type 2, whereas for neuropathy, it was 3.66 (2.98–3.82) times higher in type 2, compared with type 1 DM.

Median time before the onset of chronic complications in type 1 and 2 diabetes

In patients with type 1 DM, we noticed a statistically significant difference between disease duration until the occurrence of either of the chronic microvascular complications

(nephropathy, neuropathy, and retinopathy) (Table 3, Fig. 7). In patients with type 2 DM, a statistical significance was reached only between disease duration until the occurrence of diabetic retinopathy, while for other microvascular complications, such as nephropathy and neuropathy, there were no significant differences between the lengths of disease evolution until their onset (Fig. 8). When comparing the length of disease progression between the two patient groups, the only complication for which the difference in time span reached statistical significance was stage I and II diabetic nephropathy.

Relation between biological parameters and the duration of diabetes

A significant positive correlation between the values of biological parameters (HbA1c and CRP) and the duration of disease progression was noted for type 1 DM patients. This positive correlation was also observed for the whole patient cohort, comprising both types of DM patients, but not for type 2 diabetic patients (Table 4).

Discussion

Upon comparing type 1 and 2 DM patients, we noticed statistically significant differences regarding the age of patients, with considerably younger subjects among type 1 DM. Accordingly, in those with type 2 DM, the duration of disease was significantly higher, as was the level of HbA1c, reflecting a lower glycemic control. There were no statistically significant differences in CRP values between the two groups, as was previously demonstrated by Zaghloul et al., indicating a similar risk of diabetes-induced endothelial dysfunction [18].

We noticed statistically significant differences in HbA1c values among type 1 diabetic patients with, and without microvascular complications: significantly higher numbers were found in patients with nephropathy (with highest values in stage III), retinopathy and neuropathy, compared to those without such complications. Regarding CRP levels within the same group, we found statistically significant elevations in association with hypertension and microvascular complications, with the exception of neuropathy. Our results support those of a recently published study by Baker et al., demonstrating that CRP values increased directly with the progression of nephropathy in type 1 DM, the highest values being recorded in stage III [19]. The association between inflammatory status and progression to renal impairment was also highlighted by Overgaard et al., demonstrating the usefulness of CRP in predicting micro- and macroalbuminuria in patients with type 1 DM, over a 30 years period of follow-up [20].

The link between diabetic retinopathy and systemic inflammation, reflected by circulating CRP levels, was demonstrated in a recent study conducted on type 1 diabetic patients, but the researchers pointed out that this association does not imply a direct involvement of inflammation in the initiation of retinopathy [21]. On the other hand, the EURODIAB study demonstrated a clear association between inflammatory biomarkers and both early and late stage retinopathy, suggesting the involvement of inflammatory response in those phases [22]. An earlier study also found evidence of an association between systemic inflammation, monitored by CRP levels, and type 1 diabetic retinopathy [23]. Our results are therefore in line with those of previous studies, indicating the need for further investigation of the role of inflammation in the pathogenesis of this complication.

In our patients with type 2 DM, no significantly higher levels of HbA1c and CRP were seen in association with either arterial hypertension, or microvascular complications, although Aryan et al. observed that CRP levels may serve as predictors of the onset of micro- and macrovascular complications [24]. Longitudinal studies in type 2 diabetic patients, compared with non-diabetic subjects, demonstrated a progressively increased inflammatory response, which precedes

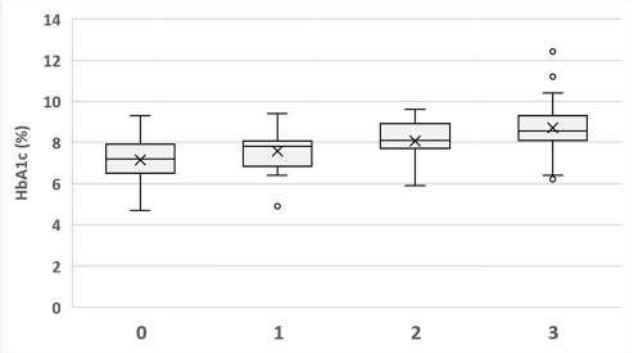

Fig. 5 Median glycated hemoglobin (HbA1c) percentage among type 1 diabetic patients without (0) and with various stages (1–3) of diabetic nephropathy

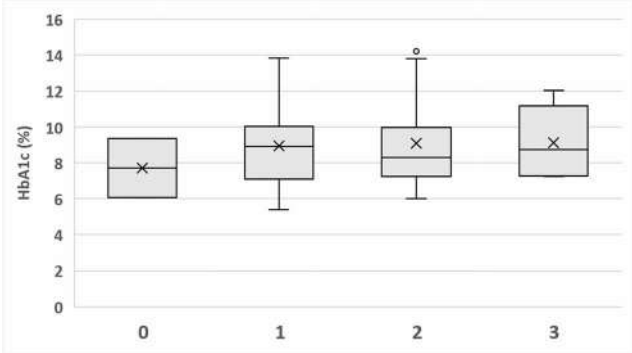

Fig. 6 Median glycated hemoglobin (HbA1c) percentage among type 2 diabetic patients without (0) and with various stages (1–3) of diabetic nephropathy

Table 3 Length of disease evolution until the onset of chronic complications in diabetic patients

		Type 1 DM					*Type 2 DM*			
		Type 1 vs type 2 DM	*Duration of diabetes (years)*				*Duration of diabetes (years)*			
		p value	*N*	*Mean*	*Median*	*p value*	*N*	*Mean*	*Median*	*p value*
Nephropathy	*Absent****	0.394**	67	5.761	5.000	**<0.010***	2	7.500	14	0.079*
	Present, stage I	**0.001****	16	5.812	4.500		28	12.214	10.500	
	Present, stage II	**0.009****	25	10.600	10.000		19	16.263	14.000	
	Present, stage III	0.766**	30	20.950	18.500		5	22.000	22.000	
Neuropathy	*Present*	0.333**	37	16.703	15.000	**<0.010****	53	14.113	14.000	0.111**
	*Absent****	**0.020****	101	7.470	6.000		1	28.000	28.000	
Retinopathy	*Present*	0.489**	24	21.188	18.000	**<0.010****	24	19.083	18.500	**<0.001****
	*Absent****	**0.013****	114	7.579	6.000		30	10.600	10.000	
Hypertension	*Absent****	0.386**	118	8.432	6.000	**<0.010***	5	14.200	7.000	0.428*
	Present, stage I	0.383**	7	18.714	14.000		3	8.667	10.000	
	Present, stage II	0,457**	13	18.962	17.000		17	16.118	15.000	
	Present, stage III	.	0	.	.		29	13.966	13.000	
Hyper-cholesterolemia	*Present*	0.789**	34	15.691	14.000	**<0.010****	45	14.889	14.000	0.280**
	*Absent****	0.159**	104	8.067	6.000		9	11.778	10.000	
Hyper-triglyceridemia	*Present*	0.328**	27	12.278	10.000	0.058**	28	13.714	13.500	0.690**
	*Absent****	**0.002****	111	9.378	7.000		26	15.077	14.000	

DM: diabetes mellitus; N: number of patients.

* Kruskal-Wallis test, significance level at 0.05

** Mann-Whitney U test, significance level at 0.05

*** Mean and median disease duration in patients without the respective complication

vascular complications [25]. Other studies pointed out to the lack of significant correlations between serum CRP levels and retinopathy in type 2 DM [26–28] and to a negative correlation between plasma leptin level, possibly associated with decreased insulin sensitivity, and acute-phase reactants in these patients [29]. Thus, in type 2 DM, the relationship between inflammatory biomarkers and the onset of chronic complications seems less straightforward than in type 1 DM. Our results are only partially supported by those of previous studies, suggesting the need for further exploration into the biomarkers' capacity of predicting chronic complications in type 2 DM.

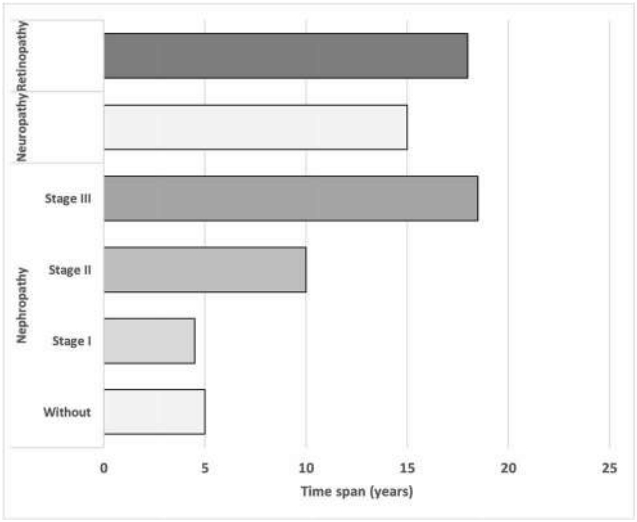

Fig. 7 Length of disease evolution until the onset of microvascular complications in patients with type 1 diabetes mellitus

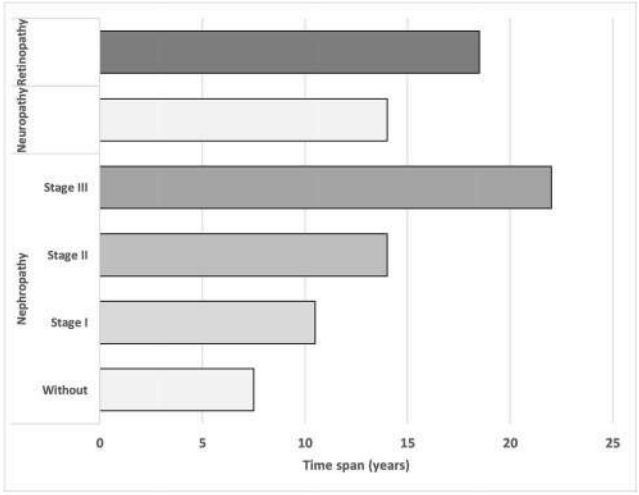

Fig. 8 Length of disease evolution until the onset of microvascular complications in patients with type 2 diabetes mellitus

Table 4 Correlation between the duration of diabetes and the level of biological parameters

	Duration of type 1 and 2 DM		*Duration of type 1 DM*		*Duration of type 2 DM*	
	Spearman's correlation coefficient	*p* value	Spearman's correlation coefficient	*p* value	Spearman's correlation coefficient	p value
Hb A1c (%)	0.308*	**0.000**	0.451*	**0.000**	−0.195	0.157
CRP mg/dL	0.197*	**0.007**	0.254*	**0.003**	0.118	0.395

DM: diabetes mellitus; HbA1c: glycated hemoglobin; CRP: C-reactive protein.

* Spearman's correlation coefficient, significance level at 0.01

Between the two types of DM, statistically significant differences were found in HbA1c levels associated with stage I nephropathy, with higher values in patients with type 2 DM, indicating a poorer glycemic control. From the perspective of the relationship between inflammation and diabetic nephropathy, a significant difference in CRP values was observed among patients with stage II diabetic nephropathy, in this case with higher concentrations in those with type 1 DM.

In the absence of diabetic retinopathy, HbA1c was significantly higher in patients with type 2, compared to those with type 1 DM, again suggesting a poorer glycemic control. CRP values in patients with retinopathy were also different, reaching the statistical significance between the two groups, with higher levels in patients with type 1, than those with type 2 DM. Taken together, these two results may show an increased tendency towards a systemic inflammatory response in some patients with type 1 DM, possibly as a result of its auto-immune origin.

There were no statistically significant differences between the two groups of patients, regarding the association of studied biomarkers with diabetic neuropathy.

Microvascular complications do occur in the progression of diabetes, regardless of its type. However, our results indicate a higher risk of complications in patients with type 2, compared to those with type 1 DM. As such, the risk of nephropathy was 1.87 times higher in patients with type 2 DM, while the risk of retinopathy was more than 2 times higher and that of neuropathy was even 3 times higher for these patients. Our results may be explained by the longer and more subtle evolution of type 2, compared to the progression of type 1 DM.

Concerning the progression of type 1 DM, stage I nephropathy occurred after a median of 4 years and stage III after a median of 18.5 years of evolution, indicating a link between disease duration and the staging of nephropathy. In the group with type 2 DM, stage I nephropathy occurred after a median of 10.5 years and the onset of stage III was situated after a median of 22 years of evolution, without significant differences in the length of disease evolution between different stages of this complication. Taken together, these results are indicative of a higher predictability in the evolution of type 1, compared with type 2 DM. On the other hand, patients with type 2 DM seem to have a longer time span until the occurrence of nephropathy, but afterwards, its progression is generally faster than in type 1 DM.

In our study, the median time of evolution until the occurrence of retinopathy (including its early stages) was 18 years in patients with type 1 and 18.5 years in those with type 2 DM, indicating an approximately equal duration in the two patient groups, without statistically significant differences. Epidemiological studies have shown that the incidence of retinopathy in patients with type 2 DM is directly related to the duration of diabetes and to the maintenance of glycemic control [30].

Similarly, the median time to the onset of neuropathy was 15 years in type 1 and 14 years in type 2 DM, without significant differences between the two patient groups.

In the entire group, a statistically significant positive correlation was noticed between either HbA1c, or CRP values, and disease duration. These biomarkers increased steadily over the period of follow-up, from diagnosis until the onset of complications. In type 1 DM patients, we also found a linear relationship between CRP and HbA1c levels, besides the correlation between each biomarker and the length of disease progression. Our results are in agreement with those of previous studies [18, 31], indicating a strong positive correlation between CRP and HbA1c, and their linear progression in type 1 DM. However, in our patients with type 2 DM, no such correlation between disease duration and the level of studied biomarkers was observed, suggesting a more variable, less predictable evolution of type 2 DM.

Although our patients were not treated with high insulin doses, we cannot exclude the potential effects of insulin therapy on CRP levels, as suggested by Bala et al., who indicated an association between high doses of insulin treatment and increased hsCRP values [32]. Additionally, we may assume that CRP, unlike hsCRP, is probably less likely to be modified by this parameter.

Conclusions

This study demonstrated the existence of a direct link between systemic inflammation, reflected by C-reactive protein values, and the timely progression of type 1 DM. In these patients, higher levels of HbA1c were indicative of more advanced nephropathy, retinopathy and peripheral neuropathy, while elevated CRP concentrations predisposed to more severe nephropathy and retinopathy.

In patients with type 2 DM, no statistical significance was found between the studied biomarkers and the occurrence of microvascular complications, although diabetic retinopathy was more common in subjects with a longer disease evolution.

Diabetic nephropathy was shown to appear sooner in patients with type 1 DM, whereas retinopathy and peripheral neuropathy had a similar pattern of timely occurrence for the two types of diabetic patients.

Compliance with ethical standards The research protocol was approved (no. 408 / 08.11.2017) by the Ethics Committee of the University of Medicine and Pharmacy Cluj-Napoca, Romania, in accordance with the revised Helsinki Declaration of 2000. All patients gave their informed written consent before any procedure was initiated.

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

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ORIGINAL ARTICLE

Hypoglycemic, hypolipidemic, and hepatoprotective effects of *Polyscias fulva* (Hiern) Harms ethanolic bark extract in streptozotocin-induced diabetic Wistar rats

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Abstract

Background *Polyscias fulva* (Hiern) Harms stem bark is used in traditional folk medicine in Kenya for diabetes mellitus and obesity management. This study sought to examine the antidiabetic effects of ethanolic stem bark extract of *P. fulva* in streptozotocin (STZ)–induced diabetic Wistar albino rats.

Methods Diabetes in rats was induced by intraperitoneal injection of STZ (50 mg/kg bwt) in experimental groups. Rats were divided into five groups ($n = 5$ per group): group 1, control; group 2, diabetic untreated rats; groups 3 and 4, diabetic rats on 200 and 400 mg/kg bwt/day of extract, respectively; and group 5, diabetic rats on metformin (100 mg/kg bwt/day). The rats received oral treatments daily for 21 days, and fasting blood glucose levels and body weights were determined weekly. Liver histopathological analysis and malondialdehyde (MDA) assay as well as serum analysis of lipid profile, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins (TP), albumin (ALB), and globulins were performed at the end of the treatment period.

Results Extract had significant hypoglycemic and hypolipidemic effects in the diabetic rats compared with diabetic untreated rats ($p < 0.05$). Serum levels of ALP, ALT, and AST were significantly lowered, while TP and ALB were elevated in the extract-treated diabetic rats compared with diabetic untreated rats. The levels of liver MDA of extract-treated rats were significantly lowered compared with those of the diabetic untreated group. Extract treatment reversed liver pathological changes observed in the diabetic untreated group.

Conclusion This study has provided insights into the potential of the stem bark of *P. fulva* as an alternative medicine for diabetes mellitus.

Keywords Antidiabetic · *Polyscias fulva* · Streptozotocin · Bark · Rat

Introduction

The people affected worldwide with diabetes mellitus (DM) was estimated in 2017 to be 451 million, and the figures were projected to rise to 693 million by 2045 [1]. Hyperglycemia has been implicated in the onset and progression of DM and causation of severe complications through various mechanisms including induction of oxidative stress, decreased nitric oxide bioavailability, glucose autoxidation, and non-enzymatic protein glycation [2, 3]. Currently, pharmacological remedies have not fully addressed DM and its associated complications [4]. This high level of DM treatment failures coupled with unpleasant side effects and enormous cost associated with diabetic therapy have generated an urgent need and desire for alternative treatments such as plant medicine [5].

Polyscias fulva (Hiern) Harms (Araliaceae) is one of the many plants widely used in traditional folk medicine in Kenya for diabetes and obesity management especially among the Nandi community, and there is need to scientifically undertake

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studies to validate claims that its stem bark has antidiabetic effects [6]. In Kenya, *P. fulva* is mainly found in the highlands and tea-growing areas and its Kenyan local names include Soiyet in the Nandi; Auoun in Keiyo and Marakwet; Mutati in Kikuyu; Aounet in Kipsigis; Mwanzu in Luhya; and Nyakom ondiek in Luo [7]. Antioxidant capacity and anti-inflammatory effects of the stem bark extract of *P. fulva* have been previously reported [8].

In this present study, we therefore sought to examine the antihyperglycemic effects of ethanolic stem bark extract of *P. fulva* in streptozotocin (STZ)–induced diabetic male Wistar albino rat model. Also, the effects of *P. fulva* bark extract on serum lipids and biochemical liver function indices as well as liver's lipid peroxidation levels and histological alterations were evaluated so as to understand the plausible mode of action of the antidiabetic potential of *P. fulva* stem bark.

Materials and methods

Plant collection and identification

The stem barks *of P. fulva* were collected in its natural habitat at Timboroa forest, Uasin Gishu County, Kenya, and were identified and authenticated by Mr. Bernard Wanjohi, a plant taxonomist at the University of Eldoret. A voucher number M.U.H/PF/0031/17 was assigned and the plant specimen kept in the herbarium at University of Eldoret.

Preparation of bark extract

The stem barks were dried at room temperature and ground when completely dry using an electric mill (Disk Mill FFc-23, China). About 500 g of the powder was extracted with 95% ethanol by maceration for 3 days [8], filtered, and evaporated to dryness at 50 °C using a rotary evaporator (Rotavapor type EL 30, model AG CH-9230, Germany). The residue obtained constituted the crude extract, and it was stored at 4 °C.

Qualitative phytochemical analysis of bark extract

The bark extract was subjected to various qualitative phytochemical tests to determine the general classes of phytochemicals present using standard procedures [9, 10].

Animals

Wistar albino male rats (*Rattus norvegicus)* weighing about 140–180 g and aged 6–8 weeks were obtained from the Department of Biological Sciences, University of Eldoret. The rats were kept in wire cages of rack type with 5 animals per cage. Standard conditions were maintained (12 h dark and 12 h light circle; 25 ± 5 °C; 40–60% humidity), and the rats were allowed to acclimatize for 1 week before experimentation. The animals were fed with standard rodent chow pellet (Unga Farmcare, East Africa Limited, Nakuru, Kenya) and given free access to drinking water.

Induction of diabetes

Rats were fasted for 18 h and on access to drinking water ad libitum. Diabetes was induced for the experimental groups by single intraperitoneal injection of freshly prepared STZ (Wako Pure Chemical Industries Ltd., Japan) at a concentration of 50 mg/kg bwt [11] in 100 μl ice-cold 0.1 M sodium citrate buffer (pH 4.5). The normal group was injected with 100 μl of 0.1 M citrate buffer. The rats were given 5% glucose solution overnight to overcome drug-induced hypoglycemia. Fasting blood glucose (FBS) was determined using a glucometer (Wellion CALLA Light, Med Trust, Germany) from tail vein blood. Rats that had FBS ≥ 13.9 mmol/l after 5 days were considered diabetic.

Experimental design

The formula for calculation of sample size for the comparison of two groups that was used was according to Charan and Kantharia [12]. Male Wistar albino rats were randomly divided into 5 groups of 5 animals each as follows:

Group 1: Normal control rats administered with 100 μl distilled water (vehicle)

Group 2: STZ-induced diabetic rats untreated but administered with 100 μl distilled water (vehicle)

Group 3: STZ-induced diabetic rats treated with 100 μl of 200 mg/kg bwt/day of extract

Group 4: STZ-induced diabetic rats treated with 100 μl of 400 mg/kg bwt/day of extract

Group 5: STZ-induced diabetic rats treated with 100 μl of 100 mg/kg bwt/day of metformin (standard drug)

Animal treatment

A 100 μl water suspension of the bark crude extract of *P. fulva* at a concentration of 200 and 400 mg/kg bwt/day and metformin (Glucophage, Lipha Pharma Ltd., UK) at 100 mg/kg bwt/day was orally administered daily for 21 days according to Cheng et al. [13]. The basis for the administration of the standard drug, metformin, is its novel mechanism for plasma glucose-lowering action in STZ-induced diabetic rats. FBS and fasting body weights (FBWTS) for each rat was taken on day 0, 7, 14, and 21. Dosing was adjusted weekly as per the weekly body weights recorded. Following an overnight fast after the 21 days treatment period, the rats were euthanized under mild anesthesia of chloroform to minimize stress and pain during sacrificing and blood was collected through cardiac puncture. Serum was separated by centrifugation

(centrifuge model EBA 21 from Hettich Company Limited, Germany) and frozen at − 20 °C for analysis of biochemical parameters. The liver was excised and the first portion was frozen at − 20 °C for lipid peroxidation analysis, while the second portion was fixed in 10% formalin for histopathological analysis.

Serum analysis of lipid profile and liver function indices

Total cholesterol (T.CHOL), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) were analyzed in serum and for lipid profile. For liver function tests, serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total proteins (TP), albumin (ALB), and globulins (GLB) were analyzed. These tests were done according to the standard operating procedures of COBAS INTEGRA 400 plus auto-analyzer (Roche Diagnostics, Mannheim, Germany).

Lipid peroxidation assay in liver tissues

Determination of malondialdehyde (MDA) levels, an index of lipid peroxidation in liver tissues, was done as described by Alam et al. [14].

Histopathological analysis of liver tissues

Processing of liver tissues was done according to the standard operating procedures of STP 120 automatic tissue processor. After tissue processing, a microtome (SLEE medical model GmbH) was used to cut into 4-μm-thick paraffin sections and then stained with hematoxylin and eosin [15]. Specimens were then examined for histopathological changes under a light microscope at × 40 (model CX21FSI, Olympus Corporation, Japan). The examination, analysis, and interpretation of the results were done by a histopathologist.

Statistical analysis

All quantitative data was expressed as mean ± standard error mean (SEM). Statistical analysis was by paired Student's *t* test and ANOVA. The value of $p < 0.05$ was considered to be statistically significant.

Results

Qualitative phytochemical analysis

Tannins, anthraquinones, terpenoids, saponins, flavonoids, and steroids were detected in the crude bark ethanolic extract of *P. fulva* upon qualitative phytochemicals analysis while alkaloids were absent.

Fasting blood sugar and body weight

Before treatment (day 0), all STZ-injected rats were diabetic with FBS levels significantly higher than normal control rats ($p < 0.001$) as shown in Table 1. However, upon treatment with 200 and 400 mg/kg of *P. fulva* bark extract, and 100 mg/kg of metformin for 21 days, there was significant reduction in the mean FBS levels when compared with diabetic untreated group ($p = 0.001$). The mean body weights for the normal control rats had an overall trend of increase across the study period while the diabetic induced groups had a declining trend (Table 1).

Serum lipid profile parameters

T.CHOL levels for the normal control rats were significantly higher than induced diabetic untreated group ($p < 0.001$) as shown in Fig. 1. In contrast, a significant reduction in T.CHOL was recorded in diabetic rats treated with 200 mg/kg of *P. fulva* when compared with the diabetic untreated group (Fig. 1). The diabetic untreated rats had a significant decrease in HDL-C levels compared with the normal control rats (Fig. 1). A significant increase in HDL-C was recorded in the diabetic rats treated with 200 as well as 400 mg/kg of *P. fulva* compared with the diabetic untreated rats. Diabetic rats treated with 200 and 400 mg/kg of *P. fulva* as well as 100 mg/kg of metformin recorded a significant decrease in LDL-C levels when compared with diabetic untreated rats. TGs levels in the diabetic untreated rats recorded a significant higher level as compared with normal control rats. Significant lower levels of TGs were recorded in diabetic rats treated with 200 and 400 mg/kg of *P. fulva* and 100 mg/kg of metformin when compared with diabetic untreated rats.

Liver function serum enzymes indices

The diabetic untreated group showed significant higher ALP, AST, and ALT levels as compared with normal control rats ($p = 0.002$, 0.001, and 0.001, respectively) as shown in Fig. 2. Among the treatment groups, significant lowest mean of ALP was recorded in diabetic rats treated with 400 mg/kg of *P. fulva* compared with diabetic untreated rats ($p = 0.002$). On the other hand, significant higher ALP levels were recorded in diabetic rats treated with 100 mg/kg of metformin when compared with diabetic untreated rats. The levels of AST and ALT were significantly reduced when diabetic rats were treated with 200 and 400 mg/kg of *P. fulva* and 100 mg/kg of metformin as compared with the diabetic untreated rats.

Table 1 Effect of *Polyscias fulva* bark extract on FBS and FBWTS

Experimental group	Duration (day)	FBS (mmol/l)	FBWTS (g)
Normal control	0	4.68 ± 0.17	151.18 ± 5.54
	7	4.44 ± 0.21	154.20 ± 5.32
	14	4.92 ± 0.32	158.60 ± 4.85
	21	4.48 ± 0.24	165.56 ± 4.47
Diabetic untreated	0	23.22 ± 2.56*	160.88 ± 5.33
	7	24.18 ± 1.85*	150.74 ± 5.06
	14	25.38 ± 1.78*	147.52 ± 4.87
	21	25.35 ± 1.14*	142.27 ± 4.49
Diabetic + 200 mg/kg extract	0	25.64 ± 1.70	158.50 ± 3.76
	7	20.30 ± 1.52#	149.06 ± 3.45
	14	17.54 ± 1.04#	146.46 ± 3.29
	21	15.76 ± 0.45#	144.58 ± 3.16
Diabetic + 400 mg/kg extract	0	25.54 ± 1.75	161.20 ± 2.31
	7	17.96 ± 0.86#	152.96 ± 3.52
	14	15.24 ± 0.63#	149.16 ± 3.47
	21	9.70 ± 0.69#	148.82 ± 3.55
Diabetic + 100 mg/kg metformin	0	30.22 ± 1.02#	166.78 ± 6.16
	7	20.48 ± 0.81#	158.74 ± 6.26
	14	15.88 ± 1.23#	155.24 ± 6.05
	21	11.08 ± 0.81#	151.52 ± 5.54

Values represent mean ± SEM; $n = 5$. *Significant difference compared with normal control, # significant difference compared with diabetic untreated group ($p < 0.05$, ANOVA). *STZ* streptozotocin, *FBS* fasting blood sugar, *FBWTS* fasting body weights

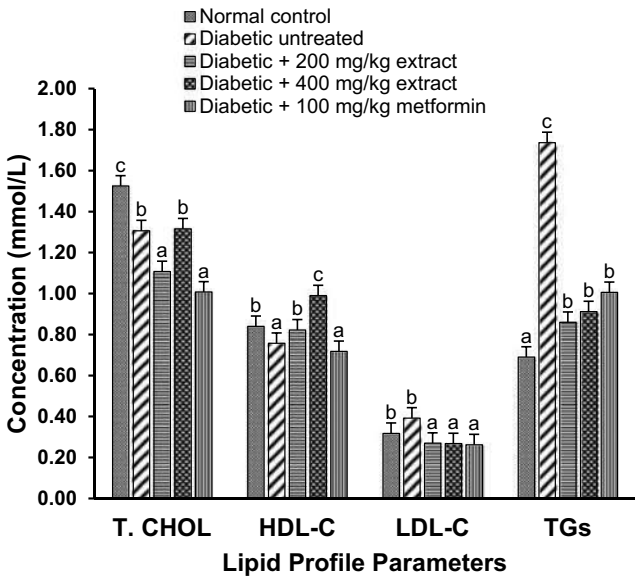

Fig. 1 Effect of *Polyscias fulva* bark extract on serum lipid profile parameters. Values represent mean ± SEM; $n = 5$. Mean values with different letters in bar graphs show statistically significant differences ($p < 0.05$, Student's t test). Total cholesterol (T.CHOL), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (TGs)

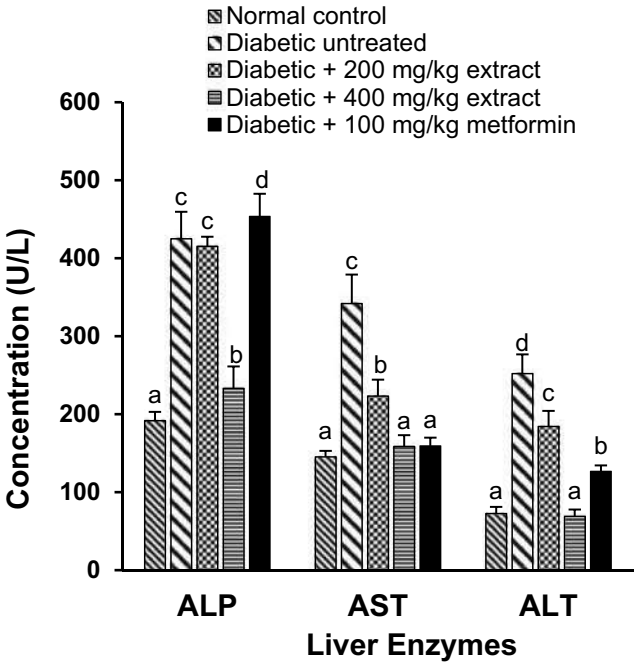

Fig. 2 Effect of *Polyscias fulva* bark extract on serum liver enzymes. Values represent mean ± SEM; $n = 5$. Mean values with different letters in bar graphs show statistically significant differences ($p < 0.05$, Student's t test). Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT)

Liver function serum proteins indices

As shown in Fig. 3, a significant increase in TP levels was recorded in the diabetic rats treated with 400 mg/kg of *P. fulva* compared with diabetic untreated rats ($p = 0.043$). The diabetic rats treated with 400 mg/kg of *P. fulva* extract recorded significant higher ALB levels than the induced untreated rats. For GLB, we found an insignificant difference among treated groups although the diabetic untreated group had higher GLB levels compared with the normal control group.

Lipid peroxidation in liver tissues

There was a significant increase in malondialdehyde levels by 57.2%, an index of lipid peroxidation, in liver tissues of diabetic untreated rats when compared with the normal control ($p = 0.008$) as shown in Fig. 4. However, diabetic rats treated with 200 and 400 mg/kg *P. fulva* and 100 mg/kg of metformin showed a significant reduction by 62.3%, 78.6%, and 49.7%, respectively, in lipid peroxidation when compared with the diabetic untreated group.

Liver histopathological analysis

Normal control group of rats showed no pathological changes as we observed normal morphology of hepatocytes and organized hepatic cell with no inflammation, fatty degeneration, and proliferation (Fig. 5a). Diabetic untreated group showed pathological changes of mild periportal chronic inflammation (arrow in Fig. 5b). Diabetic rats treated with 200 mg/kg of *P. fulva* showed improved hepatic architecture but with very mild bile duct proliferation (arrows in Fig. 5c) while those treated with 400 mg/kg of *P. fulva* showed normal hepatic architecture with prominent nucleoli (arrows in Fig. 5d) and those treated with 100 mg/kg of metformin showed normal hepatocytes morphology with prominent nucleoli (arrows in Fig. 5e).

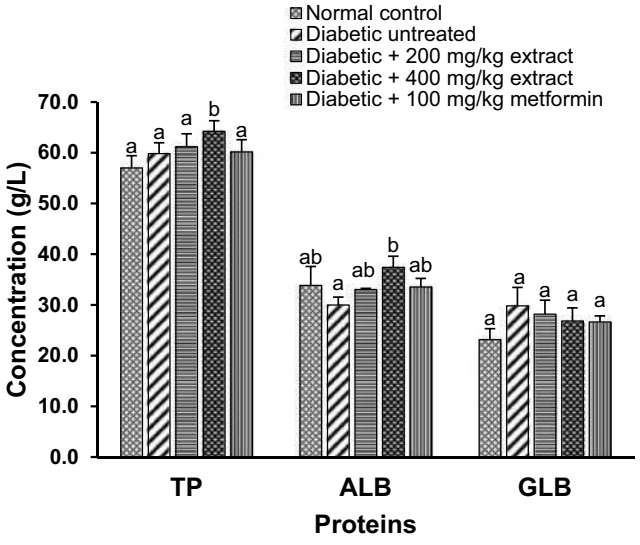

Fig. 3 Effect of *Polyscias fulva* bark extract on serum proteins. Values represent mean ± SEM; $n = 5$. Mean values with different letters in bar graphs show statistically significant differences ($p < 0.05$, Student's t test). Total proteins (TP), albumin (ALB), globulins (GLB)

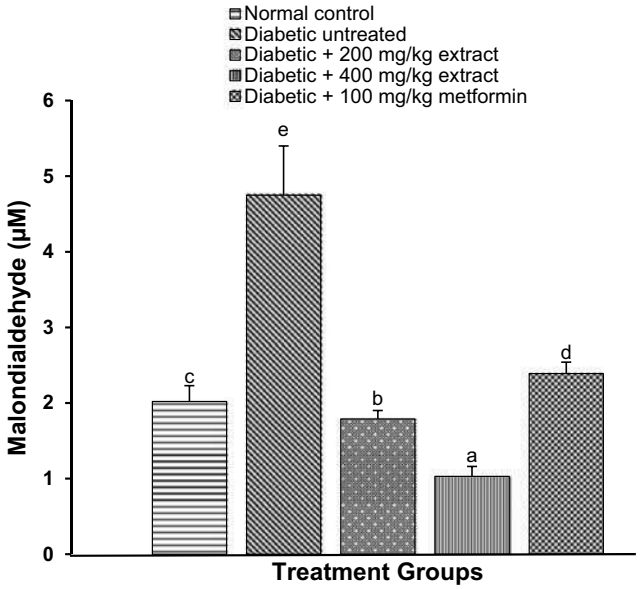

Fig. 4 Effect of *Polyscias fulva* bark extract on lipid peroxidation in liver tissues. Values represent mean ± SEM; $n = 5$. Mean values with different letters in bar graphs show statistically significant differences ($p < 0.05$, Student's t test)

Discussion

In the present study, STZ successfully induced hyperglycemia in rats, and this is postulated to be through STZ selectively destroying the pancreatic insulin secreting β-cells, leaving less active β-cells resulting in a diabetic state [11]. On the other hand, our study showed that the stem bark extract of *P. fulva* has antihyperglycemic effects in the diabetic rats which were comparable with the effects of the standard antidiabetic drug, metformin. The biochemical mechanism of anti-hyperglycemic actions of *P. fulva* bark extract might be due to an insulin mimetic effect on muscle and adipose tissues by either stimulating glucose uptake and metabolism [16], by inhibiting hepatic gluconeogenesis [17] and glycogenolysis [18], and by stimulation or regeneration process of remnant β-cells due to reduced oxidative stress [3]. These plausible anti-hyperglycemic mechanistic effects of *P. fulva* extract could also be partly attributable to the phytochemicals rich in the extract such as flavonoids, tannins, and saponins which we found present after our qualitative phytochemical analysis of the extract. The observed body weight loss in diabetic rats

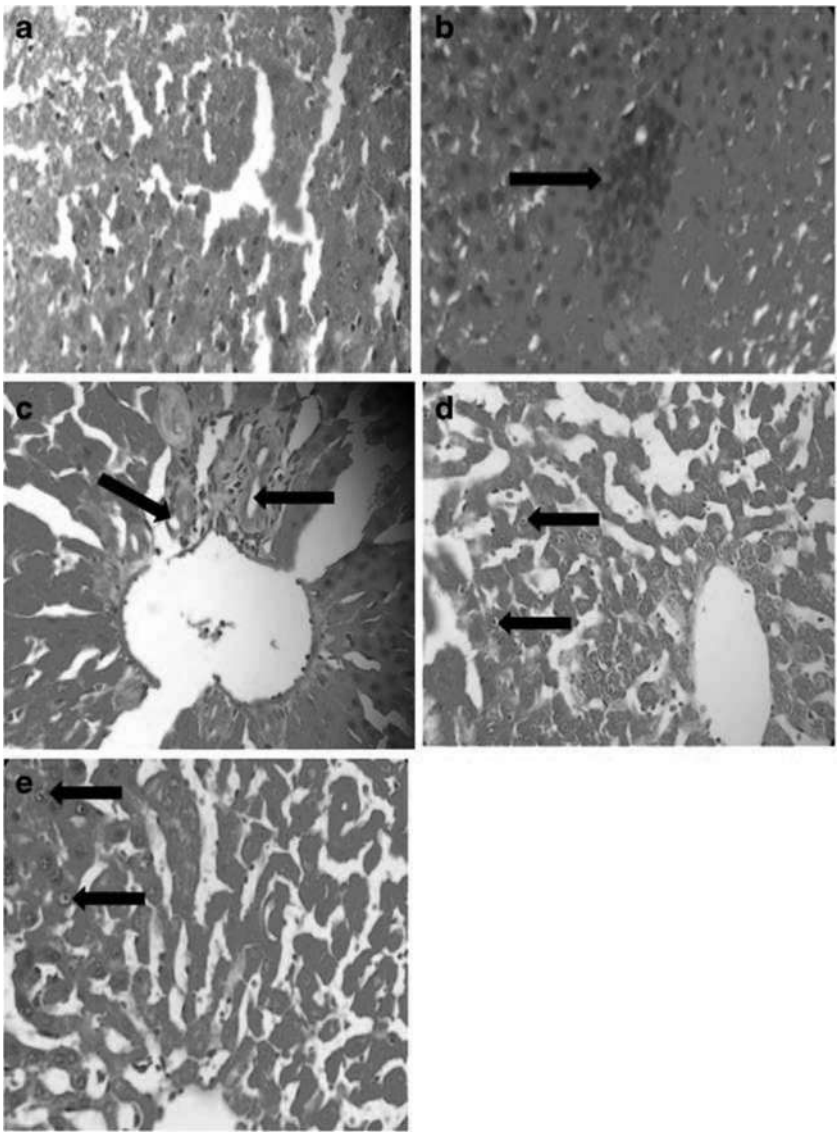

Fig. 5 Representative photomicrographs of liver sections of normal control and experimental rats (hematoxylin and eosin stained × 40). (a) Normal control, normal hepatocytes; (b) diabetic untreated rats; *arrow*, mild periportal chronic inflammation; (c) diabetic rats treated with 200 mg/kg of *Polyscias fulva* bark extract; *arrows*, mild bile duct proliferation; (d) diabetic rats treated with 400 mg/kg of *Polyscias fulva* bark extract; *arrows*, prominent nucleoli; (e) diabetic rats treated with 100 mg/kg of metformin; *arrows*, prominent nucleoli

in this study could be due to increased muscle wasting [19], dehydration, loss of carbohydrates, and the excessive breakdown of tissue proteins and fat [20].

Hypertriglyceridemia was notable in the diabetic untreated rats versus the normal control rats while a significant drop in TGs level after treatment with bark extract of *P. fulva* and metformin was also noted. STZ-induced diabetes mostly involves abnormal lipid metabolism associated with the insulin deficiency [21]. This reported hypotriglyceridemic alongside other moderate hypolipidemic effects of *P. fulva* bark extract in the diabetic state recorded in this study mechanistically is possibly imparted by the extract's phytochemicals. The reduction in LDL-C in metformin-treated group could be due to the drug's effect of potentially activating AMPK that suppresses FADS genes leading to reduction in LDL-C levels [22]. AMPK in its active form exhibits an anti-lipogenic effect and acts by suppressing hepatic expression of lipogenic enzymes (Acetyl-CoA Carboxylase 1-ACC1 and FAS). Further, active AMPK phosphorylates and inhibits ACC1/2 leading to decreased production of malonyl-CoA levels which releases the inhibitory effect on carnitine palmitoyltransferase 1A (CPT1A) and therefore favors fatty acid oxidation [23].

Lipid peroxidation is an autocatalytic free-radical-mediated destructive process whereby poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides including conjugated dienes and MDA [24]. In this present study, the results showed that STZ-induced diabetic rats were susceptible to lipid peroxidation versus the normal control. Prolonged exposure to hyperglycemia in diabetic state increases the generation of free radicals and reduces capacities of the antioxidant defense system which leads to oxidative stress [25]. Treatment of diabetic rats with *P. fulva* stem bark extract led to reduced lipid peroxidation. This reduction of lipid peroxidation by *P. fulva* extract could

be explained in part by its phytochemicals with known antioxidants [8].

DM is frequently associated with the elevated activities of liver toxicity marker enzymes such as ALT, AST, and ALP in serum, which might be mostly due to the outflow of these enzymes from the liver's cytosol into the bloodstream emanating from hepatocellular damage [20]. Administration of *P. fulva* bark extract and metformin reduced the AST and ALT levels in diabetic condition and a reduction of ALP in diabetic rats treated with *P. fulva* ethanolic bark extract at the highest dose. The high levels of ALP in diabetic rats treated with 100 mg/kg metformin could be due to diabetes-induced damage to the liver tissues as also observed in other studies [26]. Additionally, increased activities of ALP could have resulted from STZ that may have caused hepatic damage and distortion in membrane integrity of the cell [27]. The levels of TP and ALB were on the other hand elevated in the extract-treated diabetic rats compared with diabetic untreated rats that may be indicative of reduced biosynthetic ability of the liver for serum proteins in the untreated diabetic state [28]. *P. fulva* extract could have trapped the oxygen-related free radicals and therefore hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidation processes leading to MDA generation as indicated in our results on lipid peroxidation. *P. fulva* extract could also have increased the hepatic and blood content of glutathione since higher content of glutathione in blood and liver would lead to a better tissue protection against oxidative stress [29] hence reduction in hepatotoxic effects of hyperglycemia and STZ effects.

The pathological changes observed in the liver tissues of the diabetic untreated rats could have been due to injury to the liver hepatocytes resulting from effects of STZ-induced diabetes, chronic hyperglycemia, and lipid peroxidation. The ameliorating effect of *P. fulva* extract on the derangement of serum liver function indices and its hypoglycemic, hypolipidemic, and reduction in liver's lipid peroxidation in the diabetic rats in the present study supports our improved histopathological observations. The hepatoprotective effects of *P. fulva* are also in part attributable to the rich phytochemicals in the bark extract that we found present and that has been shown to have antioxidant properties [8]. Our findings are in agreement with several studies that have shown histopathological changes in liver tissues of diabetic untreated rats, while the architecture of the liver in diabetic rats treated with plant extracts was near or similar to that of normal liver, indicating that the degenerative changes initiated by DM are improved/reversed by plant extracts in diabetic rats [30].

In conclusion, this study has shown that the phytochemical-rich ethanolic extract of *P. fulva* crude stem bark has hypoglycemic, hypolipidemic, and hepatoprotective effects in STZ-induced diabetic rats. These findings have provided important insights into the potential of *P. fulva* stem bark extract for its use as an alternative medicine in the management of diabetes mellitus.

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

Compliance with ethical standards

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

Research involving animals Ethical approval for this study was granted by the University of Eastern Africa, Baraton's Research Ethics Committee (Reference; REC: UEAB/14/3/17). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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ORIGINAL ARTICLE

The impact of limited and strategic blood glucose monitoring on metabolic control in a type 1 diabetes clinic in Central India

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Abstract

Background Self-monitoring of blood glucose (SMBG) is an essential component of type 1 diabetes (T1D) management and typically involves several daily tests. However, due to high cost, SMBG supplies are often unavailable in low-resource settings. This study assessed whether the use of two SMBG tests per day improves glycemic control, measured by a change in HbA1c, in youth with T1D followed at the DREAM Trust (DT) in Nagpur, India.

Methods Single-site prospective cohort study of youth ≤ 23 years of age with T1D ≥ 1 year followed by DT, who were provided with SMBG meters and two test strips per day. Patients received education regarding SMBG and how to respond to blood glucose values and trends. They were followed every 3 months with HbA1c and questionnaires for a total of 21 months.

Results HbA1c declined significantly from 10.2 ± 2.5% (88 ± 4 mmol/mol) at baseline to 9.5 ± 2.4% (80 ± 3 mmol/mol) at 21 months ($p < 0.001$). In univariable analysis, change in HbA1c was associated with adherence to insulin dosing, number of patient education sessions, household income, and holding a below the poverty line certificate. In multivariable analysis, only adherence to insulin dosing was a significant predictor for a decrease in HbA1c. There was no increase in diabetes-related acute complications.

Conclusions The use of two SMBG test strips per day for the management of T1D in a low-resource setting was safe, and over the 21 months following its introduction, there was a clinically and statistically significant decrease in HbA1c.

Keywords Blood glucose self-monitoring · Diabetes mellitus, type 1 · Glycated hemoglobin A1c · Insulin · Poverty

Introduction

Self-monitoring of blood glucose (SMBG) is an essential component of type 1 diabetes (T1D) management and is associated with improved glycemic control and reduced risk of both acute and long-term complications of diabetes [1]. Unfortunately, due to high cost, SMBG supplies are often unavailable in low-resource settings [2].

This has historically been the case at the Diabetes Research Education and Management (DREAM) Trust (DT), a nongovernmental organization and registered charity in Nagpur, India (Maharashtra State) that offers free healthcare and insulin to underprivileged children with T1D. Our previously published descriptive study of DT's patient population in 2011 showed a median of four SMBG tests in 30 days, associated with a median HbA1c of 10.4% (90.2 mmol/mol) [3]. In late 2012, the International Diabetes Federation (IDF) Life for a Child

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Program began supplying DT patients with two SMBG test strips per day.

The primary purpose of this study was to assess whether the addition of two SMBG tests per day (from none) can improve glycemic control in youth with T1D in a low-resource setting, specifically by measuring a change in HbA1c over 21 months.

Materials and methods

We conducted a single-site prospective cohort study of youth ≤ 23 years of age with T1D (diagnosed ≥ 1 year ago) followed by DT (for ≥ 1 year), who were provided with SMBG meters and two test strips per day by Life for a Child (November 2012–September 2015). All DT patients meeting inclusion criteria and receiving SMBG meters and strips (Nipro Diagnostics) were invited to participate in this study (patients received diabetes monitoring supplies regardless of study participation). The study was divided into two phases over a 21-month period:

1. Phase 1 (0–9 months): Instructions for SMBG and insulin dose adjustments were provided by DT team (without external input). Patients were instructed to monitor BG before a meal or bedtime twice daily (exact timing not specified). They were also asked to increase insulin doses by 2 units for BG > 16.7 mmol/L (300 mg/dL), and overall insulin dose adjustments were made during clinic visits. Data was collected at baseline (including participant characteristics) and then every 3 months for HbA1C (Bio-Rad D-10 high-pressure liquid chromatography method), insulin doses, acute complications (DKA and severe hypoglycemia), and height and weight. At baseline, rate of acute complications was based on recall of number of events over the last year.
2. Phase 2 (9–21 months): A suggested approach to strategic SMBG use and patient education was provided to DT staff by collaborators from the Children's Hospital of Eastern Ontario (CHEO), Ottawa, Canada. Specific recommendations that were implemented by the DT team include the following:

    a. Strategic SMBG: In resource-rich settings, patients typically monitor blood glucose (BG) before each meal and before bed, for a minimum of four SMBG tests per day. To replicate this pattern with only 50% of the necessary SMBG strips, participants were instructed to alternate the timing of SMBG tests daily: day 1, test BG before breakfast and before lunch; and day 2, test BG before supper and before bed. To support this strategic approach to SMBG, participants received logbooks that indicated the times of day to check BG each day.
    b. Management of hypoglycemia: If BG < 4 mmol/L (< 70 mg/dL), patients were instructed to take 15 g of simple, rapidly absorbed carbohydrate.
    c. Management of hyperglycemia: If BG > 14 mmol/L (> 250 mg/dL) before a meal, patients were instructed to give a higher dose of short-acting insulin. The correction dose was provided at the time of their DT visit (verbally or in writing for literate patients) and was equivalent to the patient's regular insulin dose plus 5% of their total daily insulin dose.
    d. Insulin dose adjustments: Patients/families were instructed to contact DT by phone to receive instructions regarding insulin dose adjustments in the event that BG was high for three consecutive readings at the same time of day or low at the same time of day for two readings in any given week:

        1. For three unexplained consecutive high BG readings at the same time of day, DT instructed patient to increase appropriate insulin dose by 10%.
        2. For two unexplained low BG readings at the same time of the day within a week, DT instructed patient to decrease appropriate insulin dose by 20%.

    e. Patients/parents were asked to record in their logbook: BG readings, insulin dose adjustments/modifications, and symptomatic hypoglycemia (treated) if BG was not tested.

Data was collected every 3 months as in phase 1, with additional data collection regarding adherence to SMBG and insulin dosing.

This study received institutional research ethics board approvals at CHEO (REB #12/10E) and DT, and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants by DT staff. If participants were too young to consent to study participation, informed consent was obtained from their parents/ legal guardians, and assent was obtained from the child.

Statistical analysis

Demographic and clinical characteristics were summarized using mean and standard deviation or median and interquartile range for continuous variables, and frequency and percent for categorical variables. The mean HbA1c at 0, 9, and 21 months was compared using paired *t* tests. Mean differences, together with a 95% confidence interval, were also reported.

The primary analysis consisted of examining predictors of change in HbA1c between baseline and 21 months. This was achieved by conducting a univariable analysis followed by a multivariable analysis. For the univariable analysis,

continuous variables were assessed with the Spearman correlation, while for categorical variables, the Wilcoxon or Kruskal-Wallis test was used, as appropriate. Variables examined were patient age, sex, religion, caste, duration of T1D (at baseline), total number of insulin dose adjustments in clinic (0–21 months), change in the total daily dose of insulin per kg (0 vs. 21 months), method of insulin storage (refrigerator or clay pot), adherence to BG testing (at 21-month visit), adherence to insulin dosing (at 21-month visit), median number of patient education sessions attended (0–21 months), number of missed clinic visits (0–21 months), self-reported household income, self-reported possession of a below the poverty line certificate issued by the relevant authorities [4], patient level of education, and parental levels of education. Variables with p values less than 0.1 were included in a multivariable ordinary least squares linear regression model.

Secondary analyses included evaluation of safety, adherence to education sessions, and adherence to diabetes management recommendations. The incidence of acute diabetes-related complications pre- and post-BG monitoring was assessed using Poisson models, and 95% confidence intervals were reported. Adherence to BG testing was determined by dividing the number of tests completed between 18- and 21-month clinic visits by the expected number of tests to be completed. Adherence to insulin dosing was based on reported adherence between the 18- and 21-month clinic visits. Both were summarized using frequency and percent. For each patient, the total number of occurrences for each type of education was summed across the clinic visits. As most patients had the same number of education sessions for each type of education, each patient's attendance was summarized by the median number of sessions attended.

Two-sided p values less than 0.05 were considered statistically significant. All statistical analyses were performed using R version 3.4.2 [5].

Results

Two hundred forty patients were eligible for the study; however, only 191 participants attended all three of the baseline, 9-, and 21-month visits allowing for HbA1c collection and comparison (group 1). Baseline demographic and clinical characteristics are presented in Table 1. There was a significant decline in HbA1c from baseline 10.2 ± 2.5% (88 ± 4 mmol/mol) to 21 months 9.5 ± 2.4% (80 ± 3 mmol/mol), $p < 0.001$ (Table 2). There was also a significant decline in HbA1c from baseline to 9 months 9.8 ± 2.3% (84 ± 2 mmol/mol) ($p = 0.01$) and from 9 to 21 months ($p = 0.03$). In addition, we conducted an analysis on a second group of participants ($n = 152$), who missed at most one visit during the study (group 2). This more adherent group showed a similar pattern of findings with regard to change in HbA1c over the course of the study (Table 2), and therefore, they were not analyzed separately.

Adherence to SMBG and insulin dosing is presented in Table 3. The adherence to SMBG was moderate (51–90% tests completed) to excellent (> 90% tests completed) in 74.5% of participants. About half of participants had excellent adherence to insulin dosing (with no missed doses); however, the other half missed multiple doses, with 6.5% missing a third or more. It is important to note that none of the participants followed the instruction to give a correction dose of short-acting insulin ahead of a meal for hyperglycemia.

Insulin dose adjustments were made both in-clinic and between-clinic visits. The number of in-clinic adjustments (out of a total of seven visits, assessed at visits 3–21 months) varied from 0 (1.0%) to 7 (2.6%), with 65.4% of patients having 4 or more in-clinic insulin dose adjustments made during the study. The number of insulin dose adjustments between clinic visits varied from 0 (41.4%) to 10 (1.0%), indicating that most patients (58.6%) had at least one insulin dose adjustment made between clinic visits, with a median number of 2 adjustments over the course of 12 months (assessed at visits 12–21 months).

In univariable analysis, change in HbA1c between 0 and 21 months was associated with adherence to insulin dosing (assessed at 21-month visit) ($p < 0.001$), number of patient education sessions (0–21 months) ($p = 0.03$), household income ($p = 0.03$), and holding a below the poverty line certificate ($p = 0.02$). In multivariable analysis, using variables with $p < 0.10$ on univariable analysis, only adherence to insulin dosing was a significant predictor for change in HbA1c, with participants who missed > 30 doses in 3 months showing a rise in HbA1c (Table 4). When missed number of insulin doses was excluded from the model, not holding a below the poverty line certificate emerged as a significant predictor of decline in HbA1c from 0 to 21 months ($p = 0.03$).

There was no statistically significant increase in acute diabetes-related complications with the introduction of BG monitoring (Table 5).

Discussion

In this study, we found that HbA1c decreased significantly following the addition of two SMBG test strips per day (from none) over a 21-month period in a low-resource setting, without an increase in acute diabetes-related complications. Decline in HbA1c was independently associated with adherence to insulin administration.

International guidelines emphasize the importance of regular SMBG. The 2018 International Society of Pediatric and Adolescent Diabetes (ISPAD) Guidelines recommend that children should have access to technology and materials for

Table 1 Baseline demographic and clinical patient characteristics (n = 191)

Characteristic	Mean ± SD, median (IQR) or N (%)
Age (years)	15.9 ± 4.7
Female	106 (55.5%)
Religion	
Hindu	136 (72.0%)
Buddhist	39 (20.6%)
Muslim	13 (6.9%)
Christian	1 (0.5%)
Caste	
Scheduled caste	40 (21.2%)
Other backward caste	48 (25.4%)
Scheduled tribe	2 (1.1%)
Other caste	81 (42.9%)
No caste	18 (9.5%)
Duration of T1D (years)	6.5 ± 4.1
Total insulin dose (units/kg/day)	1.1 ± 0.3
Education level (years)	
Participant	9.5 ± 3.9
Mother	10.1 ± 3.9
Father	11.0 ± 3.9
Weekly household income (rupees)	1800 (1200, 2500)
Holding a below the poverty line certificate	75 (40.3%)
Insulin storage	
Refrigerator	135 (70.7%)
Clay pot	21 (11.0%)
Other	35 (18.3%)
Microvascular complications of diabetes	9 (4.7%)
Cardiovascular disease (including hypertension)	2 (1.0%)
Associated complications of diabetes	
Hypothyroidism	40 (20.9%)
Other	5 (2.6%)

SMBG to test enough to optimize diabetes care and that this may require 6–10 SMBG measurements per day [1].

This frequency of monitoring can be achieved in high-resource settings, and it is well documented that increased frequency of SMBG is associated with improved glycemic control in high-income countries. A 2011 study of BG monitoring in children and adolescents with T1D in Austria and Germany (n = 26,723) found that SMBG was significantly associated with improved metabolic control, with a 0.20% drop in HbA1c for one additional BG test per day ($p < 0.0001$), controlling for age, gender, diabetes duration, year of treatment, insulin regimen, insulin dose, BMI SDS, and center difference [6]. They also found that in adolescents > 12 years, HbA1c improved distinctly with 2 or more BG measurements per day [6]. Findings from the T1D exchange similarly indicate a strong independent association between SMBG and glycemic control, across socioeconomic demographics [7].

SMBG supplies are, however, often inaccessible or unaffordable in low-resource settings [2], as was the case at DT for many years. ISPAD recommends that diabetes center personnel should advocate to nations, states, and healthcare funders to ensure that children and adolescents with diabetes have adequate glucose monitoring supplies [1]. Data examining the impact of SMBG on HbA1c in low- and middle-income countries are, however, limited. Over the past 10 years, a few studies looking at T1D management in low-resource settings in Africa and South America have suggested benefit from SMBG on glycemic control [6, 8–12]. A recent study by Chopra et al. showed a significant decline in HbA1c in an Indian pediatric T1D clinic with the provision of both insulin and SMBG test strips (average of 3.6 per day) for 1 year [13]. Our study adds to this body of literature by demonstrating a clinically and statistically significant improvement in HbA1c with the use of 2 SMBG strips per day (from none) when added to a regimen that already included regular access to insulin, in patients followed at the DT. This is an important finding that can be used to support advocacy efforts for improving access to SMBG supplies in other low-resource settings, even if affordability limits the ability to provide the optimal number of daily test strips.

Table 2 Comparison of absolute and change in A1C at 0, 9, and 21 months (n = 191)

Time point	Group 1 (n = 191)[a]			Group 2 (n = 152)[b]		
	A1C % (mean ± SD)	Mean difference (95% CI)	p value	A1C % (mean ± SD)	Mean difference (95% CI)	p value
Baseline	10.2 ± 2.5			10.3 ± 2.5		
9 months	9.8 ± 2.3	vs baseline: 0.39 (0.11, 0.67)	0.01	9.7 ± 2.2	vs baseline: 0.60 (0.29, 0.91)	< 0.001
21 months	9.5 ± 2.4	vs baseline: 0.66 (0.32, 1.01)	< 0.001	9.4 ± 2.3	vs baseline: 0.88 (0.51, 1.26)	< 0.001
		vs 9 months: 0.27 (0.02, 0.53)	0.03		vs 9 months: 0.28 (0.00, 0.56)	0.05

[a] Group 1 = participants with HbA1c at 0, 9, and 21 months

[b] Group 2 = participants with HbA1c at 0, 9, and 21 months who missed ≤ 1 clinic visit during study period

Table 3 Adherence to BG monitoring and insulin dosing 18–21 months ($n = 191$)

Characteristic	*n* (missing)	*N* (%)
Adherence to BG monitoring	191 (85)	
Excellent (> 90%)		23 (21.7%)
Moderate (> 50–90%)		56 (52.8%)
Poor (> 10–50%)		27 (25.5%)
Very poor (≤ 10%)		0 (0.0%)
Number of correction doses of short-acting insulin given before a meal for BG > 14 mmol/L (250 mg/dL)	191 (30)	0 (0.0%)
Number of missed insulin doses (in 3 months)	191 (21)	
0		82 (48.2%)
1–10		44 (25.9%)
11–20		22 (12.9%)
21–30		11 (6.5%)
> 30		11 (6.5%)

Our study also aimed to determine if external support and education about SMBG use are essential to the success of the provision of this resource to T1D clinics in low-resource settings. We found that HbA1c declined significantly in the first 9 months of our study *without* external support or education and then continued to decline over the subsequent 12 months with the addition of external support and education. Our results suggest that there is a benefit to simply providing SMBG supplies to clinics and patients (without the need to provide additional external support/education) although this is likely

Table 4 Multivariable analysis between select variables[a] and change in HbA1c (0–21 months) ($N = 165$)

Variable	*n*	Change in HbA1c from 0 to 21 months Median (IQR)[b]	Adjusted coefficient (95% CI)`	*p* value
Number of missed insulin doses[c]				0.003
0	80	− 0.7 (− 2.0, − 0.1)	0 (reference)	
1–10	41	− 1.1 (−2.6, − 0.1)	0.21 (−0.68, 1.09)	
11–20	22	− 0.1 (− 1.9, 0.9)	0.51 (−0.60, 1.61)	
21–30	11	− 1.2 (− 1.6, 1.0)	0.42 (−1.06, 1.90)	
> 30	11	3.1 (0.7, 4.0)	3.12 (1.59, 4.64)	
Number of patient education sessions				1.00
1	1	0.9 (0.9, 0.9)	0 (reference)	
2	16	0.4 (− 0.7, 1.5)	0.18 (− 5.52, 5.87)	
3	55	− 0.6 (− 2.0, 0.4)	0.35 (− 5.34, 6.03)	
4	93	− 1.0 (− 2.0, 0.5)	0.34 (− 5.45, 6.13)	
Number of missed clinic visits				0.57
0	83	− 0.9 (− 1.9, 0.3)	0 (reference)	
1	50	− 0.7 (− 2.4, 0.1)	0.21 (− 0.82, 1.24)	
2	23	0.3 (− 1.4, 1.5)	1.12 (− 0.44, 2.69)	
3	6	0.6 (− 0.8, 3.2)	1.47 (− 1.05, 3.99)	
4	3	0.9 (0.4, 1.0)	1.72 (− 1.79, 5.22)	
Household income (per 100 rupees/week)	165		0.00 (− 0.04, 0.04)	0.94
Holding a below the poverty line certificate				0.16
No	102	− 0.9 (− 2.5, 0.3)	0 (reference)	
Yes	63	− 0.5 (− 1.3, 1.1)	0.66 (− 0.26, 1.58)	

[a] Variables selected based on a $p < 0.1$ from univariable analysis

[b] For discrete predictors only

[c] Over 3 months (18–21 months)

dependent upon the baseline knowledge and experience of each individual healthcare team. There may also be an additional benefit to enhanced education to the diabetes team about how to strategically test BG and use the information for insulin dose adjustments as suggested by the decline in A1C from nine to 21 months in our study; however, our study was not designed with a control group and therefore cannot specifically answer this question. Chopra et al. designed a randomized controlled trial to assess the impact of patient-directed education. They randomized participants in an Indian pediatric T1D clinic to either receive, or not, enhanced diabetes education via intensive telephone contact by a diabetes educator. All participants received insulin and SMBG supplies for 1 year. Interestingly, they found that the group that received the educational intervention improved knowledge, but not HbA1c. HbA1C improved in both groups equally with the provision of insulin and SMBG supplies alone [13]. Our study differs from Chopra's in that the educational intervention was aimed at the diabetes care team vs. patients. Also, DT was already providing insulin to patients at baseline (not a new intervention), but patients did not have prior access to SMBG supplies.

Despite the availability of SMBG supplies and insulin in our study, there remain concerns around adherence to T1D management. Though adherence to SMBG was fair, adherence to insulin dosing was the main determinant of change in HbA1c during the study period. Specifically, patients who missed a significant number of insulin doses (> 30 in 3 months) had an increase in HbA1C of more than 3% over 21 months. This variable obscured all others included in multivariable regression analysis, including many social determinants of health. This suggests that targeting interventions to assess and address barriers to insulin administration may further help to improve glycemic control independent of social and economic conditions.

It is also worth noting that none of the participants in our study gave correction doses of short-acting insulin for pre-meal hyperglycemia (as per instructions provided in Phase 2) and that there were relatively few contacts with DT between visits for insulin dose adjustments. These two findings highlight further areas for improvement in patient education about how to best use SMBG data. We observed improvement in glycemic control despite many participants not adhering to management recommendation. We would therefore expect an even greater improvement in HbA1c with improved adherence to these aspects of T1D management, even with limited BG monitoring. Interestingly, however, the subset of patients (group 2, $n = 152$) with greater adherence to clinic visits (missing ≤ 1 during the study period) did not show a statistically significant difference in improvement in glycemic control when compared to the group as a whole (Table 2).

We acknowledge certain limitations of this study, including incomplete data for all 240 participants who started the study (39 attended the baseline visit but missed the 9- or 21-month visit). This might have led to a selection bias by including the 191 patients who were more adherent to diabetes follow-up in the analysis. This was not a randomized controlled trial, limiting the strength of data. However, in designing this study, we did not feel that it was ethical to withhold SMBG supplies from half of the participants given the likelihood of improved glycemic control. Finally, baseline number of acute diabetes-related complications was determined by patient report of events in the last year, which may be subject to recall bias; however, these events are significant enough that we would expect that most parents would remember their occurrence in the last year.

In conclusion, HbA1c decreased significantly following the addition of two BG test strips per day (from none) over a 21-month period in children and youth with T1D followed by DT in India, without increasing the risk of acute adverse events such as hypoglycemia. This is important data that should be used in advocating for funding of SMBG supplies for children with T1D in other low-resource settings even if resources only permit the provision of a limited number (two) of daily test strips. The results are consistent with data from other countries using an "intermediate care" approach to T1D

Table 5 Diabetes-related acute complication rate pre- and post-BG monitoring ($n = 191$)

Characteristic	# of cases per total PYs[a]	Incidence rate per 100 PYs[a]	Ratio post/pre (95% CI)	*p* value
DKA episodes				
Pre-BG monitoring	4/191	2.09	Reference	
Post-BG monitoring	15/334.2	4.49	2.14 (0.68, 8.87)	0.25
Severe hypoglycemia episodes				
Pre-BG monitoring	4/191	2.09	Reference	
Post-BG monitoring	8/334.2	2.39	1.14 (0.31, 5.19)	1.00
Skin infections, secondary to BG testing				
Pre-BG monitoring	1/47.8	2.09	Reference	
Post-BG monitoring	0/334.2	0.00	0.00 (0.00, 5.57)	0.25

[a] PYs = person years

management in young people [14]. Adherence to insulin administration was also independently associated with HbA1c, emphasizing its critical importance in glycemic control. Further analysis of context-specific barriers may be helpful in developing interventions to further optimize the use of SMBG and ensure adequate insulin dosing throughout the day.

Authors' contributions Caroline Zuijdwijk was involved in study conception and design, data interpretation, and writing the manuscript. Sharad Pendsey was involved in study conception and design and facilitated the collection of data in his clinic. James Ron was involved in study conception and design, as well as data interpretation. Graham Ogle provided scholarly input and was involved in data interpretation. Amisha Agarwal and Nick Barrowman were involved in data analysis and interpretation. Seema Chalkhore and Sanket Pendsey were involved in study design, as well as data collection and recording. Alexandra Ahmet was involved in study conception and design and data interpretation. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Compliance with ethical standards

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

Informed consent Informed consent was obtained from all participants by DT staff. If participants were too young to consent to study participation, informed consent was obtained from their parents/ legal guardians, and assent was obtained from the child.

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ORIGINAL ARTICLE

Diagnostic performance of HbA1c for detecting prediabetes and diabetes in Turkish adults

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Abstract

Background We evaluated the diagnostic performance of HbA1c in diabetes and prediabetes and compared its efficiency in a model where HbA1c is used as a screening test.

Material and method Records of 945 patients who had undergone simultaneous OGTT and HbA1c were evaluated. American Diabetes Association (ADA) criteria were used for diagnosis. Agreement between OGTT and HbA1c was evaluated. Using OGTT as the gold standard, the diagnostic performance of HbA1c was evaluated with ROC analysis and optimum cutoffs were determined. Then, the group was rearranged as if HbA1c was performed first as a screening test with new cutoffs and if OGTT was performed for diagnosis among suspected individuals.

Results According to OGTT, 53 (5.6%) patients were diabetic and 247 were prediabetic; 18 of diabetics (34%) and 94 of prediabetics (38%) were diagnosed by HbA1c with present ADA cutoffs. The kappa coefficient for agreement between OGTT and HbA1c was 0.756 for diabetes and 0.336 for prediabetes. In ROC analysis, optimal HbA1c cutoff points were 38 mmol/mol (AUC 0.953) for diabetes and 35.5 mmol/mol (AUC 0.673) for prediabetes. In a new model after initial screening with HbA1c, 398 patients with HbA1c > 35.5 mmol/mol were taken for further OGTT. Fifty-three of diabetics (100%) and 199 of prediabetics (81%) were diagnosed. Prediabetic 48 patients (19%) were missed.

Conclusion HbA1c showed poor performance in diagnosis of diabetes and prediabetes. Instead, using HbA1c as a screening test with well-defined cutoff may offer practical and economical advantages greatly reducing the number of OGTTs required.

Keywords Diabetes mellitus · Diagnosis · Glucose tolerance test · Glycated hemoglobin A · Prediabetes

Introduction

In the current guidelines, diabetes is diagnosed either the fasting plasma glucose (FPG) concentration of ≥ 7.0 mmol/L or the 2-h plasma glucose (2-h PG) concentration of ≥ 11.1 mmol/L after a 75-g oral glucose tolerance test (OGTT) Table 1 [1]. In 2010, ADA recommended HbA1c as another tool using a level of ≥ 48 mmol/mol for diagnosing diabetes and 39–46 mmol/mol for identifying high-risk individuals for diabetes in future (i.e., prediabetes) [2]. Unfortunately, FPG, 2-h PG OGTT, and HbA1c do not verify each other in many cases either with diabetes or prediabetes [3]. Agreement of HbA1c is poor either with FPG and 2-h OGTT. Various studies have confirmed that patients who fill the criteria of 2-h OGTT for diagnosis of diabetes have near-normal HbA1c levels and might not be detected if OGTT was not performed [4]. OGTT is also efficiently used to diagnose insulin resistance in different clinical situations and HbA1c alone has no use in patients with metabolic syndrome [5]. Although OGTT is the gold standard, it has several limitations. It requires preparation of the patient, it lacks repeatability, and ıt is a time-consuming and burdensome diagnostic tool [6, 7]. Analytic performance of HbA1c testing is better, with less preanalytic instability, < 2% day-to-day within-person variability, overall expressing glycemic exposure, and risk for long-term complications [8]. It is also efficiently used in follow-up of diabetics, in spite of the consequences in diagnosis. Much work has been done to use it as a comparable diagnostic test, most studies recommended different HbA1c cutpoints, ranging from 37.7 to 53 mmol/mol, for diagnosis of diabetes. Variabilities were

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Table 1 Criteria for the diagnosis of diabetes and prediabetes

Diagnosis	FPG	OGTT 2-h PG	HbA1c
Normoglycemia	< 100 mg/dL (< 5.6 mmol/L)	100–125 mg/dL (5.6–6.9 mmol/L)	< 5.7% (< 39 mmol/L)
Prediabetes	< 140 mg/dL (< 7.8 mmol/L)	140–199 mg/dL (7.8–11 mmol/L)	5.7–6.4% (39–47 mmol/mol)
Diabetes	≥ 126 mg/dL (≥ 7.0 mmol/L)	≥ 200 mg/dL (11.1 mmol/L)	≥ 6.5% (≥ 48 mmol/mol)

FPG, fasting plasma glucose

In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing

attributed to factors such as age, ethnicity, genetic basis, and erythrocyte life-span altering glycosylation rate and turnover of hemoglobin [9–11]. We evaluated the diagnostic performance of HbA1c in diabetes and prediabetes and compared the efficiency of a simulated model where HbA1c is used as an initial screening test on the same patients' group.

Material and methods

Patient selection

We retrospectively evaluated the medical records of 945 individuals who attended outpatient clinics of Endocrinology, Internal Medicine and Family Medicine at Dr Lütfi Kırdar Kartal Training and Research Hospital between January 2014 and February 2018. The patients had undergone simultaneous OGTT and HbA1c testing for evaluation of diabetes and prediabetes.

OGTT testing procedure

Exclusion criteria for OGTT testing included states of pregnancy, lactation, acute severe illness, hospitalization in preceding 2 weeks, low hemoglobin concentration (< 12 g/dL in women; < 13 g/dL in men), hemoglobin disorders, hemolytic anemia, major blood loss or blood transfusion in the past 6 months, serum creatinine ≥ 2 mg/dL, connective tissue disease, thyroid disease, and concomitant drug therapy known to cause hyperglycemia such as corticosteroids, diuretics, and nicotinic acid [12]. Tests were performed between 07.00 and 09.00 in the morning after 3 days of unrestricted diet (containing at least 150 g of carbohydrate per day) and activity, after at least 8-h fasting only in ambulatory outpatients. Venous blood samples were drawn first. FPG > 7 mmol/L was diagnosed as diabetics and remaining participants subsequently ingested 75 g anhydrous glucose dissolved in 300 mL water in 5 min. Venous blood was collected again for 2-h PG measurement [12]. Patients were classified as normoglycemia, IGT, and diabetes according to their 2-h PG values. All patients were once more classified according to their HbA1c results Table 1. All diagnosis was based on ADA recommendations.

Glucose measurements were done with the hexokinase method, on AU5800 Chemistry Analyzer (Beckman Coulter, Brea, CA, USA). HbA1c was measured by cation-exchange high-performance liquid chromatography Bio-Rad Variant II Turbo instrument (Bio-Rad Laboratories, Hercules, USA) This method was certified by the National Glycohemoglobin Standardization Program (NGSP) and traceable to the Diabetes Control and Complications Trial (DCCT).

Statistical analysis

Agreement between HbA1c and OGTT was tested with Kappa statistics. The kappa coefficient is scaled between 0 (poor) and 1 (perfect agreement), and intermediate values are interpreted as fair (0.21–0.40), moderate (0.41–0.60), or substantial agreement (0.61–0.80) [12]. The diagnostic performance of HbA1c was investigated by AUC-ROC analysis referring to OGTT as the gold standard and optimal HbA1c cutoffs were determined. All statistical analyses were performed with MedCalc Statistical Software (version 12, MedCalc Software, Mariakerke, Belgium). $p < 0.05$ was considered statistically significant.

Results

Records of 945 patients, 154 males (16.2%) and 791 females (83.7%) with a mean ± SD age of 36.48 ± 13.43 years, were retrospectively evaluated. These patients had undergone OGTT and HbA1c testing simultaneously. The study flow chart is shown in Fig. 1. According to OGTT results, 26 patients had FPG > 7 mmol/L and 27 patients had OGTT 2-h PG > 11 mmol/L making a total of 53 (5.6 %) diabetics. A total of 163 (17%) patients had impaired fasting glycemia (IFG) values and 63 of them had diabetic or IGT OGTT 2-h PG results. Also, 147 (15.5%) patients had impaired glucose tolerance (IGT) during OGTT and this made a total of 247 prediabetes. All study groups diagnosed according to ADA

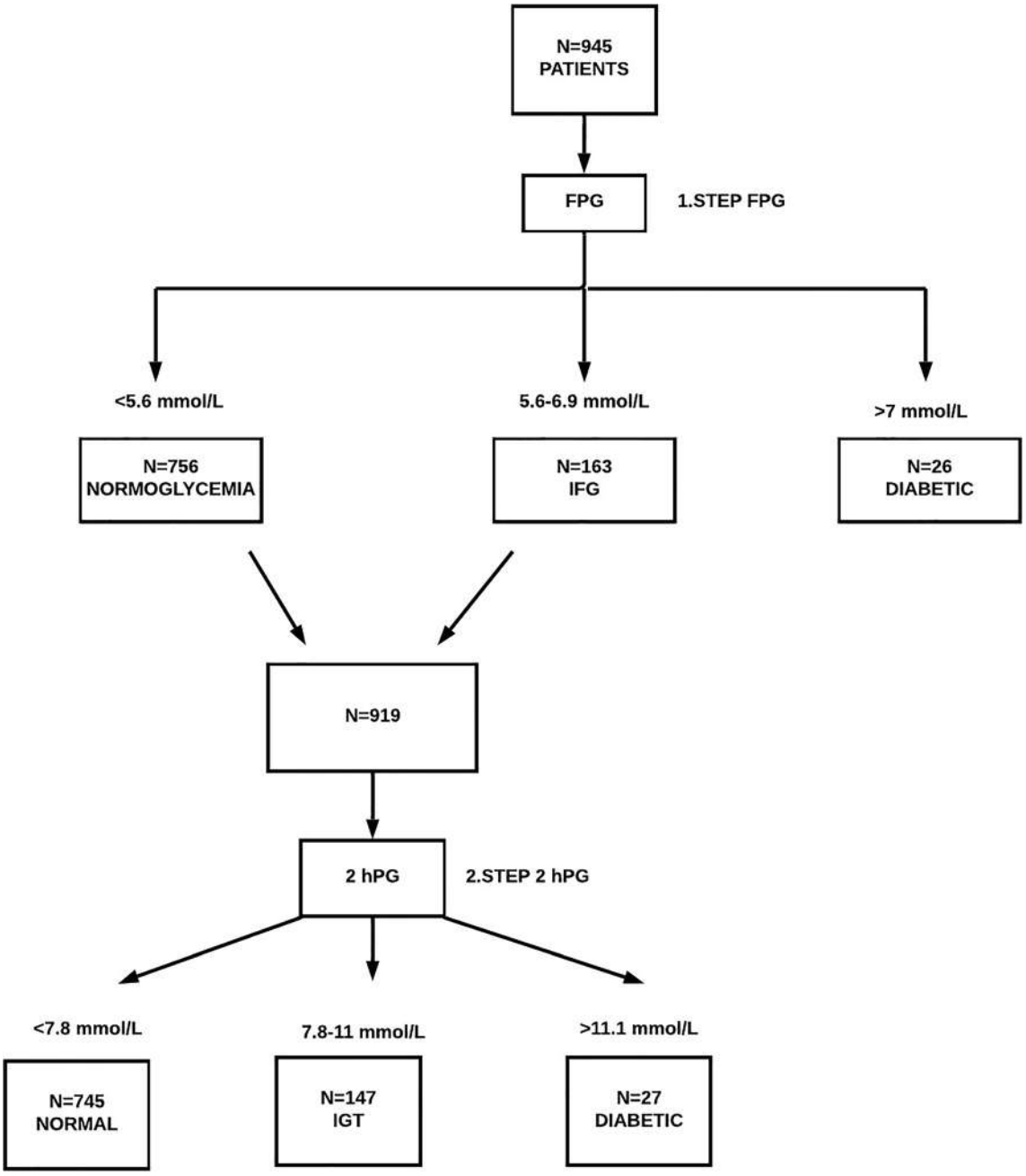

Fig. 1 Study flow chart. FPG, fasting plasma glucose; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance

criteria are given in Table 2. Only 18 (34%) of diabetics and 94 (38%) of prediabetics were diagnosed by HbA1c testing with present ADA cutoffs. The kappa coefficient for agreement between OGTT and HbA1c was 0.336 for prediabetes, 0.756 for diabetes which was interpreted as fair and substantial respectively. In ROC analysis, optimal HbA1c cutoff points were found as 38 mmol/mol (AUC 0.953; 0.934–0.967 CI) for diabetes and 35.5 mmol/mol (AUC 0.673; 0.641–0.704 CI) for prediabetes (Figs. 2 and 3, respectively).

The total group was rearranged in a new model where HbA1c was used for screening with a cutoff of 35.5 mmol/mol calculated in this study. In our simulation model, if 945 patients were first screened with HbA1c and results were classified according to the new optimum cutoff, 547 patients would be considered normal while 398 patients would undergo further OGTT testing for diagnosis. In the FPG phase of OGTT, 26 patients and the 2-h PG phase, 27 patients (totally 100%) would be diagnosed as diabetics. Of the remaining 345 patients, 199 were prediabetic; this number was 81% of formerly identified prediabetics. This model seemed efficient enough to diagnose all diabetics and 81% of prediabetics with applying OGTT to only 42% of patients. Flow chart of simulated model is shown in Fig. 4.

Table 2 All study groups diagnosed according to the ADA criteria

		Diagnosis based on HbA1c%		
		Normoglycemia (no., %)	Prediabetes (no., %)	Diabetes (no., %)
Diagnosis based on OGTT	Normoglycemia (no.,%)	585 (62%)	58 (6.1%)	2 (0.2%)
	Prediabetes (no.,%)	149 (15.8%)	94 (9.9%)	4 (0.4%)
	Diabetes (no.,%)	9 (0.9%)	26 (2.7%)	18 (1.9%)

Normoglycemia (FPG < 5.6 mmol/L or 2-h PG < 7.8 mmol/L) (HbA1c < 38.8 mmol/mol)

Prediabetes (FBG 5.6–6.9 mmol/L or 2-h PG 7.8–11 mmol/L) (HbA1c 38.8–46.4 mmol/mol)

Diabetes (FPG ≥ 7 mmo/L or 2-h PG 11.1 mmol/L) (HbA1c > 46.4 mmol/mol)

HbA1c, hemoglobin A1c; *no.*, number

Discussion

The present study revealed a fair and substantial agreement between OGTT and HbA1c for prediabetes and diabetes respectively. Taking OGTT testing as the reference, optimal HbA1c cutoff points were found as 38 mmol/mol (AUC 0.953; 0.934–0.967 CI), with the sensitivity of 85.45% and specificity of 90.23% for diabetes and 35.5 mmol/mol (AUC, 0.673; 0.641–0.704 CI) with the sensitivity of 49.3% and specificity of 79.32% for prediabetes. HbA1c threshold of 47.5 mmol/mol was based on the cutoff point for detection of retinopathy [8]. Using HbA1c criteria, only 1/3–1/10 of affected individuals could be detected [13]. On the other hand, HbA1c offers analytic superiority over OGTT; in cases of easy patient preparation, high preanalytical stability and less intraindividual and day-to-day variations [3]. Several studies are investigating the role of HbA1c for diagnosis of DM, which is compared with the OGTT. Sensitivity and specificity values for HbA1c vary according to different ethnic groups. Differences in hemoglobin concentration, glycation rate, lifecycle, or quantity of erythrocytes related to genetics affect these values [14]. The usefulness of HbA1c for diagnosis of diabetes is influenced by genetic factors [15]. Colagiuri et al. evaluated the diagnostic efficiency of HbA1 in a large population ($N = 10{,}447$) without diabetes. In their study, a cutoff value of 34 mmol/mol for HbA1c had a sensitivity of 78.7% with a specificity of 82.8% [16].

In a study by Giniş et al., HbA1c cutoff value for the diagnosis of DM was recommended as 43 mmol/mol with a sensitivity of 81.8% and specificity of 80%; PPV and

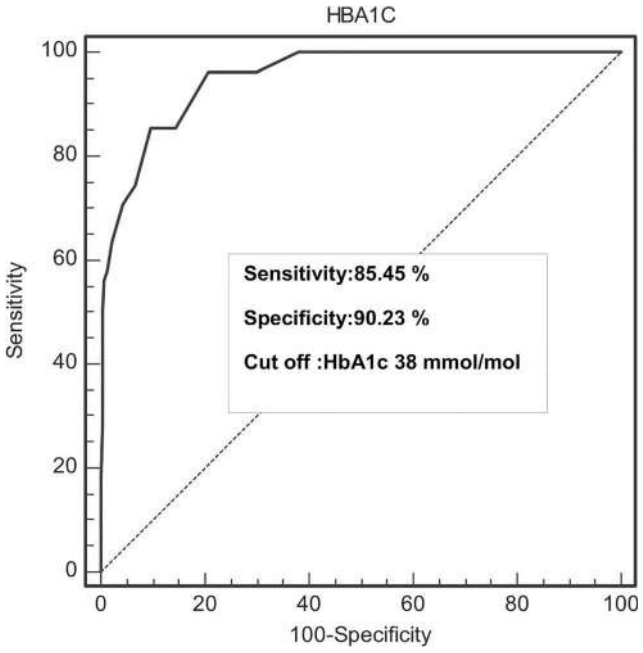

Fig. 2 The receiver operating characteristic curve for HbA1c in the diagnosis of type 2 DM, which corresponds to the AUC (95% CI) of 0.953 (0.934–0.967). HbA1c, hemoglobin A1c; AUC, area under the receiver operating characteristic curve; CI, confidence interval

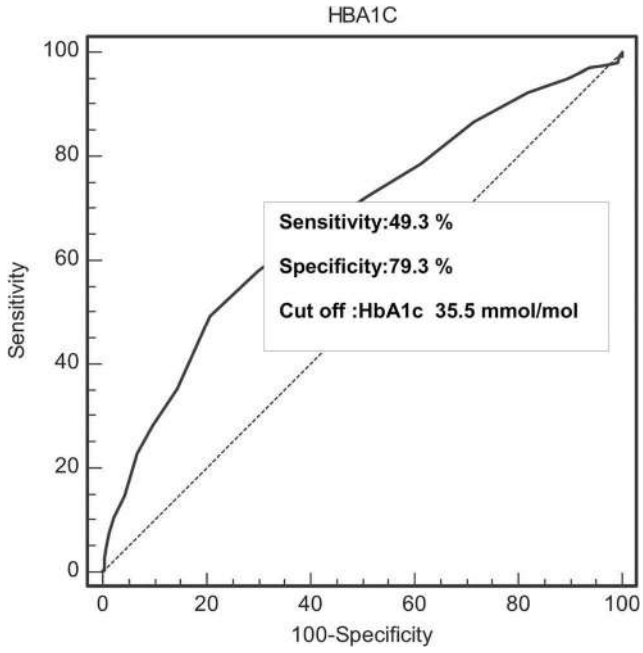

Fig. 3 The receiver operating characteristic curve for HbA1c ın the diagnosis of prediabetes, which corresponds to the AUC (95% CI) of 0.668 (0.638–0.697). HbA1c, hemoglobin A1c; AUC, area under the receiver operating characteristic curve; CI, confidence interval

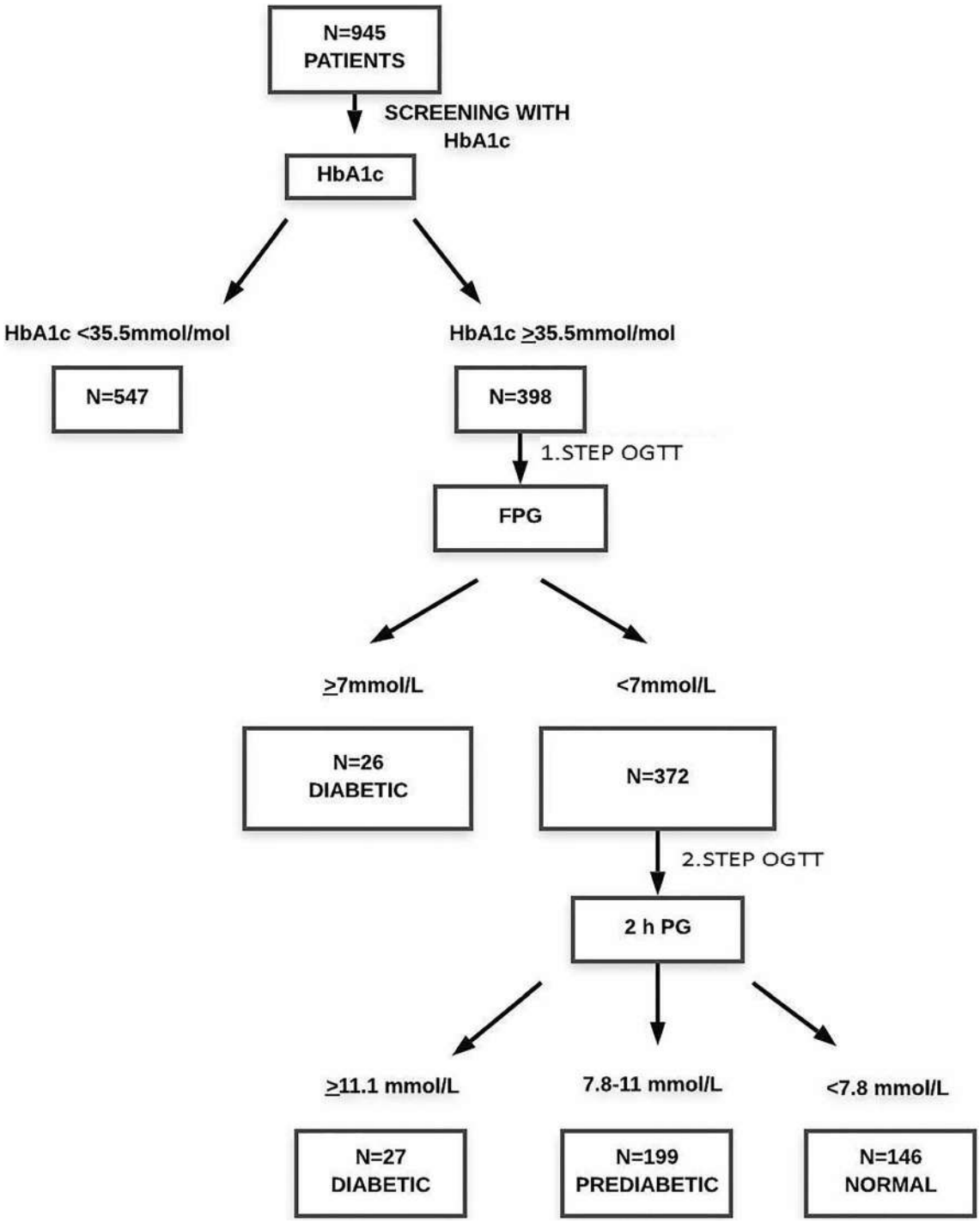

Fig. 4 Flow chart of simulated model

NPV for HbA1c were 80.2% and 81.05%, respectively [17]. A sensitivity of 56.8% and specificity of 89.2% were calculated for a cutoff value of 48 mmol/mol. Their cut-off, sensitivity, specificity, PPV, and NPV values were different from us but they found lower cutoffs than ADA similar to our study. They screened a smaller number of participants ($n = 295$) and used latex agglutination inhibition method in their study while our study included a large number of patients (945) and HPLC method that is certified by the NGSP and standardized or traceable to the DCCT reference assay was used. It seems it is better to determine and apply populational cutoffs for rather than applying universal ones.

Applying 38 mmol/mol as a new cutoff, we tried a simulation where we used HbA1c, not for diagnosis but screening. As a result with 945 HbA1c testings for screening with a

cutoff of 35.5 mmol/mol, the number of patients requiring OGTT reduced to 398 patients (42%) of the original. As a result, we could identify all of the diabetics and 81% of prediabetics. For those missing IFG patients (19%), repeating analysis of FPG with suitable intervals may be recommended if they are in risk groups and/or show clinical symptoms. The estimated prevalence of even diabetes is in the range of 8–12% and can remain silent up to raising with complications if proper screening procedures cannot be proposed.

This study included a large number of Turkish patients who underwent simultaneous OGTT and HbA1c for an accurate comparison of two tests. Eighty percent of the study population consisted females which is the limitation of the study. We compared diagnostic efficiencies of HbA1c with current cutoffs and in a new model where HbA1c is used as an initial screening test on the same patients' group. Optimum cutoff we found in this study was far away from ADA recommended cutoffs. In this study, we made a simulation as if HbA1c was used as a screening test in this patient population. We observed that this model might offer clinical and economic benefits with better diagnostic efficiency. HbA1c is strongly recommended in follow-up of diabetic patients but the use of it as a diagnostic tool remains controversial.

Compliance with ethical standards The authors declare that they have no conflict of interest.

Research involving human participants Since this is a retrospective data analysis without using individual patient characteristic, a particular consent could not be obtained for the study. But permission was taken from our ethical committee and number was given.

Statement on consent for participation The study was approved by the Ethics Review Committee of the Dr Lütfi KırdarKartal Research and Training Hospital (permission number and date: 2018/514/130/6 29.05.18).

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ORIGINAL ARTICLE

Optimal reference values for insulin sensitivity indices in Iranian healthy females: a population-based study

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Abstract

Purpose To define reference values for various insulin sensitivity (IS) indices in healthy women in a population-based study of the five provinces in Iran

Methods In this cross-sectional study, 250 normal-weighted women without hypertension, diabetes, polycystic ovary syndrome, hormonal disorders, pregnancy, or menopause were evaluated in this study. After a 12-h fasting, blood samples were obtained from all participants, levels of metabolic markers (fasting plasma glucose, lipid profile, insulin, etc.) were measured using enzymatic colorimetric and ultrasensitive enzyme-linked radioimmunoassay methods. Multiple insulin sensitivity indices were calculated using well-known predetermined formulas. *K*-means cluster analysis was used to determine reference values for each insulin sensitivity index.

Results The threshold values of homeostasis model assessment (HOMA-%β, HOMA-βcell) and fasting insulin resistance index (FIRI) were 400.41, 198.55, and 2.57 corresponding to 97th, 93rd, and 92nd percentiles, respectively. Reference values of quantitative insulin sensitivity check index (QUICKI) and TG/HDL-c were 0.44 and 4.40, both of which relate to 92nd percentile. The recently introduced single-point insulin sensitivity estimator (SPISE) was analyzed and showed a threshold of 10.10 (87th percentile) in this study. Threshold values of reciprocal insulin (1/Insulin) and glucose-to-insulin ratio (G/I) were 0.51 (95th percentile) and 76.91 (99th percentile), respectively.

Conclusion This study determined reference values for various insulin sensitivity indices in healthy Iranian women. These values can be used to define insulin sensitivity in women in epidemiological and clinical studies.

Keywords Insulin sensitivity · Cluster analysis · Reference value · Population study

Introduction

Insulin sensitivity/resistance plays an important role in the pathophysiology of obesity, diabetes mellitus, and polycystic ovary syndrome [1–3]. Increased risk of cardiovascular diseases (CVD), including coronary heart disease and stroke, and hypertension has been shown to be associated with high insulin levels [4]. Insulin sensitivity (IS) is closely associated with insulin secretion, which corresponds significantly in glucose dysregulation and obesity [5, 6]. Therefore, knowledge of the insulin sensitivity/resistance profile of individuals might be helpful in determining their CVD risks among other known CVD risk factors.

The gold standard method for measuring insulin sensitivity (IS) is the euglycemic clamp [7]. In comparison with surrogate markers, the euglycemic clamp method requires more time and financial resources. Therefore, many epidemiological studies nowadays use various surrogate markers instead of the former to assess and calculate IS. The quantitative insulin sensitivity check index (QUICKI), homeostasis model assessment (HOMA), fasting insulin resistance index (FIRI), reciprocal insulin, and single-point insulin sensitivity estimator (SPISE) are examples of such markers [8, 9].

There are few studies that explore specific reference values of surrogate markers to determine IS. Moreover, the cutoff

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values of insulin sensitivity/resistance are not homologous across different populations and ethnicities [10]. Tohidi et al., in a population-based study, showed reference values of insulin and insulin resistance indices (HOMA-IR and QUICKI) in men and women [11]. In this study, we aimed to explored national reference values for various IS indices in healthy women in a population-based study of women in five provinces of Iran, which are a good representative of all Iranian women [12].

Materials and methods

Subjects

The subjects of this study were recruited from Iranian PCOS Prevalence Study, which is a large population-based study on prevalence and clinical aspects of polycystic ovary syndrome. It originally consists of more than 1700 reproductive age women in urban areas of five randomly selected provinces in various geographic regions, i.e., Ghazvin, Kermanshah, Golestan, Khuzestan, and Hormozgan [13, 14]. For the present study, women with diabetes, hypertension, and body mass index (BMI) ≥ 25 kg/m^2 and those with polycystic ovary syndrome (PCOS), or idiopathic hyperandrogenism, were excluded. Finally, 250 healthy women were selected for the current study (Fig. 1).

Measurements

Weight of each subject was measured while she had worn minimal clothes using digital scale (Seca 707, Hanover, MD, USA). Height was measured in standing, normal posture of the shoulders with a tape meter. Two measures of systolic and diastolic blood pressures (SBP and DBP, respectively) from the right arm were done under standard conditions while seated and the means of each two SBP and DPB were calculated. A blood sample was obtained from each subject at 7–9 A.M. after a 12-h overnight fasting.

Fasting plasma glucose (FPG) was measured using an enzymatic colorimetric method with glucose oxidase (inter- and intra-assay coefficients of variation (CV) were 2.2%). Serum insulin concentration was measured using the ultrasensitive enzyme-linked radioimmunoassay method (Mercodia, Uppsala, Sweden) with a covariance < 4%. The level of triglycerides was determined using enzymatic colorimetric method with glycerol oxidase (inter- and intra-assay CVs were 0.6 and 1.6%, respectively). Total cholesterol (TC) level was measured by enzymatic colorimetric method with cholesterol esterase and cholesterol oxidase (inter- and intra-assay CVs were 0.5 and 2%, respectively). High-density lipoprotein cholesterol (HDL-c) level was determined after precipitation of apolipoprotein β with phosphotungstic acid in a similar enzymatic colorimetric method (method sensitivity and CV were 1 mg/dl and 2.1%, respectively). After internal qualification, all samples were analyzed.

Definitions

Based on National Institute of Health (NIH) [15], Rotterdam [16], and Androgen Excess Society (AES) [17] criteria, PCOS was defined as either (1) combination of chronic anovulation and clinical hyperandrogenism and/or hyperandrogenemia, or (2) at least two of the following: oligo/anovulation, hyperandrogenemia, and/or hyperandrogenism, polycystic ovaries on ultrasonography. Diabetes was defined as fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l), 2-hPG $\geq$ 200 mg/dl (11.1 mmol/l), or taking glucose-lowering drugs. Hypertension was defined as either systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or using anti-hypertensive medications.

The markers used in this study were reciprocal insulin [18], glucose-to-insulin ratio (G/I) [19], HOMA-%β [20], HOMA-βcell [21], QUICKI [21], FIRI [21], triglyceride-to-HDL-c ratio, and SPISE [9]. Their corresponding formulas are as follows:

$$\text{Reciprocal insulin} = 1/\text{fasting insulin (mU/l)}$$

$$\text{G/I} = \text{fasting plasma glucose (mmol/l)}/\text{fasting insulin (mU/l)}$$

$$\text{HOMA}-\%\beta = \text{fasting plasma insulin (mU/l)} \times 20/(\text{fasting plasma glucose (mmol/l)}-3.5)$$

$$\text{HOMA}-\beta\text{cell} = \text{fasting plasma insulin (mU/l)} \times 20/(\text{fasting plasma glucose (mmol/l)}-3)$$

$$\text{QUICKI} = 1/(\log(\text{fasting insulin (mU/l)}) + \log(\text{fasting plasma glucose (mg/dl)}))$$

$$\text{FIRI} = \text{fasting glucose} \times \text{fasting insulin}/25$$

$$\text{Triglyceride}-\text{to}-\text{HDL}-\text{c ratio} = \text{Triglyceride}/\text{HDL}-\text{c}$$

$$\text{SPISE} = 600 \times \text{HDL}-\text{c}^{0.185}/\left(\text{triglyceride}^{0.2} \times \text{BMI}^{1.338}\right)$$

where HDL-c and triglyceride units were expressed in milligrams per deciliter and BMI unit was expressed in kilograms per square meter.

Statistical analyses

The objective of clustering is to partition the original data points into clusters, in which the data points are internally homogenous but different from those of other clusters. In brief, in *K*-means cluster analysis, a desired number of clusters (i.e., "*K*") was suggested. Second, data points were assigned to clusters to which they are the closest. Third, the mean of each cluster was updated based on the data points assigned in

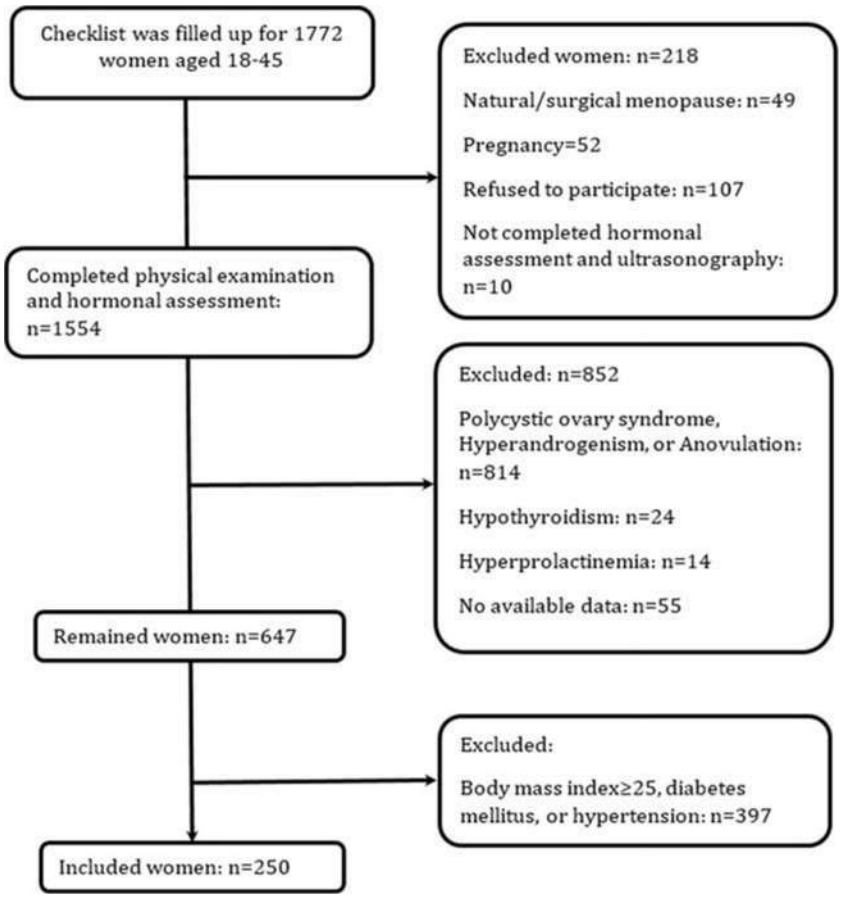

Fig. 1 Study flowchart

each cluster. Finally, these steps are repeated until no changes have been observed in clusters [22]. The exact number of "*k*" depended on the variable which was used for clustering. This method was used to determine the best threshold for each index [23], which is simple and effective that have made *K*-means one of the most popular choices among various approaches for clustering [24].

Statistical analysis was performed using software package STATA (version 12; STATA Inc., College Station, TX, USA) and using IBM SPSS Statistics for Windows version 16.

Results

Two hundred and fifty women were included in this study. The mean ± SD of age and BMI were 31.8 ± 7.7 years and 22.0 ± 2.3 kg/m^2, respectively. Table 1 shows characteristics of the women participated in this study. The mean ± SD of waist circumference and FPG was 74.0 ± 8.2 cm and 85.8 ± 11.5 mg/dl, respectively. The median (IQR 25, 75) of insulin was 5.6 (4.0, 9.4) mIU/l.

The *K*-means method resulted in categorization and obtaining the best reference value of each variable. The mean; median; and 5th, 10th, 90th, and 95th percentiles of each indices are shown in Table 2. It also shows the reference value of each IS index based on the *K*-means analysis. According to Table 2, the reference value of the QUICKI was 0.44 corresponding to the 92nd percentile in the study participants. The reference value of SPISE index of insulin sensitivity was 10.10 which corresponds to the 87th percentile.

Table 1 Baseline descriptive statistics of the study participants (n = 250)

Variable	Mean ± SD	Variable	Mean ± SD
Age (years)	31.8 ± 7.7	SBP (mmHg)	105.9 ± 11.5
Weight (kg)	56.2 ± 7.3	DBP (mmHg)	66.4 ± 9.3
Height (cm)	159.7 ± 6.3	FPG (mg/dl)	85.8 ± 11.5
BMI (kg/m^2)	22.0 ± 2.3	Cholesterol (mg/dl)	167.2 ± 33.9
WC (cm)	74.0 ± 8.2	LDL-c (mg/dl)	97.4 ± 29.9
HC (cm)	95.0 ± 8.3	HDL-c (mg/dl)	49.0 ± 11.7
WHR	0.78 ± 0.08	Triglycerides (mg/dl)	89.5 (71.0, 122.0)[a]
WHtR	0.46 ± 0.05	Insulin (mIU/l)	5.6 (4.0, 9.4)[a]

BMI, body mass index; *WC*, waist circumference; *HC*, hip circumference; *WHR*, waist-to-hip ratio; *WHtR*, waist-to-height ratio; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *FPG*, fasting plasma sugar; *LDL-c*, low-density lipoprotein cholesterol; *HDL-c*, high-density lipoprotein cholesterol

[a] Data are shown as median (IQR 25, 75)

Table 2 Overall descriptive statistics of the surrogate markers of insulin sensitivity and related cutoff values ($n = 250$)

Marker		HOMA-%β	HOMA-βcell	FIRI	QUICKI	TG/HDL-c	SPISE	1/Insulin	G/I
Mean		148.91	99.72	1.38	0.38	2.56	8.12	0.22	18.65
Median		100.72	71.61	1.04	0.37	1.88	7.81	0.18	14.68
Standard deviation		229.98	140.35	1.13	0.04	3.80	1.74	0.17	16.34
Percentiles	5th	24.02	18.36	0.34	0.32	0.85	5.98	0.06	5.40
	10th	34.14	25.00	0.46	0.33	0.95	6.26	0.08	6.67
	90th	285.31	179.73	2.44	0.43	4.11	10.32	0.42	35.57
	95th	382.27	244.25	3.15	0.46	5.63	11.31	0.54	49.16
Cutoff (percentile)		400.41 97th	198.55 93rd	2.57 92nd	0.44 92nd	4.40 92nd	10.10 87th	0.54 95th	76.91 99th

HOMA, homeostasis model assessment; *FIRI*, fasting insulin resistance index; *QUICKI*, quantitative insulin sensitivity check index; *TG/HDL-c*, triglycerides-to-high-density-lipoprotein-cholesterol ratio; *SPISE*, single-point insulin sensitivity estimator; *G/I*, fasting plasma glucose-to-insulin ratio

Analyses of HOMA-βcell and HOMA-%β, which are indices of insulin sensitivity as well as pancreatic beta-cell function, resulted in threshold values of 198.55 (93rd percentile) and 400.41 (97th percentile), respectively.

Discussion

The literature has yet to document the single best surrogate method, instead of the gold standard euglycemic clamp, for identification of insulin sensitivity in a general population. Some studies, however, suggested and tested some of those surrogate markers, i.e., QUICKI, Matsuda index, Cederholm index, Avignon index, glucose-to-insulin ratio, FIRI, etc. [7, 8]. They also have shown promising results in using these surrogate markers to define insulin sensitivity/resistance when there is shortage of time and money to use the gold standard method. Moreover, determining the exact reference values of each marker in each population is of great importance. To do this, the *K*-means cluster analysis is a useful method, which categorizes distinct clusters that are internally homogenous but heterogeneous between clusters. To identify the normative reference values, the single minimum value of the higher cluster is chosen to be the best estimate [25]. The *K*-means is a simple and efficient tool that is widely used to divide data into that clusters [26]. It is most useful when the outliers are deleted from the data, as we did in our analysis.

This population-based study presents the reference values of insulin sensitivity variables in healthy women of five provinces in Iran using the *K*-means cluster analysis. The threshold values of HOMA-%β, HOMA-βcell, and FIRI were 400.41, 198.55, and 2.57 corresponding to 97th, 93rd, and 92nd percentiles, respectively. Analyses of the QUICKI and TG/HDL-c variables resulted in the following thresholds: 0.44 and 4.40, both of which relate to 92nd percentile of the corresponding variable. The recently introduced single-point insulin sensitivity estimator (SPISE) was analyzed and showed a threshold of 10.10 (87th percentile) in this study. Finally, threshold values of reciprocal insulin (1/Insulin) and glucose-to-insulin ratio (G/I) were 0.51 and 76.91, corresponding to 95th and 99th percentiles, respectively.

There are some studies that evaluated insulin sensitivity indices in Iran. Esteghamati et al. in a study in Tehran illustrated the threshold of the HOMA-IR index in men and women determining lower limit of higher quintile of the HOMA-IR in men and women [27]. In another study, Tohidi et al. depicted age- and sex-specific cutoff values of fasting insulin, HOMA-IR, and QUICKI of 309 healthy women and men [11]. They recruited healthy male and female participants of the Tehran Lipid and Glucose Study, which is an ongoing cohort in one province of Iran, i.e., Tehran. They resulted that 95% reference value of the QUICKI was 0.33–0.42, a finding similar to our study. In comparison with these studies, we provided a larger spectrum of women who live in five different provinces of Iran [13] and used the *K*-means cluster analysis to determine the reference values of insulin sensitivity indices. Moon et al., in a Korean study on healthy people, showed different results [28]. Although they investigated different indices than those of our study, genetic variables and the distribution of age and BMI in their study participants are different than those in our population. Another study by McAuley et al. in New Zealand also illustrated a cutoff of 12.2 mIU/l for insulin to screen insulin sensitivity in the general population [29]. Based on the differences seen in the insulin sensitivity indices in other populations, our results might not be generalized to other populations.

In fact, determining insulin values without insulin sensitivity could not guide thoroughly to glucose regulation, as its pathophysiology is vastly monitored by the correlation of these two components [5, 6, 30]. Therefore, we need to determine insulin sensitivity to know the risk of dysregulated glucose metabolism, obesity, and other cardiometabolic risks.

The gold standard method is the euglycemic clamp; however, its use is limited due to time- and labor-consuming nature of the technique in clinical setting and epidemiological studies [31]. Also, surrogate markers of insulin sensitivity could provide sufficient information in many clinical and epidemiological studies.

Some of the surrogate markers use insulin in the calculation formula: reciprocal insulin, glucose-to-insulin ratio, HOMA-%β, and HOMA-βcell. There are indices of insulin sensitivity that do not have insulin such as TG/HDL-c and SPISE. Although the latter indices do not require insulin measurement, they have resulted in predicting insulin sensitivity [9].

Our study has a number of strengths and weaknesses. We have evaluated a large group of healthy women living in five provinces of Iran to determine the reference values of a wide variety of insulin sensitivity indices. We have analyzed these variables in a population-based setting. We used explicit and restricted inclusion criteria for selection of healthy women subjects. Moreover, we have reported various indices for insulin sensitivity that could be helpful for further population-based studies. Nevertheless, our study suffers from not using the gold standard method to determine insulin sensitivity, which could be of interest in future studies.

In this population-based study, we have determined the reference of different surrogate markers of insulin sensitivity. We used a two-step cluster and the *K*-means cluster analyses to do that. Further studies are needed to validate these reference values and their applicability.

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Compliance with ethical standards

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

Ethical consent Informed written consents were obtained from all participants, and ethical review board of Research Institute for Endocrine Sciences approved the study protocol.

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ORIGINAL ARTICLE

A case-control study of mental health status of diabetic patients seen in Calabar, Nigeria

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Abstract

Background There has been a rise in burden and risk factors for development and progression of diabetes mellitus in developing countries. The associated multisystemic complications and the burden of care potentially impair mental health status of sufferers. This study was aimed at assessing mental health status among diabetic patients in a developing country setting.

Methods Case-control study design was used, and subjects were recruited using random sampling method. Cases were recruited from medical wards and clinics in the University of Calabar Teaching Hospital, Calabar, for 14 weeks beginning mid-April through mid-July 2019. Age- and sex-matched non-diabetic normotensive controls were civil servants. Validated and pretested PHQ-9 instrument was used to assess mental health status, and clinical depression was defined as score $\geq$ 10. p value was set at 0.05.

Results One hundred and sixty-five diabetic patients and their matched controls were studied, with male/female ratio of 1:0.9. Mean age was 54.9 ± 9.6 years. Prevalence of clinical depression was 17.2% and 23.1% for male and female diabetics, respectively. For both male and female subjects, significantly higher proportion of cases compared with controls had clinical depression ($p < 0.05$). Among female cases, presence of comorbid hypertension, BMI, level of blood pressure, and duration of diabetes were directly associated with depression ($p < 0.05$). Among male cases, older age, occasional consumption of alcohol, and better glycemic control were associated with depression ($p < 0.05$).

Conclusion There is high prevalence of depression among diabetic males and females, with potential untoward effects on outcome of care. More robust collaboration between diabetologist and mental health specialist is therefore recommended to address this unmet need.

Keywords Diabetes mellitus · Mental health · Depression · Calabar · Nigeria

Introduction

By 2030, an estimated 366 million people will be living with type 2 diabetes characterized by impaired regulation of blood glucose [1]. The chronic disease currently constitutes at least 10% of international health expenditure [2], and patients have to endure or cope with potentially complex lifestyle modifications and compliance with medications [3]. Psychologically stable diabetic patients are more able and likely to sustainably provide better self-care, as well as access available healthcare services for prevention of disease progression [3]. The situation however becomes different or worse when systemic complications set in, potentially leading to depression which has been found to be common among diabetic patients. The presence of depression may result from the burden of care, as well as contribute to progression of disease [4]. Consequently, diabetes and depression comorbidity is associated with more severe and complicated disease, worse prognosis, higher cost of care, and lower quality of life [5].

Globally, all age groups, including adolescents and adults, are continually plagued by chronic disease-induced stress, depression, and anxiety, with type 2 diabetes mellitus, hypertension, and dyslipidemia as leading causes of psychological

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comorbidity [6, 7]. Studies have reported high prevalence of depression among diabetic patients. Among diabetics, depression prevalence rates of 44.7%, 49.5%, and 50.0% were reported in Ethiopia [8], India [9], and Australia [10], respectively. A systematic review of 19 studies on mental disorders among diabetics found twofold increased odds of depression among type 2 diabetics compared with general population [11]. A population-based study of mental health status among 846 cohorts in the Netherlands found 3.15 times increased odds of depression among type 2 diabetics [12]. Potential sources of depression among diabetics include distress associated with self-care, deprivation from living an apparently normal life like individuals in general population, and chronic pain [5, 8]. In some settings, stigma, discrimination, and excessive empathy associated with being diabetic may significantly contribute to depression [13]. Diabetes therefore contributes to onset and severity of depression in different ways for different patients, depending on degree of presence and severity of certain factors.

Depression and psychiatric problems often coexist with diabetes [11]. Yet, much of these conditions remained underdiagnosed and under-treated in many settings [4, 11]. In many developing countries especially in sub-Saharan Africa, the psychological well-being of diabetics is largely neglected, perhaps due to higher prioritization of attainment of optimum glycemic control for reduction in risk of physical complications [14]. There is therefore growing concern and research interest in the psychological consequences of diabetes, with key interest in depression in resource-poor settings. Previous study conducted two decades ago by Coker et al., in Nigeria, reported prevalence rate of 4% [15]. More recent study in North-Central Nigeria [16] and North-East [17] Nigeria found prevalence rate of 19.4% and 31.6%, suggesting rising burden of depression among diabetics in the region. There is therefore urgent need for screening and treatment of depression among diabetic patients in developing countries. This study was situated within the context of paucity of literature on psychological impact of diabetes in developing countries. It was aimed at assessing pattern and determinants of depression among diabetic patients in a developing country setting, with focus on the differential effect of gender. The current study was aimed at assessing the prevalence and pattern of these psychological problems, in diabetic population as compared with age- and sex-matched controls. Awareness and management of these conditions will improve patient outcome.

Materials and methods

Study duration was 14 weeks beginning mid-April through mid-July 2019. Cases were recruited from medical wards and clinic in the UCTH, Calabar.

Cases were adult patients (18 years and above) with at least 12-month duration of being diagnosed of type 2 diabetes mellitus. Selected diabetic patients were those who had been diagnosed of type 1 diabetes by a consultant diabetologist using standard laboratory and clinical criteria. Patients with type 1 diabetes, gestational diabetes, and previously diagnosed mental disorder were excluded from the study.

Sample size of 160 for each group was determined using formula for case-control studies [18] as follows:

$$N = \frac{r+1}{r}\frac{(p^*)(1-p^*)(Z_\beta + Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

where r = ratio of cases and control = 1; 95% alpha level of significance = 1.96; 80% power = 0.84; p^* = 24.1% average prevalence of depression comparing cases and control, assuming 15% effect size of difference in proportion (p1-p2), obtained from previous study which found 31.6% prevalence of depression among diabetics in Nigeria [17]); and 20% nonresponse rate.

Systematic ransom sampling technique was used to recruit cases into the study. Sampling frame consisted of the clinic attendance register, from where subjects were recruited using a sampling interval. The sampling interval was calculated by dividing the expected weekly number of diabetic patients (as obtained from records) by the allocated sample size per week. With expected study duration of 14 weeks, the allocated sample size for each week was 160 divided by 14, which yielded approximately 12 subjects. With estimated weekly clinic uptake of 61 diabetic patients, an approximate sampling interval of 5 was calculated (61/12).

The first subject was selected through balloting among the early (first to fifth) clinic attendees, and subsequent subjects were selected using the calculated sampling interval, with the clinic register as the sampling frame. If a subject was ineligible or did not consent to participating, the next patient on the register was contacted, and recruitment using the sampling interval continued from that point. Sampling continued until sample size was completed.

Age- and sex-matched non-diabetic normotensive controls were recruited from active and retired civil servants in Calabar Municipality. Multistage sampling method was used to recruit controls into the study. In the first phase, simple random sampling technique by balloting was used to select 4 out of 12 civil service ministries. In the second stage, systematic random sampling was used to recruit subjects into the study using the personnel register as sampling frame. Sampling interval of approximately 3 (110/40) was calculated by dividing the estimated number of personnel in the selected ministries (approximately 110) by the allocated sample size per selected ministry

(160/4 = 40). The first subject was selected through balloting among the first to third personnel on the register, and subsequent subjects were selected using the calculated sampling interval. If a subject was ineligible or did not consent to participating, the next personnel on the register was contacted, and recruitment using the sampling interval continued from that point. Sampling continued until sample size was completed.

Medical records and interviewer administration using structured questionnaire were used to obtain quantitative data on sociodemographic, anthropometric, laboratory, clinical, and mental health characteristics of subjects. Validated and pretested Patient Health Questionnaire (PHQ-9) instrument was used to assess mental health status, with higher scores indicating impaired mental health status. PHQ-9 is a preferred instrument in this study due to ease of its use, as well as its validity and reliability for assessing presence and severity of depression in diverse populations. Hence, it is a sensitive and specific instrument commonly used to screen for depression among diabetics. Total PHQ-9 score ranges from 0 to 27, with 4-point Likert scale ranging from "not at all" being 0 to "nearly every day" being 3. Scores are graded as minimal (0–4), mild (5–9), moderate (10–14), moderately severe (15–19), and severe (20–27) forms of depression. Independent t test was used to compare scores, while chi-square and Fisher's exact tests were used to compare categories of mental health status between study groups. Mann-Whitney *U* nonparametric test was used to compare medians between study groups for continuous variables with skewed (non-normal) distribution.

Results

Sociodemographic, anthropometric, and clinical characteristics

With response rate of 95.4%, complete data was obtained from 330 subjects comprising equal proportion of diabetic cases and age-/sex-matched normotensive non-diabetic controls. Male/female ratio was 1:0.9, and mean age was 54.9 ± 9.6 years ranging from 36 to 74 years. Approximately two thirds (66.0%) of subjects were within 51–70 years old (Table 1). Most subjects were married (80.9%), had at least secondary level of education (88.8%), and never smoked (72.4%). Roman Catholic (41.2%) was the commonest religion. Compared with controls, cases had significantly higher proportion of being widowed, having lower level of education, previous smoking history, and consuming alcohol occasionally ($p = 0.00$).

Mean BMI was 26.7 ± 4.7, ranging from 18.8 to 41.5 kg/m^2, and most subjects (61.2%) were either overweight or obese. Compared with controls, significantly higher proportion of cases were overweight and obese ($p = 0.00$). One hundred and forty-one cases (85.5%) were hypertensive. Median (IQR) duration of hypertension was 6.0 (4.0) years, ranging from 1 to 15 years, and median (IQR) duration of diabetes was 6.0 (5.0) years, ranging from 1 to 20 years. Mean systolic, diastolic, and mean arterial blood pressures were 131.7 ± 15.9 mmHg, 81.4 ± 10.6 mmHg, and 98.2 ± 11.6 mmHg, respectively. Approximately a quarter (25.8%) and a one third (34.2%) reported having sexual problems and being asked about their sexual life by their medical doctor, respectively. Admitting presence of sexual problems and being asked about sexual life was proportionally commoner among cases compared with controls ($p < 0.05$). Fifty-one subjects (15.5%) used supplements to enhance their sexual life, but there was no significant difference in proportion comparing cases and controls ($p > 0.05$).

Distribution of responses to PHQ-9 instrument

For at least more than half the days, female cases compared with controls had significantly higher prevalence of trouble falling/staying asleep, feeling tired/having little energy, poor appetite/overeating, having trouble concentrating, and having suicidal thoughts ($p = 0.00$, Table 2). For at least more than half the days, male cases compared with controls had significantly higher prevalence of all items assessed, except for interest in doing things and suicidal thoughts ($p = 0.00$).

Distribution of mental health status

Among female subjects, 138 (88.5%) had some form of depression (Table 3). Most depressed female subjects (72.4%) had minimal (42.9%) and mild (29.5%) forms of depression. Moderate and moderately severe depression was found in 14.1% and 1.9%, respectively. No subject was severely depressed. Significantly higher proportion of cases compared with controls had at least moderate depression, while more controls compared with cases had none/mild depression ($p < 0.05$). Among male subjects, 147 (84.5%) had some form of depression. Most depressed male subjects (74.2%) had minimal (55.2%) and mild (19.0%) forms of depression. Moderate and moderately severe depression was found in 5.2% each. No subject was severely depressed. Significantly higher proportion of cases compared with controls had at least moderate depression, while more controls compared with cases had none/mild depression ($p < 0.05$).

Factors associated with mental health status among cases

Among female cases, clinical (moderate) depression was significantly commoner among those that were unmarried, had

Table 1 Sociodemographic, anthropometric, and clinical characteristics of subjects ($n = 330$)

Variable	Diabetic case *n* (%)	Non-diabetic control *n* (%)	Total *n* (%)	Chi-square (*p* value)
Sex				
Male	87 (52.7)	87 (52.7)	174 (52.7)	1.00
Female	78 (47.3)	78 (47.3)	156 (47.3)	
Age groups (in years)				
≤ 40	14 (8.5)	14 (8.5)	28 (8.5)	1.00
41–50	33 (20.0)	33 (20.0)	66 (20.0)	
51–50	72 (43.6)	72 (43.6)	144 (43.6)	
61–70	37 (22.4)	37 (22.4)	74 (22.4)	
> 70	9 (5.5)	9 (5.5)	18 (5.5)	
Marital status				
Married	122 (74.0)	145 (87.9)	267 (80.9)	0.00
Single	7 (4.2)	7 (4.2)	14 (4.2)	
Divorced/separated	15 (9.1)	10 (6.1)	25 (7.6)	
Widowed	21 (12.7)	3 (1.8)	24 (7.3)	
Educational level				
Primary or less	33 (20.0)	4 (2.4)	37 (11.2)	0.00
At least secondary	132 (80.0)	161 (97.6)	293 (88.8)	
Religion				
Pentecostal	74 (44.9)	51 (30.9)	125 (37.9)	0.00
Orthodox	22 (13.3)	28 (17.0)	50 (15.2)	
Catholic	53 (32.1)	83 (50.3)	136 (41.2)	
Others	16 (9.7)	3 (1.8)	19 (5.8)	
Smoking status				
Yes (currently)	6 (3.6)	0 (0.0)	6 (1.8)	0.00
Smoked previously but stopped	57 (34.5)	28 (17.0)	85 (25.8)	
Never	102 (61.9)	137 (83.0)	239 (72.4)	
Consume alcohol				
Frequently	12 (7.3)	7 (4.2)	19 (5.8)	0.00
Occasionally	21 (12.7)	47 (28.5)	68 (20.6)	
Rarely	67 (40.6)	60 (36.4)	127 (38.5)	
Never	65 (39.4)	51 (30.9)	116 (35.2)	
BMI category				
Normal	41 (24.8)	87 (52.7)	128 (38.8)	0.00
Overweight	85 (51.5)	75 (45.5)	160 (48.5)	
Obese	39 (23.6)	3 (1.8)	42 (12.7)	
Comorbid hypertension				
Yes	141 (85.5)	0 (0.0)	141 (42.7)	0.00
No	24 (14.5)	165 (100)	189 (59.3)	
Have problem with sexual life				
Yes	59 (35.8)	26 (15.8)	85 (25.8)	0.00
No	106 (64.2)	139 (84.2)	245 (74.2)	
Use supplements for sexual life				
Yes	30 (18.2)	21 (12.7)	51 (15.5)	0.17
No	135 (81.8)	144 (87.3)	279 (84.5)	

comorbid hypertension, and reported having problems with their sexual life ($p < 0.05$, Table 4). Other factors did not significantly influence mental health status ($p > 0.05$, Table 4). Among male cases, clinical (moderate) depression was significantly commoner among those that were older than 50 years, unmarried, orthodox religion, consumed alcohol occasionally, and reported having problems with their sexual life ($p < 0.05$, Table 5).

Table 2 Distribution of responses to PHQ-9 questions assessing mental status by gender ($n = 330$)

Variable	Females		($n = 156$)		Males		($n = 174$)	
	Not at all *n* (%)	Several days *n* (%)	> half the days *n* (%)	Nearly everyday *n* (%)	Not at all *n* (%)	Several days *n* (%)	> half the days *n* (%)	Nearly everyday *n* (%)
Little interest or pleasure in doing things								
Case	35 (44.9)	33 (42.3)	3 (3.8)	7 (9.0)	54 (62.1)	30 (34.5)	3 (3.4)	0 (0.0)
Control	41 (52.6)	23 (29.5)	8 (10.3)	6 (7.7)	51 (58.6)	33 (37.9)	0 (0.0)	3 (3.4)
Fisher's exact (*p* value)	0.20				0.10			
Feeling down, depressed, or hopeless								
Case	50 (64.1)	22 (28.2)	6 (7.7)	0 (0.0)	66 (75.9)	12 (13.8)	6 (6.9)	3 (3.4)
Control	47 (60.3)	22 (28.2)	7 (9.0)	2 (2.6)	18 (20.7)	66 (75.9)	0 (0.0)	3 (3.4)
Fisher's exact (*p* value)	0.54				0.00			
Trouble falling/staying asleep or too sleepy								
Case	39 (50.0)	21 (26.9)	14 (17.9)	4 (5.1)	36 (41.4)	33 (37.9)	18 (20.7)	–
Control	43 (55.1)	28 (35.9)	0 (0.0)	7 (9.0)	66 (75.9)	18 (20.7)	3 (3.4)	–
Fisher's exact (*p* value)	0.00				0.00			
Feeling tired or having little energy								
Case	41 (52.6)	16 (20.5)	21 (26.9)	–	48 (55.2)	30 (34.5)	9 (10.3)	–
Control	20 (25.6)	54 (69.2)	4 (5.1)	–	33 (37.9)	51 (58.6)	3 (3.4)	–
Fisher's exact (*p* value)	0.00				0.00			
Poor appetite or overeating								
Case	46 (59.0)	6 (7.7)	15 (19.2)	11 (14.1)	45 (51.7)	21 (24.1)	12 (13.8)	9 (10.3)
Control	53 (67.9)	21 (26.9)	4 (5.1)	0 (0.0)	57 (65.5)	27 (31.0)	3 (3.4)	0 (0.0)
Fisher's exact (*p* value)	0.00				0.00			
Feeling bad about yourself as a failure								
Case	52 (66.7)	23 (29.5)	3 (3.8)	0 (0.0)	69 (79.3)	15 (17.2)	3 (3.4)	0 (0.0)
Control	61 (78.2)	14 (17.9)	0 (0.0)	3 (3.8)	48 (55.2)	36 (41.4)	0 (0.0)	3 (3.4)
Fisher's exact (*p* value)	0.03				0.00			
Trouble concentrating on doing things								
Case	32 (41.0)	32 (41.0)	10 (12.8)	4 (5.1)	54 (62.1)	21 (24.1)	9 (10.3)	3 (3.4)
Control	65 (83.3)	10 (12.8)	3 (3.8)	0 (0.0)	57 (65.5)	30 (34.5)	0 (0.0)	0 (0.0)
Fisher's exact (*p* value)	0.00				0.00			
Moving/speaking too slowly or restless								
Case	59 (75.6)	7 (9.0)	12 (15.4)	0 (0.0)	69 (79.3)	12 (13.8)	6 (6.9)	–
Control	60 (76.9)	8 (10.3)	6 (7.7)	4 (5.1)	54 (62.1)	30 (34.5)	3 (3.4)	–
Fisher's exact (*p* value)	0.11				0.01			
Thoughts of suicide								
Case	56 (71.8)	15 (19.2)	7 (9.0)	–	75 (86.2)	9 (50.0)	3 (3.4)	–
Control	78 (100)	0 (0.0)	0 (0.0)	–	78 (89.7)	9 (50.0)	0 (0.0)	–
Fisher's exact (*p* value)	0.00				0.22			

Correlation between clinical parameters and PHQ score among cases

Among female cases, BMI, duration of hypertension, duration of diabetes, blood pressure levels, total cholesterol, LDL cholesterol, and triglyceride levels had significant direct correlation with PHQ score ($p < 0.05$, Table 6). Age, fasting blood sugar, HbA1c, and HDL cholesterol levels did not significantly correlate with PHQ score ($p > 0.05$). Among male cases, age, duration of hypertension, and total cholesterol level had significant direct correlation with PHQ score ($p < 0.05$). Fasting blood sugar as well as levels of HbA1c were found to be significantly indirectly correlated with PHQ score ($p < 0.05$). BMI, blood pressure levels, duration of diabetes, levels of HDL cholesterol, LDL cholesterol, and triglycerides did not significantly correlate with PHQ score ($p > 0.05$).

Table 3 Comparison of distribution of mental health status by gender ($n = 156$)

Variable	Case *n* (%)	Control *n* (%)	Total *n* (%)	Test statistic (*p* value)
Females ($n = 156$)				
Mental health status subgroups (score)				
No depression (score of 0)	11 (14.1)	7 (9.0)	18 (11.5)	Fisher's
Minimal depression (1–4)	23 (29.5)	44 (56.4)	67 (42.9)	exact
Mild depression (5–9)	26 (33.3)	20 (25.6)	46 (29.5)	(0.01)
Moderate depression (10–14)	15 (19.2)	7 (9.0)	22 (14.1)	
Moderately severe depression (15–19)	3 (3.8)	0 (0.0)	3 (1.9)	
Severe depression (20–27)	–	–	–	
Mental health status main groups (score)				
None to mild depression (0–9)	60 (76.9)	71 (91.0)	131 (84.0)	$\chi = 5.8$
At least moderate depression (≥ 10)	18 (23.1)	7 (9.0)	25 (16.0)	(0.02)
PHQ score (median (IQR))	5.00 (6.00)	2.00 (3.00)	4.00(4.00)	$p = 0.02$
Males ($n = 174$)				
Mental health status subgroups				
No depression (score of 0)	18 (20.7)	9 (10.3)	27 (15.5)	Fisher's
Minimal depression (1–4)	45 (51.7)	51 (58.6)	96 (55.2)	exact
Mild depression (5–9)	9 (10.3)	24 (27.6)	33 (19.0)	(0.00)
Moderate depression (10–14)	9 (10.3)	0 (0.0)	9 (5.2)	
Moderately severe depression (15–19)	6 (6.9)	3 (3.4)	9 (5.2)	
Severe depression (20–27)	–	–	–	
Mental health status main groups				
None to mild depression (0–9)	72 (82.8)	84 (96.6)	156 (89.7)	Fisher's
At least moderate depression (≥ 10)	15 (17.2)	3 (3.4)	18 (10.3)	(0.00)
PHQ score (median (IQR))	3.00 (5.00)	4.00 (3.00)	3.50 (5.00)	$p = 0.16$

Discussion

This study found 17.2% and 23.1% prevalence of clinical depression for male and female diabetics, respectively. This finding is in tune with similar study in Ife, South West Nigeria [19], and Jos, North-Central Nigeria [16], which found 20.0% and 19.4% prevalence of depression among diabetic outpatients, respectively. However, similar studies in India [20] and Saudi Arabia [21] reported higher prevalence rates of 27.5% and 40.0%, respectively. Differences in rates may be attributable to differences in ethnicity, geography, religion, sociocultural, and other sociodemographic and lifestyle characteristics [5].

Significantly higher prevalence of depression was found among cases compared with controls, for both males and females (Table 3). Distress resulting from ceaseless need for self-monitoring, care, and follow-up visits may contribute to depression among diabetic patients [22]. There is also a pathophysiologic basis, since neurotransmitters for depression are found to be higher during hyperglycemia characteristic of poorly controlled diabetes mellitus [23]. Common pathway for both depression and diabetes includes physical inactivity, poor diet, poor sleep pattern, low social status, and chronic stress. In particular, chronic exposure to stress is associated with diabetes and depression via the sympathetic nervous system (SNS) and hypothalamus-pituitary-adrenal axis (HPA-axis) [24]. Other factors may however have modified the relationship between diabetes and depression, including personality traits, availability of social support, coping mechanisms, health beliefs, and level of spirituality [25]. Unfortunately, this study was limited by lack of assessment of the presence and role of these factors, especially considering the multifactorial etiologic basis for diabetes and depression.

In this study, significantly higher proportion of diabetics compared with controls were overweight or obese (Table 1). Obesity may contribute to the occurrence and progression of depression. Also, overeating, as potential symptom of depression, may lead to obesity, resulting in increased insulin resistance and worse clinical state among diabetics [4]. Regular assessment of BMI may therefore be useful for early identification of onset and progression of obesity and for more effective management of depression and glucose regulation among diabetics [26]. Unfortunately, in most developing country settings, regular assessment of BMI and blood pressure is only done during clinic visits which may be infrequent or even missed by many patients due to several individual-,

Table 4 Factors associated with mental health status among female cases ($n = 78$)

Variable	None/mild depression *n* (%)	≥ Moderate depression *n* (%)	Total *n* (%)	Chi-square (*p* value)
Age groups (in years)				
≤ 50	25 (86.2)	4 (13.8)	29 (100)	Fisher's
> 50	35 (71.4)	14 (28.6)	49 (100)	(0.13)
Marital status				
Married	46 (76.7)	7 (38.9)	53 (100)	χ =9.1
Unmarried	14 (23.3)	11 (61.1)	25 (100)	(0.00)
Educational level				
Primary or none	17 (81.0)	4 (19.0)	21 (100)	Fisher's
At least secondary	43 (71.7)	14 (77.8)	57 (100)	(0.61)
Religion				
Pentecostal	18 (69.2)	8 (30.8)	26 (100)	Fisher's
Orthodox	10 (16.7)	0 (0.0)	10 (100)	(0.09)
Catholic	25 (71.4)	10 (28.6)	35 (100)	
Others	7 (100)	0 (0.0)	7 (100)	
Smoking status				
Smoked previously but stopped	3 (100)	0 (0.0)	3 (100)	Fisher's
Never	57 (76.0)	18 (24.0)	75 (100)	(0.33)
Consume alcohol				
Rarely	17 (28.3)	8 (44.4)	25 (100)	χ =1.65
Never	43 (71.7)	10 (55.6)	53 (100)	(0.20)
Comorbid hypertension				
Yes	45 (75.0)	18 (28.6)	63 (100)	Fisher's
No	15 (100)	0 (0.0)	15 (100)	(0.02)
BMI category				
Normal	19 (82.6)	4 (17.4)	23 (100)	Fisher's
Overweight	23 (74.2)	8 (25.8)	31 (100)	(0.74)
Obese	18 (75.0)	6 (25.0)	24 (100)	
Have problem with sexual life				
Yes	11 (42.3)	15 (57.7)	26 (100)	Fisher's
No	49 (94.2)	3 (5.8)	52 (100)	(0.00)
Doctor ever asked about sexual life				
Yes	37 (77.1)	11 (22.9)	48 (100)	χ =0.01
No	23 (76.7)	7 (23.3)	30 (100)	(0.97)
Use supplements to enhance sexual life				
Yes	3 (50.0)	3 (50.0)	6 (100)	Fisher's
No	57 (79.2)	15 (20.8)	72 (100)	(0.10)

community-, and facility-based factors [27]. This scenario may contribute to build-up of risk factors for depression and diabetes disease progression.

There was indirect correlation between key laboratory parameters (such as fasting blood sugar and HbA1c) and PHQ-9 score but with statistical significance only for males (Table 6). In other words, poor glycemic control was associated with decreased risk of depression among male diabetics. This suggests that male unlike female diabetics may significantly be more involved in stress-relieving activities which potentially minimizes level of depression but increases risk of poor glycemic control. Alcohol consumption, which is one of such activities, was found to be more prevalent among males compared with females in this study (Tables 4 and 5). Lack of significant association found among females, however, is in tune with previous cross-sectional study among 6226 participants in Maryland, USA, where degree of depression was not found to vary with level of glycemic control [28]. Also, perhaps increase in cardiometabolic risk profile with age may be counteracted by better adoption of strategies for coping with

Table 5 Factors associated with mental health status among male diabetics ($n = 87$)

Variable	None/mild depression *n* (%)	≥ Moderate depression *n* (%)	Total *n* (%)	Chi-square (*p* value)
Age groups (in years)				
≤ 50	18 (100)	0 (0.0)	18 (100)	Fisher's
> 50	54 (78.3)	15 (21.7)	69 (100)	(0.03)
Marital status				
Married	60 (87.1)	9 (13.0)	69 (100)	χ =4.12
Unmarried	12 (66.7)	6 (33.3)	18 (100)	(0.04)
Educational level				
Primary or none	9 (75.0)	3 (25.0)	12 (100)	Fisher's
At least secondary	63 (84.0)	12 (16.0)	75 (100)	(0.44)
Religion				
Pentecostal	66 (88.0)	9 (12.0)	75 (100)	Fisher's
Orthodox	12 (66.7)	6 (33.3)	18 (100)	exact
Catholic	66 (95.7)	3 (4.3)	69 (100)	(0.01)
Others	12 (100)	0 (0.0)	12 (100)	
Smoking status				
Yes (currently)	6 (100)	0 (0.0)	6 (100)	Fisher's
Smoked previously but stopped	45 (83.3)	9 (16.7)	54 (100)	exact
Never	21 (77.8)	6 (22.2)	27 (100)	(0.42)
Consume alcohol				
Frequently	12 (100)	0 (0.0)	12 (100)	Fisher's
Occasionally	12 (57.1)	9 (42.9)	21 (100)	exact
Rarely	36 (85.7)	6 (14.3)	42 (100)	(0.00)
Never	12 (100)	0 (0.0)	12 (100)	
Comorbid hypertension				
Yes	66 (84.6)	12 (15.4)	78 (100)	Fisher's
No	6 (66.7)	3 (33.3)	9 (100)	(0.18)
BMI category				
Normal	15 (83.3)	3 (16.7)	18 (100)	Fisher's
Overweight	42 (77.8)	12 (22.2)	54 (100)	exact
Obese	15 (100)	0 (0.0)	15 (100)	(0.13)
Have problem with erection?				
Yes	18 (54.5)	15 (45.5)	33 (100)	Fisher's
No	54 (100)	0 (0.0)	54 (100)	(0.00)
Doctor ever asked about sexual life				
Yes	36 (85.7)	6 (14.3)	42 (100)	χ =0.50
No	36 (80.0)	9 (20.0)	45 (100)	(0.48)
Use supplements to enhance sexual life				
Yes	18 (75.0)	6 (25.0)	24 (100)	χ =1.4
No	54 (85.7)	9 (14.3)	63 (100)	(0.24)

potential sources of depression among females compared with males [29].

Presence and longer duration of hypertension were found to be associated with depression for both male and female diabetics. Similar findings were reported by multicenter cross-sectional studies in Bangladesh [30], which found significant increased risk of depression among diabetic subjects with comorbid hypertension. Such comorbidity may contribute to onset and progression of depression via higher risk of systemic complications, cost of healthcare, and pill burden [31]. Longer duration of diabetes also found to be associated with depression in this study may be attributed to increased risk of systemic complications and cumulative cost of care, as supported by reports from a prospective study in the UK [32].

Table 6 Correlation between clinical parameters and PHQ score among cases by gender (n = 165)

Variable	Females (n = 78)		Males (n = 87)	
	Pearson coefficient	p value	Pearson coefficient	p value
Age	0.06	0.63	0.24	0.00
BMI (kg/m2)	0.38	0.00	− 0.05	0.52
Duration of hypertension (in years)	0.55	0.00	0.56	0.00
Systolic blood pressure (mmHg)	0.37	0.00	− 0.04	0.64
Diastolic blood pressure (mmHg)	0.35	0.00	− 0.04	0.63
Mean blood pressure (mmHg)	0.38	0.00	− 0.04	0.62
Duration of diabetes (in years)	0.56	0.00	0.19	0.09
Fasting blood sugar (mmol/l)	− 0.19	0.10	− 0.33	0.00
HbA1c level	0.20	0.08	− 0.30	0.01
Total cholesterol (mmol/l)	0.30	0.01	0.27	0.01
HDL cholesterol (mmol/l)	− 0.19	0.10	− 0.16	0.14
LDL cholesterol (mmol/l)	0.27	0.02	− 0.10	0.34
Triglycerides (mmol/l)	0.40	0.00	0.10	0.43

There may be greater adverse mental health impact of long duration of chronic diseases in developing economies such as Nigeria, where budgetary allocation to health sector and access to health insurance have remained unacceptably poor [33].

There is higher prevalence of depression among diabetic patients compared with general population in the study setting. Comparing current rates with reports from previous studies in similar settings, there appears to be rising prevalence rate of depression among diabetics in Nigeria. Unfortunately, most cases of depression among diabetics are usually undiagnosed and therefore left unmanaged. There is therefore need for multidisciplinary approach and team responsible for diabetic care and management toward improved quality of life in developing countries.

It is key for diabetologist to recognize, treat, and prevent onset and progression of depression and other forms of psychological impairment in their patients, in collaboration with psychiatrist, clinical psychologist, and social workers. While the endocrinologist is primarily responsible for clinical management, the mental health specialist should be responsible for screening, monitoring, and management of psychological state of all diabetic patients. Due to challenge of poor compliance with referral in many settings, co-location of endocrinology and mental health outpatient clinic services in public hospitals may be required, to ensure improved access to available services. In other words, outpatient diabetic and psychiatric clinic days, times, and location may have to be the same to ensure screening and access to mental healthcare services for all diabetics. Further research on the psychological impact of diabetes and the feasibility and impact of collocating diabetic and mental health outpatient clinics in similar and dissimilar developing country setting is recommended.

Author contribution A U—Improved on initial work concept; supervised data collection; wrote initial manuscript

A A—Provided initial conceptualization of the work; reviewed manuscript

E O—Supervised data collection; reviewed manuscript

O O—Analyzed data; reviewed manuscript

Compliance with ethical standards

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

Ethical approval Case-control study design was used, and subjects were recruited using systematic random sampling method following ethical approval from the University of Calabar Teaching Hospital (UCTH) research ethics committee.

Informed consent Informed consent was obtained before data collection, and p value was set at 0.05.

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ORIGINAL ARTICLE

A survey on intensity of statin therapy among diabetes mellitus patients in secondary care practice

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Abstract

Objectives To assess the prevalence of statin usage, intensity of statin therapy, and serum LDL cholesterol levels achieved in clinical diabetes mellitus population to analyze whether the recommendations of American College of Cardiology/American Heart Association's cholesterol guidelines 2013 (ACC/AHA cholesterol guidelines 2013) are achieved in them.

Methods Fasting lipid profile values, prevalence of statin usage, and intensity of statin therapy among 306 diabetes mellitus patients in the age group of 40 to 75 years, visiting the medicine department of a secondary care hospital, were noted. Results were analyzed as per the ACC/AHA cholesterol guidelines 2013 using Microsoft excel 2016.

Results Out of 294 diabetes mellitus patients who qualified for statin therapy, only 68% (199) are on statins. In this group of patients, 22% (44) are on low-intensity, 77% on moderate-intensity, and 1% (3) are on high-intensity statin therapy. When the serum LDL cholesterol levels of the diabetic patients were analyzed, only 21% (64) of them could achieve the level of < 70 mg/dl, below which the risk of developing cardiovascular disease is low.

Conclusions Dyslipidemia, a risk factor for the development and progression of atherosclerosis among diabetes mellitus patients, is not being treated in secondary care practice as per the ACC/AHA cholesterol guidelines of 2013. Initiating moderate-intensity statin therapy among non-statin users and shifting patients on low-intensity to moderate-intensity statin therapy would help these patients in achieving the desired serum LDL cholesterol level of < 70 mg/dl.

Keywords ACC/AHA cholesterol guidelines 2013 · Diabetes mellitus · Intensity of statin therapy · Serum LDL cholesterol level

Introduction

Diabetes mellitus is considered as a well-established independent risk factor for cardiovascular diseases (CVD). Risk of death from stroke and heart disease among diabetic patients is 2 to 4 times higher when compared with non-diabetic individuals [1]. Hyperglycemia alone cannot explain the higher cardiovascular risk in diabetes mellitus patients, simply because aggressive glycemic control need not necessarily lead to decrease in the incidence of cardiovascular events [2]. Therefore, in order to manage vascular complications associated with diabetes, an approach is to address additional risk factors that lead to the development as well as progression of atherosclerosis rather than just managing the blood glucose levels. Dyslipidemia is commonly seen in diabetes and is an important forecaster of cardiovascular risk. Diabetic dyslipidemia is characterized by changes in the quantity and quality of lipids and lipoproteins with increased serum triglycerides, reduced high-density lipoprotein (HDL)-cholesterol, and a swing towards small dense low-density lipoprotein (LDL) [3].

Diabetic dyslipidemia predicts future cardiovascular events and can be controlled primarily with statins. Target of statin therapy is to decrease quantities of serum LDL cholesterol and other apolipoprotein B-containing lipoproteins [4]. Large randomized control trials (RCTs) have demonstrated that statins are unambiguously beneficial to the patients. It reduces the occurrence of major CVD events in diabetes mellitus patients with or without recognized CVD [5]. Statins act by reducing the cholesterol biosynthesis and increasing the clearance of LDL from plasma by the upregulation of hepatic LDL receptors [6, 7].

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LDL concentrations are not always higher in type 2 diabetics when compared with non-diabetic individuals [8]. At a given concentration of LDL cholesterol in diabetic patients, there is more number of LDL particles, and these particles are referred to as small dense LDL particles [9]. During the early and critical step of atherosclerosis, these small dense LDL particles are taken up by scavenger receptors, thus making them more atherogenic [10]. Current data says that LDL cholesterol remains a robust independent predictor of CVD in diabetic patients, even when it is below the National Cholesterol Education Program target of 130 mg/dl. The strong heart study observed that with every percent of 10 mg/dl increase in LDL cholesterol, there is a 12% increase in CVD risk in diabetic subjects [11].

Before the release of ACC/AHA cholesterol guidelines of 2013, LDL cholesterol goals were < 100 mg/dl for patients with diabetes mellitus who are known to be CVD risk factor equivalent [12]. The guidelines revised in 2013 identified four groups of patients who shall benefit from statin therapy and recommended patients aged 40 to 75 years with diabetes mellitus and LDL cholesterol levels of 70–189 mg/dl to be treated with statins of moderate- or high-intensity statin therapy [13]. The committee also declared that risk among diabetic patients varies and recommend at least moderate-intensity statin therapy for all patients with diabetes between the ages of 40 and 75 irrespective of the risk. If the diabetic patients in this age group have an estimated 10-year atherosclerotic cardiovascular disease (ASCVD) risk of greater than 7.5%, the guidelines recommend high-intensity statin therapy.

In addition, the 2013 guidelines no longer specify a particular goal for serum LDL cholesterol but instead recommend monitoring it to determine adherence to statin therapy and anticipated therapeutic response. Maintaining the diabetes mellitus patient on at least moderate-intensity statin therapy is a mandatory requirement, and the therapy should be continued even if the serum LDL cholesterol levels fall < 70 mg/dl without bringing down the dosage of the statins [14].

In this context, this study is carried out to assess how the lipid levels are managed in patients with diabetes mellitus after the announcement of the ACC/AHA cholesterol guidelines of 2013. It intended to find out whether these patients with serum LDL cholesterol levels of > 70 mg/dl are treated with statins and among the statin group of patients, what percentage is on moderate-intensity and high-intensity statin therapy as recommended by the guidelines. It also explored whether the subgroup of diabetic patients who are being treated for secondary prevention is receiving recommended dosage of high-intensity statin therapy.

Methods

It is a retrospective study in which the information was collected from hospital records of the diabetes mellitus patients. A total of 306 diabetes mellitus patients in the age group of 40 to 75 years who visited the medicine department of a secondary care hospital after the publication of ACC/AHA 2013 cholesterol treatment guidelines (between October 2015 and September 2017) were included in the study. All these diabetic patients were medically treated and followed up in the hospital. Demographic information like age and gender were noted. Fasting lipid profile values (total cholesterol estimated with CE-CHOD-POD, triglycerides by GPO Trinder, HDL by the direct-homogenous method using Cobas C 311 autoanalyzer, and LDL value calculated using Friedewald's formula) and the dosage of statins prescribed to the patients were recorded from the patients' hospital records. This study was approved by the hospital ethics committee (IEC Project No: 422/2015) of the university. Results were analyzed as per the ACC/AHA cholesterol guidelines 2013 using Microsoft excel 2016 and expressed in both numbers and percentages. Study outcomes included details of statin therapy including its prevalence, intensity, and serum LDL cholesterol levels existing in the diabetes mellitus patients who are being treated for primary and secondary prevention after the release of ACC/AHA cholesterol guidelines of 2013.

Results

A total of 306 diabetes mellitus patients in the age group of 40–75 years were included in the study. For the overall sample, mean (SD) age was 60.85 ± 8.9 years, 46% were male, 69% were also being treated for hypertension.

Treating all diabetes mellitus patients in the age group of 40–75 years with serum LDL cholesterol levels of 70–189 mg/dl with at least moderate-intensity statin therapy is the recommendation of the guidelines of 2013 [14]. Out of 306 diabetes mellitus patients in the age group, 12 of them have serum LDL cholesterol levels of < 70 mg/dl and therefore do not qualify for statin therapy. Among the remaining 294 patients, 199 are on statin therapy (68%) with the large proportion of diabetic patients not initiated with it (Fig. 1).

Out of 199 patients who are on statin therapy, 77% are on moderate-intensity treatment with majority of 137 patients (69%) in the lower dosage (10 mg of atorvastatin or 5 mg of

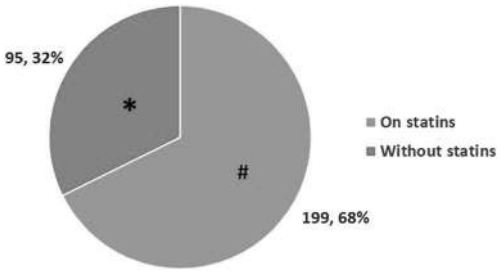

Fig. 1 Prevalence of statin therapy among diabetes mellitus patients

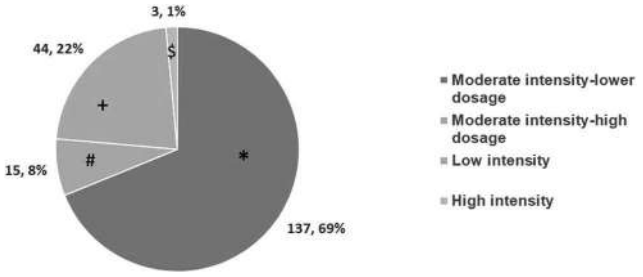

Fig. 2 Profiling of statin therapy among diabetes mellitus patients

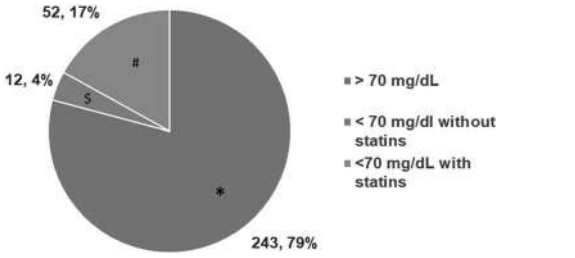

Fig. 4 Serum LDL cholesterol levels of diabetes mellitus patients

rosuvastatin or 2 mg of pitavastatin per day) and 15 patients (8%) on higher dosage (20 mg of atorvastatin or 10 mg of rosuvastatin per day) of statins. Percentage of patients on high-intensity statin therapy is just one. Forty-four diabetic patients (22%) are on low-intensity statin therapy (5 mg of atorvastatin or 1 mg of pitavastatin per day) (Fig. 2).

The 2013 cholesterol guidelines recommended high-intensity statin therapy for patients with known CVD. Out of 49 such patients with diabetes mellitus who are being treated for secondary prevention, only 4% are on high-intensity statin therapy as recommended by the guidelines. Among the remaining patients, 37 (76%) are on medium-intensity statin therapy, 3 (6%) are on low-intensity statin treatment, and 7 (14%) are not on statins (Fig. 3).

The intention of not recommending any target serum LDL cholesterol levels by the ACC/AHA cholesterol guidelines of 2013 is because of fearing under-treatment of the four high-risk groups for hyperlipidemia. The panel drafting the guidelines felt that the target levels can be reached even with low dosage of statins in some diabetic individuals and therefore recommended at least moderate-intensity of statins to all diabetic patients irrespective of the serum cholesterol levels achieved by them with statins. Ideally, if the diabetic patients with 70 to 189 mg/dl of serum LDL cholesterol are treated with the recommended dosage of medium-intensity statin therapy, majority of them should achieve the value of < 70 mg/dl. With this argument in mind, we analyzed the intensity of statin therapy among the diabetes mellitus patients with serum LDL cholesterol levels > 70 mg/dl. Among the 306 diabetes mellitus patients whose serum LDL cholesterol levels are known, 21% (64 patients) have the serum LDL cholesterol levels of < 70 mg/dl (Fig. 4). Out of 242 patients who have not reached < 70 mg/dl of LDL cholesterol, 95 are not on statins. Forty-one are on low-intensity statin therapy (5 mg atorvastatin or 1 mg of pitavastatin per day). Among the 103 patients who are on moderate-intensity statin treatment, 92 are on lower dosage (10 mg of atorvastatin or 5 mg of rosuvastatin or 2 mg of pitavastatin per day), 11 are on higher dosage of statins (20 mg of atorvastatin or 10 mg of rosuvastatin per day), and 3 are on high-intensity statin treatment (40 mg of atorvastatin per day) (Fig. 5).

Discussion

Initiating at least moderate-intensity statin therapy among all diabetes mellitus patients in the age group of 40–75 years with the serum LDL cholesterol levels of 70–189 mg/dl is a requirement under the recommendations of the ACC/AHA guidelines of 2013 in order to achieve relative reductions in serum LDL cholesterol levels. According to the guidelines, no target serum LDL cholesterol level is to be met by the patients. But, measurements of LDL cholesterol need to be done occasionally only to assess whether patients have adhered to the treatment and whether they are deriving maximum benefit from the dose of statin tolerated by them.

When the prevalence and the dosage of statin drugs prescribed to the diabetes mellitus patients were analyzed, it is revealed that only 68% of them are on statin treatment (Fig. 1). So, 32% of these patients are not at all initiated with statin therapy, which does not tally with the recommendations of the ACC/AHA 2013 cholesterol guidelines which advocate at least medium-intensity statin therapy for all diabetic patients. However, non-initiation of statin therapy among the high-risk

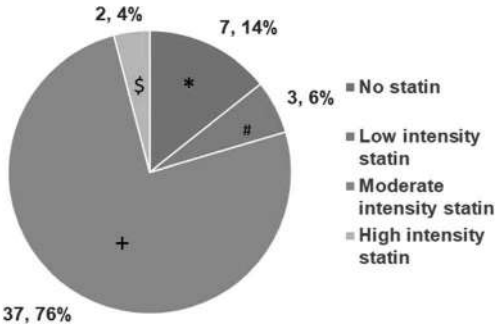

Fig. 3 Prevalence of statin therapy for secondary prevention in diabetes mellitus patients

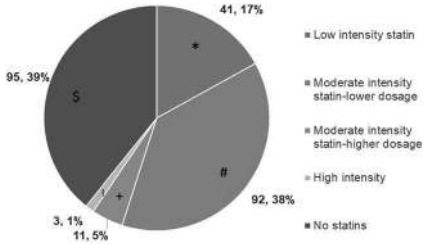

Fig. 5 Profile of statin therapy among diabetes mellitus patients with serum LDL cholesterol levels of > 70 mg/dl

group of patients or under-treatment with dosage of statin which is lower than the recommended dosage is not new and is widely reported earlier from different countries like USA, Germany, and Japan [15–17]. Among statin users, 22% are being treated with low intensity of statins (5 mg of atorvastatin or 1 mg of pitavastatin per day), which is less than the dosage recommended for the diabetes mellitus patients by the ACC/AHA 2013 cholesterol guidelines (Fig. 2). Another 77% of statin users are on moderate-intensity treatment, with 164 patients on lower dosage (10 mg of atorvastatin or 5 mg of rosuvastatin per day) and 23 of them on the higher dosage of statins (20 mg of atorvastatin or 10 mg of rosuvastatin per day), which is in conformity with the guidelines.

Earlier studies have revealed that most physicians choose to treat dyslipidemia with LDL goal and consider 70 mg/dl to be the suitable target for people at the highest risk for cardiovascular disease [18]. But, it is revealed that in spite of achieving the target level of 70 mg/dl with high-intensity statin therapy, there is a lingering CV risk in these patients [19]. Individuals with hypobetalipoproteinemia and PCSK9 mutation have LDL cholesterol in the range of 15 mg/dl and have inherited natural protection from CVD without having any adverse effects from the extremely low LDL levels, ushering in the strong belief that "lower the levels of LDL cholesterol the better is the protection" [20]. Therefore, in the absence of any clear-cut target serum LDL cholesterol levels mentioned in the guidelines, we carried out further analysis of the data, keeping in mind the serum LDL cholesterol levels of < 70 mg/dl as the value to be achieved. Such analysis of 306 patients with diabetes mellitus revealed that majority of patients (79%) have not achieved the value of < 70 mg/dl (Fig. 4), probably because of either non-initiation of statin therapy, usage of insufficient dosage of statins in them, or non-adherence to the statin therapy by the patients prescribed to them. So, majority of these patients are still at considerable risk of developing future cardiovascular event.

Next, analysis of prevalence of statin therapy and the statin dosage prescribed to diabetes mellitus patients who were not able to bring down their serum LDL cholesterol levels to < 70 mg/dl revealed that 39% are not on statins and 28% of them are on low-intensity statin therapy, which adds up to a whopping 57% of diabetes mellitus patients who are not being adequately treated to reduce the risk of developing CVD. Forty-three percent of them are on moderate-intensity statin therapy, with 38% on lower dosage of moderate-intensity statin therapy (Fig. 5). Increasing the dosage of statins from low intensity to moderate intensity and prescribing higher dosage of statins within the moderate-intensity therapy might help to bring down their serum LDL cholesterol levels to < 70 mg/dl. Moreover, 89% of diabetes mellitus patients who are not on statins have not attained the desired serum LDL cholesterol levels. (Fig. 4) So, initiating them to moderate-intensity therapy might help these patients in achieving the levels of serum LDL cholesterol to < 70 mg/dl, and thus bring down the risk of cardiovascular events in them.

There is a possibility that high-risk individuals among the diabetes mellitus patients are being under-treated with statins. One such group is the patients who have already had a previous cardiovascular event and the intention of statin prescription to them is secondary prevention. Such individuals are required to be under high-intensity statin therapy irrespective of their serum LDL cholesterol levels according to the ACC/AHA cholesterol guidelines of 2013. In our study group, 49 diabetes mellitus patients are being treated for secondary prevention and only 4% of them are being treated with high-intensity statin therapy. So, as per therecommendations of the guidelines, remaining 96% of patients need to be brought under the albeit of high intensity statin therapy. A study from the USA has reported that 67% of patients with known coronary artery disease are treated with high-intensity statin therapy by general cardiology healthcare providers [21].

Using RCTs, the 2013 cholesterol guidelines have declared that the most effective strategy for reducing ASCVD risk is with statins and also defined the four statin-benefit groups. Moderate- or high-intensity statin therapy is recommended in all populations at risk regardless of serum LDL cholesterol target levels. However, there is significant under-treatment of high-risk patient populations in many studies. It is estimated that only 58.2% of individuals with CVD and 52.0% of patients with diabetes who are older than 40 years of age were taking statins in 2010 [18]. Our study shows higher percentage of diabetes patients on statins (65%), but many of the patients among them are on low-intensity statin therapy. Similarly, when we probed the statin usage among the high-risk patients among diabetics, the intensity of statin therapy among them is not adequate. Therefore, we can summarize that a good proportion of patients with diabetes mellitus and diabetic patients for secondary prevention are being under-treated/not treated with statins.

There may be several reasons for the under-treatment of diabetes mellitus patients with statins. The reasons for this therapeutic inertia are likely to be patient preference for low dose of any drug due to fear of adverse effects like hepatotoxicity, physician concern for possible glucose intolerance at high doses, lack of belief in guidelines, and complexity of targets set in each group of patients.

As in any retrospective study, because of the availability of inadequate data, we were not able to do the assessment of 10-year risk of heart disease or stroke using the ASCVD algorithm published in ACC/AHA 2013 guidelines among the diabetic patients to ascertain what proportion of them are required to be under high-intensity statin therapy. However, it may be noted that the 2018 AHA/ACC guidelines on the management of blood cholesterol have been advocated currently, and as per these guidelines in patients with diabetes mellitus in the age group of 40 to 75 years and LDL-C level

of ≥ 70 mg/dl, moderate-intensity statin therapy needs to be started without calculating a 10-year ASCVD risk [22]. Another drawback of the study is that the percentage of serum LDL cholesterol level reduction proportional to the intensity of statin therapy in these patients could not be assessed. A multicenter survey on this topic may be needed to confirm our observation in a single-center study.

Conclusion

Our study shows that though there is a widespread use of statins in diabetics, there is a therapeutic gap between the recommendations of the ACC/AHA 2013 cholesterol guidelines for diabetic patients and what is achieved in real-world clinical practice, due to therapeutic inertia in initiating and up-titrating statin therapy and prescribing appropriate intensity of statins to the deserving patients in secondary care practice.

Compliance with ethical standards

This study was approved by the hospital ethics committee (IEC Project No: 422/2015) of the university.

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

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ORIGINAL ARTICLE

A diabetes perception study among rural and urban individuals of West Bengal, India: are we ready for the pandemic?

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Abstract

Background Type 2 diabetes is a pandemic in India, yet studies regarding knowledge, attitude, and practices in diabetes in various Indian communities are limited. It is essential to understand the current gaps in knowledge and problems with attitude and perceptions of the general population regarding diabetes to effectively plan public health policies.

Method In this study, 2163 subjects (1079 urban, 1084 rural) from West Bengal, India, with no exposure to any formal diabetes awareness including through familial experience or by virtue of their profession, participated in a structured response cross-sectional knowledge-attitude practice (KAP) survey focusing on perception about diabetes, its diagnosis, and management during FY 2013–2014.

Result Both rural and urban communities lacked complete knowledge of diabetes. Forty-nine percent of rural responders were not aware of the term "diabetes" itself. Also, among the rural responders who were familiar with the term lacked knowledge related to blood glucose monitoring and role of insulin in diabetes management. Though 74% of urban respondents were aware of insulin's use in diabetes management, 39% had misconception regarding its extended use. Responders from both urban and rural communities showed higher awareness towards general health practices like, benefits of exercise or demerits of junk food, in comparison with diabetes-specific questions.

Conclusion The study reveals the need for effective diabetes-specific health awareness campaigns to address the rise of diabetes pandemic in resource-limited country like India. There is an urgent need to address unfounded fear of adverse reaction of insulin over extended use.

Keywords Perception · Diabetes · Insulin · Lifestyle practices · Rural and urban population

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Introduction

Type 2 diabetes mellitus (DM or diabetes) is a chronic metabolic disorder characterized by high blood glucose levels. Hypertension and type 2 DM continue to be major causes of cardiovascular morbidity and mortality worldwide [1, 2]. The disease is associated with several complications [3] including retinopathy, [4] neuropathy [5], and nephropathy [6]. The disease has become a pandemic [7, 8], with an estimated 387 million people in the world living with diabetes. India, with 72.95 million individuals affected by diabetes [9], has the second largest number of people affected by diabetes in the world. The prevalence of diabetes in India has increased from 1.2 to 8.8%, between 1971 and 2017 [10]. Study conducted in 15 states of India found mean prevalence of prediabetes to be 10.3% (state-wise range, 6.0–14.7%) [11, 12]. Surveys also

suggest 10–16% in urban and 5–8% of rural population have diabetes [13, 14]. As various studies have indicated that Asian population transitions faster from pre-diabetes stage to diabetes stage than any other ethnic population [15, 16], the findings of the study are alarming from a public health point of view.

Early detection and lifestyle modifications remain the corner stone of management of individuals with pre-diabetes and diabetes. [17], [18, 19]. Efficient use of available therapies can limit the complications due to diabetes and improve quality of life. Impact of diabetes can possibly be minimized with adequate awareness, knowledge, and changes in attitudes.

Government of India has initiated various strategies to tackle non-communicable diseases. One of the important factors to be considered is the knowledge of diabetes in the population at large. A few published studies have suggested some knowledge of diabetes in urban areas, especially those from higher socioeconomic strata [11, 20]. A study from Kerala in India has shown that rural population has a higher prevalence of diabetes than the urban population [21].

This study was undertaken to evaluate the knowledge-attitude and perception of diabetes among both urban and rural population in West Bengal, India. Insights from our study might help assess the knowledge gaps and help formulate public health programs to tackle to menace of diabetes.

The four major domains assessed were as follows: (a) knowledge regarding symptoms, signs, and consequences of diabetes; (b) need for checking blood glucose levels at regular intervals; (c) familiarity with insulin and its role in diabetes management; and (d) awareness regarding lifestyle activities and risk factors for diabetes.

Methodology

A cross sectional study was conducted wherein 2162 adults (male: 1183 & female: 980) in both urban ($n = 1079$) and rural ($n = 1084$) areas were interviewed in December to March of the FY-2013-2014. Four wards from Tollygunge constituency in Kolkata metropolis and four villages from Rampur Gram Panchayat in Mohammad Bazar block of Birbhum district were randomly selected as urban and rural areas respectively for this study. The two sites were 250 km apart. Subjects were selected from the electoral rolls (every fifth subject). Not more than one adult representative from a single household was included for the survey. Families with members undergoing or have undergone any diabetes management (including the grandparents and first cousins), members attended diabetes awareness program within 3 months of the study, were excluded. Any family with members professionally engaged in health sector were also excluded from this study. Also, responders with household members suffering from type-2 diabetes were excluded.

A close-ended questionnaire was developed to understand the knowledge of diabetes, its signs and symptoms, consequences and management in the target population. It also evaluated the practice of blood glucose measurement among the target population. The questionnaire was validated using standardized protocol in both settings by prior pilot studies. The questionnaire was prepared on the basis of input from an expert committee in metabolic health. The questions were then translated in to local language and vetted by the expert committee with a focus on objectivity of question and their sequence. The questionnaire was then tested in a pilot study, and the results were evaluated by the same committee. The recommendation of the committee was fully accepted and modifications according to dialect of the study sites were introduced according to the recommendations of local coordinators. The initial questionnaire was reviewed on the basis of the pilot study, and modifications regarding wording and translations were made accordingly prior to final data collection. The data from pilot study was not included in the final data set. All the ethical considerations under Helsinki declaration were maintained for the study. A written consent was taken from every individual. The confidentiality of personal information of the participants was maintained throughout the study.

Undergraduate students with basic idea about field survey and data collection were engaged as field surveyors in both settings. Training workshop on study protocol and study tool was organized for the surveyors followed by the pilot study prior to final data collection. Data was validated by appropriate cross-checking of data by a data monitoring body.

Descriptive statistics was used to compare the perception level between different socio-demographic categories of the studied population. The chi-square test for independence/association was performed to understand whether the responses regarding diabetes perception are associated with urban or rural origins of the responders using Minitab version-17 (Minitab, Inc. State College, Pennsylvania.).

Results

Demographic characteristics

A total of 2163 individuals from urban and rural areas (urban $n = 1079$ & rural $n = 1084$) participated in the study. Demographic characteristics are shown in Table 1. The difference in education and income level among the urban and rural groups are possibly due to difference in localities. The population had similar profile in age distribution and occupation. The urban group had high frequency of responders who were students. This distinct difference in frequency can also be attributed to the social practices in the specific locality.

Table 1 Demographic characteristics and gender differences

	Urban	Rural	Total
Age group			
< 30	505 (47%)	407 (38%)	912
31–54	432 (40%)	511 (47%)	943
> 55	137 (13%)	166 (15%)	303
Sex			
Male	592 (55%)	591 (55%)	1183
Female	487 (45%)	493 (45%)	980
Educational status			
Graduate	389 (37%)	122 (12%)	511
Senior secondary	238 (23%)	114 (11%)	352
Secondary	220 (21%)	239 (23%)	459
Upper primary	160 (15%)	344 (33%)	504
Primary	38 (4%)	227 (22%)	265
Occupation			
Service	290 (28%)	341 (32%)	631
Self employed	150 (14%)	296 (28%)	446
Student	322 (31%)	64 (6%)	386
Housewife	255 (25%)	350 (33%)	604
Retired	18 (2%)	13 (1%)	31
Unemployed	4 (< 1%)	3 (< 1%)	7
Family income level (monthly) in INR			
1–10,000	427 (65%)	703 (91%)	1130
10–20,000	145 (22%)	56 (7%)	201
> 20,000	89 (13%)	16 (2%)	105

Knowledge about symptoms and consequences of diabetes

Diabetes is marked by sustained elevated blood glucose levels [22]. To understand level of awareness regarding diabetes, its symptom and consequences, a set of three questions were asked to each respondent. The questions and their responses are shown in Table 2, questions 1 through 3. Overall, the rural group showed poor response rate with 49% reporting they were even unfamiliar about the term "diabetes." The urban group showed higher awareness to the topic. However, the response profile to question 2 indicates lack of knowledge or confusion regarding key symptom of diabetes in urban group too as seen by the responses of question #2.

Blood glucose level checking practices

Regular monitoring of blood glucose levels is important for diabetes and prediabetes screening [22] and early intervention. To understand the level of awareness regarding their familiarity with the process, they were asked whether they have measured blood glucose ever (Table 2, question no. 4). Both urban and rural groups predominantly did not measure blood glucose levels at any frequency. Responders who had their blood glucose tested did it predominantly on physician's advice. Higher proportion of urban group responders had their blood glucose tested.

Perception of insulin in diabetes management

Insulin is an integral part of diabetes management [17]. A set of three questions were asked to the responders regarding use of insulin in diabetes management and effect of insulin on long-term use. The results are shown in Supplementary Table, questions 5 through 7. Fifty-seven percent of the rural responders who were familiar with the term diabetes revealed that they had no knowledge of insulin use in diabetes. Urban group had higher proportion of responders with knowledge of insulin in diabetes. However, 39% of the urban responders were misinformed about the effect of continued use of insulin in diabetes management.

Perception regarding lifestyle factors affecting diabetes

Studies have shown that diabetes is mainly a lifestyle disease. Reduction of insulin levels followed by carbohydrate and fat rich diet (junk food) can lead to central fat deposition and contribute to "belly fat" [23]. Conversely, exercise leads to activation of muscles that can utilize the blood glucose, resulting in lowering of blood glucose levels [18, 19]. Tobacco or alcohol consumption releases reactive oxygen species that can damage beta cells in pancreatic islet resulting in reduced secretion of insulin [24]. To understand how the respondents perceive lifestyle habits as factors affecting diabetes, a set of four questions were asked. The results are shown in Table 2a, questions 8 through 11. The rural group had improved response to this set of questions compared with other question sets as the "do not know" response's proportion was lower than 50%. However, a significant fraction of rural group (~ 14%) failed to link factors like central obesity and consumption of alcohol and smoking nicotine to diabetes. Though the urban group performed better than rural group in identifying lifestyle risk factor, compared with other set of questions, more "do not know" responses were recorded in this section. Though, the urban group showed high level of awareness regarding benefit of exercise in diabetes affected people, the overall awareness regarding central obesity and consumption of junk food, alcohol, and smoking was lower compared with its response in other sections.

Discussion

The, ICMR-INDIAB study, that covered 15 Indian states in phase-I, reported that the prediabetes: diabetes ratio is

Table 2 Response to the survey questions segregated according to rural and urban responders

#	Survey question	Response								*p* value
		Rural ($n = 1084$)				Urban ($n = 1078$)				
		Yes	No	Un[&]	Mis[$]	Yes	No	Un[&]	Mis[$]	
1.	Idea about "diabetes"	554 (51%)	526 (49%)	2 (< 1%)	2 (< 1%)	863 (80%)	206 (19%)	0 (0%)	10 (1%)	$p < 0.001$
2.	Normal blood glucose level can be diabetic	94 (9%)	361 (33%)	615 (57%)	14 (1%)	315 (29%)	362 (34%)	375 (35%)	27 (3%)	$p < 0.001$
3.	Diabetes leading to other diseases	395 (36%)	43 (4%)	637 (59%)	9 (1%)	732 (68%)	53 (5%)	290 (27%)	4 (< 1%)	$p < 0.001$
4.	Ever measured blood glucose?	204 (19%)	694 (64%)	168 (15%)	18 (2%)	417 (39%)	628 (58%)	23 (2%)	11 (1%)	$p < 0.001$
5.	Insulin used in diabetes treatment?	192 (18%)	90 (8%)	721 (67%)	81 (7%)	829 (77%)	69 (6%)	175 (16%)	6 (1%)	$p < 0.001$
6.	Insulin replace other medicine in extreme diabetes	173 (16%)	49 (5%)	807 (74%)	55 (5%)	838 (78%)	24 (2%)	208 (19%)	9 (1%)	$p < 0.001$
7.	Regular use of insulin harmful for the body	93 (9%)	126 (12%)	857 (79%)	8 (1%)	309 (29%)	372 (34%)	387 (36%)	11 (1%)	$p < 0.001$
8.	Excess body weight with belly fat causes diabetes	448 (41%)	161 (15%)	463 (43%)	12 (1%)	690 (64%)	103 (10%)	283 (26%)	3 (< 1%)	$p < 0.001$
9.	Regular exercise good for people suffering from diabetes	588 (54%)	40 (4%)	449 (41%)	7 (1%)	946 (88%)	32 (3%)	99 (9%)	2 (< 1%)	$p < 0.001$
10.	Smoking and alcohol consumption increase blood glucose levels	393 (36%)	151 (14%)	533 (49%)	7 (1%)	716 (66%)	86 (8%)	271 (25%)	6 (1%)	$p < 0.001$
11.	Junk food good for health	76 (7%)	790 (73%)	213 (20%)	5 (< 1%)	111 (10%)	629 (58%)	337 (31%)	2 (< 1%)	$p < 0.001$

[&] "Un" indicates participants responded "do not know"

[$] "Mis" indicates participants did not respond

reaching a plateau in urban areas [11], while prevalence of diabetes is rising among urban low socio-economic section and rural high socioeconomic sections. As the metabolic disorders are increasing in new sociodemographic sections, it is important to understand the knowledge levels in these sections and create policies to raise awareness. The data collection for this study was conducted in the similar period as that of ICMR-INDIAB study. This looked more in behavioral barriers that may be one of the causal effects of the increasing prevalence of metabolic disorders.

The current study was conducted at two sites that are more than 250 km apart. Tollygunge constituency is a part of Kolkata Municipal Corporation area, while Rampur Gram Panchayat is under Mahammad Bazar Block, a rural administrative division, in Birbhum district. Tollygunge is considered one of the most affluent localities in Kolkata, the largest city in the eastern India with multiple educational institutes and health facilities (both private and public) available, whereas Rampur Gram Panchayat has only 2 middle school with nearest high school within 10 km and only one primary health center for 6 villages of Gram Panchayat [25]. The objective was to investigate the perception of these two communities and evaluate the knowledge gaps regarding diabetes.

This study, in conjunction with published literature, reveals that even in this information rich era where the whole world is thought as a global village, "diabetes" is mostly an unknown term in rural areas of India. One of the revealing feature of this study was the high proportion of rural population who were not familiar with the term "diabetes" or its local term. A study conducted in rural village of Kolar district in Karnatka, India, observed similar response pattern from rural respondents [26]. However, the rural population showed moderate knowledge of lifestyle habits that may lead to metabolic disorders (Table 2 questions 8–10). This indicates there might be a fraction of rural population that is not specifically aware of diabetes management, but has knowledge about healthy lifestyle practices. Further investigation is needed to find out the source of this knowledge and utilize it for diabetes-specific awareness.

The study indicates that the perceptions tested were associated with the site of investigation as the test of association was significant ($p < 0.001$) for all the questions. Forty-nine percent of the rural responders and 19% of urban responders failed to identify the term "diabetes." The response pattern of Q2 in Supplementary Table indicates that even the responders in the community who were aware of the term diabetes were not very confident about the symptoms. A significant proportion (69% rural and 56% urban) had not taken the blood glucose test. Thirty percent of rural responders and 21% of urban responders, who responded yes to Q1 Table 2, were not fully aware of the impact of diabetes on other health issues. These results indicate that the community is not ready to address the initial symptoms of type 2 diabetes. Effective intervention in these metabolic diseases is only possible if optimal community cooperation is available.

The knowledge about general health practices to counter metabolic syndromes was better than diabetes-specific knowledge. Fifty-four percent of rural responders and 88% of urban responders perceived exercise to be helpful for people suffering from diabetes (Table 2). Among the responders aware of diabetes, the percentage went up to 83% (rural) and 92% (urban) (supplementary Table). Also, a major proportion of both groups identified junk food (deep fried or highly processed carbohydrate rich food) to be bad for health. However, more than 36% of urban responders and 59% of rural responders either did not perceive excess weight with central obesity as a risk factor for diabetes (Table 2). A similar proportion also did not consider smoking and alcohol consumption can contribute to the risk of diabetes. Thus, there was basic level of awareness regarding practices that can help in countering metabolic diseases; the knowledge was not enough when it came to complex risk factors. The awareness regarding risk factors was higher among the group that was aware of the term diabetes. Sixty-one percent and 55% of those rural responders identified belly fat and smoking and alcohol consumption as a risk factor. For urban group, the positive response rate was 70% and 71% respectively. (Supplementary Table).

Insulin has a critical role in management of diabetes, including type 2 diabetes mellitus. Though urban responders had moderate knowledge regarding insulin, the rural responders (even the ones who were familiar with diabetes term) were not much aware of use of insulin even in persistent diabetes with high blood glucose levels. (Table 2 Q-5, 6 and supplementary Table Q- 5, 6). This indicates that the rural population has not been aware of diabetes management strategies. With the rise of diabetes prevalence in the rural communities, special attention is needed to inform the community of diabetes management procedures and especially use of insulin. Though the urban population was moderately aware of the insulin-based diabetes management, the group overall was not sure about its safety for long-term use. One-third of the urban responders perceived continued use of insulin as detrimental to the body. This perception may be a barrier in long-term diabetes management as insulin hesitancy might arise among the patients. Effective knowledge dissemination process needs to be devised to raise awareness regarding long-term usage of insulin in diabetes management.

The study was designed to understand perception of the target population regarding diabetes awareness. Follow-up studies regarding metabolic profiling and diabetes- prediabetes prevalence need to be undertaken for estimation of severity of the disease in the population and link it with preparedness of the population in tackling new wave of non-communicable diseases.

The study was consciously designed to avoid stakeholders who might be familiar with diabetes. This included people suffering from diabetes themselves. However, other studies have revealed that even this group suffers from lack of perception regarding diabetes care and management [27, 28]. Our previous study with families of hepatitis patients showed similar knowledge level in that group too [29]. Comparison of our study with these studies showed that the people suffering from diabetes had higher awareness regarding diabetes symptom, their medical management. However, they lacked knowledge regarding personal care and concepts like hypoglycemia. Thus, our decision of excluding the exposed population provided a better perspective of knowledge in the populace that has never encountered diabetes.

Effective partnerships between public and private organizations are required. The role of media needs to be evaluated in raising awareness about healthy lifestyle practices. Though both rural and urban areas can be sensitized by the media, role of different forms of media for area specific sensitization should be evaluated. Effectiveness of the new-age communication tools (devices like smartphones and systems like app-based platforms) that have been highly accepted in both rural and urban Indian communities and are already used as knowledge source, needs to be investigated to sustainably disseminate verifiable and beneficial healthy lifestyle and diabetes-specific information. The strategies need to be in coordination with the government initiatives on population screening for non-communicable diseases [30].

Conclusion

Diabetes is a silent killer. Not only it can have life threatening impact by itself but also it has debilitating effects on various organs and organ systems. It is not curable but controllable. Effective diabetes management is the need of the day for a resource-limited country like India where the prevalence of diabetes is on rise. However, our study shows that a significant proportion of the population is unaware of the term “diabetes”. Effective management of diabetes can only occur if a culture of health awareness and well-being is developed and integrated in the fabric of Indian society. The current study was aimed to understand the viewpoint of the society on key diabetes-related medical and lifestyle issues and to understand variations existing in urban and rural communities.

Study outcomes indicate a huge scope of improvement in perception regarding the symptoms, consequences, and management of diabetes in both rural and urban societies of Eastern India. There is a need for complete and correct information dissemination in both communities. Focus should be on (a) building knowledge base regarding metabolic diseases including diabetes, and (b) knowledge of practices that ensures early detection and intervention of diabetes. Routes of dissemination should be tailored to micro-levels of the Indian society, and strategies should consider the socio-economic status of the beneficiaries. Special attention is needed to

understand how the local communities accumulate information. The ultimate target needs to be change in health culture of the Indian society.

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Compliance with ethical standards

Conflict of interest Author Partha S. Mukherjee, Author Sujoy Ghosh, Author Pradip Mukhopadhyay, Author Kausik Das, Author Dipesh Kr. Das, Author Pabak Sarkar, Author Debdoot Bhattacharya, Author Saibal Mazumdar, and Author Kajal Chatterjee declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institute of Postgraduate Medical Education and Research (IPGME&R) Research oversight Committee, memo number–Inst/IEC/2014/776 and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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ORIGINAL ARTICLE

Correlates of fear of hypoglycemia among patients with type 1 and 2 diabetes mellitus in outpatient hospitals in Zambia

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Abstract

Background Severe hypoglycemia is a burdensome complication of diabetes mellitus that can induce fear of hypoglycemia and contribute to suboptimal glycemic control. The challenge is to achieve and maintain adequate glycemic control while avoiding episodes of severe hypoglycemia. The purpose of the study was to determine how common fear of hypoglycemia was in Zambian out-patients with diabetes and also to explore correlates of fear of hypoglycemia.

Methods One hundred fifty-seven individuals with types 1 and 2 diabetes participated in the study. Fear of Hypoglycemia Scale, Diabetes Self-Care Inventory, Problem Areas in Diabetes, and the Major Depression Inventory were completed. Multiple linear regression models were computed to assess the association between fear of hypoglycemia and psychological factors.

Results About 19% [16.3% type 1 and 12.6% type 2] of individuals with diabetes based on item endorsement expressed fear of hypoglycemia especially among individuals with type 1 diabetes. After controlling for demographic variables, diabetes self-care ($\beta = 0.24$, $p < 0.05$), and diabetes specific distress ($\beta = 0.41$, $p < 0.001$) were associated with fear of hypoglycemia.

Conclusion Fear of hypoglycemia was common and was positively associated with diabetes specific emotional distress and diabetes self-care. Interventions to avert fear of hypoglycemia are needed while optimizing glycemic control through managing diabetes care and emotion distress in individuals with diabetes.

Keywords Fear of hypoglycemia · Hypoglycemia · Fear · HFS · Correlates

Introduction

Diabetes mellitus along with hypertension are major risk factors for cardiovascular diseases. Treating these risk factors are cost effective and can help boost productivity [1, 2]. One other neglected complication in diabetes research in Sub Saharan Africa is hypoglycemia. The American Diabetes Association defines hypoglycemia as a condition characterized by abnormally low blood glucose levels usually less than 70 mg/dl [3]. However, because of individual differences, it is vital to consult a physician on appropriate glucose targets and what level is low for each individual. The cause of hypoglycemia can be either excess of diabetes medication, for example, if too much insulin does not match the amount of carbohydrates or drinks ingested or the levels of exercise was too much for the amount of carbohydrates consumed. Too much alcohol can also be a cause for hypoglycemia [4, 5]. The symptoms of hypoglycemia caused by very low blood glucose levels (neurogenic symptoms) include shaking, sweating, drowsiness, pupil dilation, hunger, nausea, anxiety, palpitations, and headache. Other symptoms (neuroglycopenic systems) include poor

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motor coordination, confusion, negative mood state, argumentativeness and irritability, seizures, coma, and even death if severe hypoglycemia remains untreated [6, 7]. Moreover, cognitive difficulties have been linked to early illness onset and illness duration, recurrent hypoglycemic episodes, and hypoglycemia especially in children with diabetes [8, 9]. A global study involving 24 countries found that hypoglycemia is common (2.5 per person/year) in individuals with type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) using insulin therapy and that it is one of the leading reasons for hospitalization for individuals with diabetes [10]. For instance, in a study in South Africa with 43 patients that were admitted at Baragwanath Hospital in Soweto, a total of 51 episodes of hypoglycemia in a 5-month period were recorded [11]. Hypoglycemia has a burden on healthcare utilization, costs, and quality of life [12]. While data shows hypolycemia is common, little is known how common fear of hypoglycemia is in Zambia. Unlike in developed countries, many SSA countries have weak diabetes health care, as such, investigating fear of hypoglycemia and its correlates remains important as it is an indicator of glycemic control and diabetes-related quality of life.

One of the challenges in the treatment of diabetes is to achieve optimal glycemic control while avoiding episodes of hyperglycemia or hypoglycemia [6]. One major downside of near normal glycemic controls is the elevated risk for hypoglycemia [13, 14]. Given the unpleasant aspects of hypoglycemia and the potentially life-threatening nature of severe hypoglycemia, many people with diabetes have significant fears of developing hypoglycemia [15]. Over time, FoH is triggered by hypoglycemia unawareness (HU). HU is defined at the onset of neuroglycopenia before the appearance of autonomic warning symptoms [16]. Enough evidence including data from systematic reviews shows that fear of hypoglycemia (FoH) is associated with being female [17–19], being old [17, 19], young age as reported by parents for children with type 1 diabetes [20], psychosocial factors such as anxiety, stress, depression, and impaired quality of life (QoL) [11, 19], whereas mixed results were found for the association of FoH with BMI [21]. FoH is also positively associated with duration of diabetes [17, 22], diabetes complications [18], and the individual's history of hypoglycemia especially severe episodes [12, 15].

FoH can adversely affect QoL, emotional wellbeing, diabetes management, and glycemic control in people with diabetes, yet few studies have been conducted on the subject in Africa, and these studies tend to be epidemiological in nature [11, 23]. For instance, a study with a sample of 43 South African patients, reported a total of 51 episodes of hypoglycemia in a period of 5 months [11]. Hypoglycemia has been observed to range between 25 and 55% in Sub-Saharan African diabetes patients per year [23]. Given that severe hypoglycemia is one of the leading reasons for hospitalization in African patients with diabetes, studies exploring FoH and factors associated with it are warranted. Identifying FoH and specific factors associated with FoH are important for developing parsimonious interventions. The aim of this study was to investigate how common FoH is and factors related with FoH in out-patients with type 1 diabetes and type 2 diabetes mellitus.

Methods

Design

This study employed a cross-sectional design. We assessed the association between the independent variables of interest (e.g., diabetes self-care, depression, and diabetes specific emotional distress) and the dependent variable, fear of hypoglycemia while controlling demographic variables.

Study sample

The study sample comprised of outpatients with either type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) who were either on insulin or oral treatment from major hospitals in Lusaka (University Teaching Hospital), Ndola (Arthur Davison Children's Hospital, Kitwe (Kitwe Central Hospital), and Livingstone (Livingstone General Hospital). Convenience sampling was used to recruit patients in the study as long as they were at least 12 years old and were diagnosed at least 6 months before the study to allow for manifestation of psychological challenges. The exclusion criteria included anyone who was not yet on any diabetes medicine, diagnosed less than 6 months and were below 12 year of age. In total 157 patients signed the consent form and were recruited over a 1-year period.

Measures

The measures were administered in English and in 2 local languages, namely Nyanja and Bemba. Back translations were done by two native speakers in each language who were fluent in the other language and English. The translators met to discuss the translation together with the first author to discuss the translation in each language and the differences between forward and back translation versions. The goal was to maximize both linguistic and psychological equivalence. All measures were chosen for use in this study based on their good psychometric properties.

Demographic variables Age, sex, education level, SES (as evaluated using proxy measures of properties and services owned by families of participants) and diabetes type. In addition, the body mass index of the participants was calculated using height and weight.

Fear of hypoglycemia The Hypoglycaemia Fear Survey (HFS) consists of 26 items. HFS comprises two scales assessing "worries

about hypoglycaemia" and "hypoglycaemia-related behaviors." The items are rated on a 5-point scale ranging from 1 (never) to 5 (very often). A Cronbach's alpha of 0.90 suggests high internal consistency [24]. In the current study alpha was 0.80 (Lambda2 = 0.81). A pilot study on 6 adolescents with T1DM confirmed item comprehension by the participants. Extreme scores indicate FoH (possible range 26–130) [11]. In order to examine the proportion of individuals with high frequency of worries about hypoglycemia, the point scales "1 = never and 2 = rarely" were considered "rarely do," the scale "3 = sometimes" remained sometimes while the scales "4 = often and 5 very often" were considered "often do" in the current study.

Diabetes-specific emotional distress The PAID is a 20-item self-report measure used to assess diabetes-specific emotional distress, including a range of feelings such as diabetes-related anger, fear, depression, worry, and guilt. Items can be responded to on a scale from 0 (not a problem) to 4 (serious problem). An overall score for the PAID can be calculated by adding all of the item scores and multiplying by 1.25, which gives a total score ranging from 0 to 100. Higher scores indicate more distress. Reported Cronbach's alphas for the PAID ranges from 0.84 to 0.96 [25–34]. In the current study the alpha was 0.88 (Lambda2 = 0.89).

Diabetes self-care The 13 item Self-Care Inventory (SCI) is a self-report measure used to assess patients' perceptions of their adherence to diabetes self-care recommendations over the previous 1 month. Individuals rate themselves on a 5-point Likert scale that reflects on how well they followed recommendations for self-care during the past month (i.e., 1 = "never do it" to 5 = "always do this as recommended, without fail"). Higher scores indicate more optimal diabetes self-care. Cronbach's alpha for the SCI was 0.84 (Lambda2 = 0.85) for T1DM and 0.85 (lambda2 = 0.86) for T2DM [35]

Depression The Major Depression Inventory (MDI) is a 12-item self-report questionnaire used to assess depression. Items of the MDI asked of the patient to rate how long in the past 2 weeks each of the depressive symptoms was present on a six-point scale ranging from 0 "not at all" to 5 "all time". It can be used as an instrument measuring severity of depression with a range from 0 - 60. The internal consistency of the MDI appeared to be good as indicated by Cronbach alphas ranging from 0.89 to 0.94 [36, 37]. In the current study, Cronbach alpha was 0.80 (Lambda2 = 0.81).

Statistical analysis

Descriptive statistics including means (standard deviations), frequencies, and percentages were computed. We used the item endorsement criteria recommended by Hajos et al. to determine the proportion of individuals with elevated fear of hypoglycemia [38]. The Mann-Whitney *U* test was conducted to examine the mean rank differences between the two types of diabetes and individual items of the FoH scale. Independent *t* tests were conducted to examine mean difference between the two types of diabetes on the total score of the FoH Scale and on the two dimensions (behavior and worry) of the scale. Multiple linear regression models were conducted to assess the association between the total score of the FoH scale and the two dimensions of the scale as the criterion variables with other predictor variables, including diabetes self-care, diabetes specific-emotional distress, depressive symptoms, age, type of diabetes, sex, socioeconomic status (SES), and body mass index (BMI). Statistical significance was set at $p < 0.05$. BMI was computed in SPSS using the following formula: weight (kg)/[height (m)]^2.

Results

Demographic data

Of the 157 participants, 80 were females (51%). We did not find significant differences in gender composition of the patients with T1DM or T2DM. Mean age was 39 ± 17 years, ranging from 12 to 68 years. Of the total sample, 115 (73%) were adults and 42 (27%) adolescents. Table 1 shows the detailed demographic characteristics of the participants.

Proportions of fear of hypoglycemia

About 19% of the sample endorsed 4 "often" and 5 "very often" worry on the scale indicating elevated FoH. The general pattern showed that individuals with T1DM (16.3%) had more FoH than individuals with T2DM (12.6%) (Table 2), although this difference was not statistically significant on the full scale or the two dimensions: behavior and worry (Table 2). At the item level, under the behavior dimension of the FoH, only one item was statistically significant: individuals with type 1 diabetes were significantly more likely to carry fast acting sugar with them ($U = 1938.50$, $p < 0.01$) (Table 3). On the worry dimension of the FHS, appearing stupid or drunk ($U = 2347.00$, $p < 0.05$), got bad evaluation at school/work because of something that happened when blood glucose was low ($U = 1722.50$, $p < 0.001$), and having an insulin reaction ($U = 2234.50$, $p < 0.05$) were reported more in T1DM individuals compared to T2DM individuals. Making a mistake/accident while at school or work ($U = 2336.00$, $p < 0.056$) bordered on significance (Table 2).

Table 1 Demographic and clinical characteristics of 157 participants with type 1 and type 2 diabetes

Sex, *n* (%)	
Females	80 (51%)
Age, mean (SD)	39±17
Age range	12-68 years
Location of patients	
Lusaka	48 (31%)
Kitwe	60 (38%)
Ndola	35 (22%)
Livingstone	14 (9%)
Developmental stage *n* (%)	
Adolescents	42 (27%)
Adults	115 (73%)
Educational levels *n* (%)	
Adolescents (42)	
5-7th Grade (Primary school)	14 (31%)
8-12th Grade (Secondary school)	16 (38%)
Missing	14 (31%)
Adults (115)	
Primary education	10 (9%)
Secondary education	29 (25%)
Tertiary education	22 (19%)
Missing	54 (47%)
Marital status (Adults/115) *n* (%)	
Single	6 (5%)
Married	80 (70%)
Missing	29 (25%)
Type of diabetes	
Type 1	93(59)
Type 2	58 (37)
Missing (either type 1or 2)	6 (4)
BMI mean (*SD*)	25 (5) kg/m^2
Males	25 (5) kg/m^2
Females	26 (5) kg/m^2
Adolescents	22 (4) kg/m^2
Adults	27 (5) kg/m^2

Factors associated with fear of hypoglycemia

Multiple linear regression showed that after adjusting for background variables, there was a positive association between FoH and higher diabetes self-care scores ($\beta = 0.20$, $p < 0.05$). Higher levels of diabetes specific-emotional distress ($\beta = 0.40$, $p < 0.001$), and higher levels of depressive symptoms ($\beta = 0.10$, $p < 0.05$) were associated with higher FoH. Among the adjusted variables, younger age was associated with more FoH ($\beta = -0.26$, $p < 0.05$). In the second model involving the behavior dimension of the FoH, a higher diabetes self-care score was associated with more FoH ($\beta = 0.24$, $p < 0.01$). For the adjusted variables, younger age was the only one associated with increased FoH ($\beta = -0.36$, $p < 0.01$) while a higher socioeconomic status was associated with more FoH ($\beta = 0.21$, $p < 0.05$). In the third model involving the worry dimension, FoH was associated with higher diabetes-specific emotional distress ($\beta = 0.46$, $p < 0.001$) and depressive symptoms ($\beta = 0.21$, $p < 0.01$). Having T1DM (as opposed to having T2DM) was the only background variable that was associated with higher FoH-worry dimension (Table 4).

Discussion

The aim of this study was to find out to how common FoH is in people with diabetes in Zambia, and also to explore correlates of FoH. About 19% [16.3% T1DM and 12.6% T2DM] of individuals with diabetes in Zambia reported elevated FoH. Our results are slightly lower, compared to those reported in an epidemiological study (25–55%) in Sub-Saharan African T1DM patients and 798 T2DM patients in Helsinki, Finland (52.5% all, 43% men, and 62% women) [23, 39]. The mean scores for Zambians living with diabetes were somewhat lower (57.38 ± 12.34) compared to a sample of insulin-dependent diabetes patients aged 18–80 years in the USA and India. It is important to realize the potential pitfalls of the comparisons (e.g., incomparable background characteristics of samples, differential appropriateness of the instrument across the USA and Zambia, sample size or other assessment bias issues). Still, across all countries, past experience of hypoglycemia, or unrecognizable condition can cause fear. It could be that participants in our study experienced less frequent severe hypoglycemic episodes, because of deliberate suboptimal diabetes care in order to avoid hypoglycemia because hypoglycemia can result from exogenous or endogenous insulin excess alone, hence some patients do a trade-off between good self-care or avoiding hypoglycemia; unfortunately, data on severe hypoglycemic events were lacking. Hypoglycemia has long been recognized to be a major limitation in achieving good control especially in T1DM, although with the increasing use of insulin to treat T2DM, the actual prevalence of hypoglycemia is likely to escalate [40]. Moreover, our SCI mean scores show suboptimal diabetes self-care in both types of diabetes. Our data showed individuals with T1DM seemed to FoH more compared to individuals with T2DM as expected. Specifically, individuals with type 1 carried more fast-acting sugar (behavior dimension), were more worried to appear stupid or drunk, making a mistake/accident at school/work, getting a bad evaluation at school/work because of something that happened when sugar was low and they worried more on having an insulin reaction.

Table 2 Proportions of engaging in diabetes-related control measures to avoid hypoglycemia and frequent worries for hypoglycemia

Item Description	Frequency (%)			Mean rank (*p*-values)	
	Never do	Sometimes	Often do	TIDM vs. T2DM	
Behavior dimension					
Eat large snack at bed time	125(80)	19(12)	13(8)	75.83 vs.77.56	(>0.05)
Avoid being alone when sugar is likely to be low	62(40)	58(37)	37(23)	75.39 vs.78.25	(>0.05)
…little sugar to be on the safe side	94(60)	44(28)	19(12)	78.64 vs.73.25	(>0.05)
Keep my sugar high when I will be alone for a while	109(69)	17(11)	31(20)	74.35 vs.79.89	(>0.05)
Eat something as soon as I feel sign of low blood sugar	27(17)	50(32)	80(**51**)	80.84 vs. 69.65	(>0.05)
Reduce my medication when I think sugar is too low	77(50)	23(14)	57(**36**)	75.58 vs. 77.96	(>0.05)
Keep my sugar higher when I plan to be in a long activity	104(66)	23(15)	30(19)	74.45 vs.79.96	(>0.05)
Carry fast acting sugar with me	**88(56)**	**39(25)**	**30(10)**	**85.16 vs.62.86**	**(<0.01)**
Avoid a lot of exercising when I think my sugar is low	63(40)	42(27)	52(**33**)	80.04 vs.70.92	(>0.05)
Check my sugar often when I plan to be in a long activity	71(45)	26(17)	60(**38**)	80.52 vs. 70.16	(>0.05)
Worry dimension					
Not recognising/realizing I am having a reaction	71(45)	56(36)	30(19)	77.89 vs.74.35	(>0.05)
Not having food, fruit or juice with me	73(47)	56(35)	28(18)	78.58 vs.73.22	(>0.05)
Feeling dizzy or passing out in public	91(58)	44(28)	22(14)	73.33 vs. 81.50	(>0.05)
Having a reaction while asleep	84(52)	50(32)	25(16)	73.36 vs. 81.45	(>0.05)
Embarrassing myself/family in social situations	122(78)	25(16)	10(6)	79.40 vs.71.92	(>0.05)
Having a reaction while alone	92(59)	55(35)	10(6)	75.08 vs.78.75	(>0.05)
Appearing stupid or drunk	**141(90)**	**14(9)**	**2(1)**	80.76 vs. 69.78	**(<0.05)**
Losing control	95(60)	53(34)	9(6)	79.75 vs. 71.37	(>0.05)
No one being around to help me during a reaction	84(54)	58(37)	15(9)	76.15 vs.77.05	(>0.05)
Making a mistake/accident at school/work	**130(83)**	**19(12)**	**8(5)**	80.88 vs. 69.59	**(>0.05)**†
Getting a bad evaluation at school/work because something…	**123(78)**	**20(13)**	**14(9)**	87.48 vs.59.19	**(<0.001)**
Having seizures or convulsions	130(83)	22(14)	5(3)	80.41 vs.70.33	(>0.05)
Difficulty thinking clearly when responsible for others.	95(57)	49(31)	18(12)	77.68 vs. 74.64	(>0.05)
Developing long term complications from freq.low blood	115(73)	32(21)	10(6)	78.93 vs.72.67	(>0.05)
Feeling lightheaded or faint	91(58)	48(30)	18(12)	76.88 vs.75.91	(>0.05)
Having an insulin reactions	**112(71)**	**36(23)**	**9(6)**	81.97 vs.67.87	**(<0.05)**

† Marginal (0.56), scale 1&2 = "never do", 3 = "sometimes", 4 &5 = "often do". Significant differences in bold.

Table 3 Fear of hypoglycemia, PAID, DSC, and MDI mean (and SDs) for the two types of diabetes

	All types	Type 1 diabetes	Type 2 diabetes	*p*-value
Fear of hypoglycaemia (total)	57.38 (12.34)	59.02 (12.71)	55.17 (11.23)	> 0.05
Behavior dimension	25.01 (6.43)	25.42 (6.53)	24.11 (6.26)	> 0.05
Worry dimension	33.37 (8.63)	33.60 (9.08)	31.06 (7.35)	>0 .05
Diabetes self-care	44.18 (9.05)	43.10 (8.93)	45.06 (8.93)	>0 .05
PAID	40.28 (18.62)	39.72 (19.09)	42.33 (17.81)	> 0.05
Depression (MDI)	29.44 (9.18)	28.56 (9.86)	31.18 (8.16)	>0 .05

The study looked at the associations between FoH and psychological constructs in the first model, and in the second and third model, the study looked at the associations between the two dimensions of FoH (behavior and worry) and a number of psychological constructs. In the first model, FoH was associated with increased diabetes care. Significant evidence shows that improved glycemic control i.e, lowering of average glucose, is associated with increased occurrence of hypoglycemia [19, 41]. The relationship between hypoglycemia and diabetes self-care particularly glycemic control is that of "you gain one and lose one." People with diabetes need to navigate their ship between two seamonsters, one named Scylla, the other Charybdis. Scylla is sitting on a rock (hyperglycemia), the other monster Charybdis creates a whirlpool (hypoglycemia).

Increased diabetes-specific distress (PAID) was also associated with increased FoH. People living with diabetes are often confronted with stressors that can affect diabetes care and contribute to FoH. Surprisingly, depression was not associated with FoH at least in this model. Of the controlled background variables, being young was associated with FoH. Our study confirms previous findings suggesting that younger age is associated with FoH [17, 20] although elsewhere older people showed more fear [15, 19, 42].

The behavior dimension of the FoH was only associated with diabetes self-care. However, among background variables, higher socioeconomic status and younger age of patients was associated with FoH. The behavior dimension of FoH involves crucial self-care activities useful for glycemic control. Perhaps patients from high SES engage in social activities that distract them from self-care activities such as drinking alcohol which has been found to be a risk factor. Moreover, the best way to control hypoglycemia is through a diet similar to that used to control diabetes mellitus: a reduction in simple sugars, a large intake of complex carbohydrates, and frequent feedings. It could be that those from high SES have easy access to candy, sodas, and even fruit juices (which manufacturers often sweeten with lots of sugar) all high in sugar and should be avoided. Future research should explore this association further.

Not surprising, the worry dimension of the FoH was associated with diabetes-specific distress and depressive symptoms. Hypoglycemia adversely alters mood, and recurrent hypoglycemia elevates anxiety and depression [43]. Depressive symptoms and selective serotonin reuptake inhibitors are associated with progressively increased risks of hypoglycemia [44].

An interesting pattern of association emerged from our data. The behavior dimension of the FoH was associated with more

Table 4 Predictors of fear for hypoglycemia

	Model 1: FoH total scale		Model 2: FoH Behavior dimension		Model 3: Worry dimension	
Variables	*Beta*	*p-value*	*Beta*	*p-value*	*Beta*	*p-value*
Age	-.26	0.02*	-.36	0.00***	-.10	0.33
Being female	.02	0.79	-.06	0.47	.08	0.33
Having type 2 diabetes	.10	0.30	.06	0.53	-.18	0.04*
Socioeconomic status	.06	0.47	.20	0.02*	-.07	0.41
Body mass index	.14	0.17	.07	0.49	.14	0.14
Diabetes self-care	.20	0.02*	.24	0.01**	.12	0.16
Diabetes-specific emotional distress	.41	0.001***	.15	0.13	.46	0.001***
Depressive symptoms	.19	0.17	.09	0.36	.21	0.02*
	R^2 = .27, Adj *R*2 = .22		R^2 = .18, Adj *R*2 = .12		R^2 = .33, Adj *R*2 = .28	

*p <0.05, **p <0.01, ***p < 0.001

constructs such as diabetes self-care activities and socioeconomic status whereas the worry dimension relates to more psychological constructs such as depression and diabetes specific distress. Young people were more likely to have fear of hypoglycemia based on the behavior dimensions of the scale while individuals with T2DM (all adults in this study) were likely to have FoH based on the worry dimension of the scale. At the item level, having T1DM compared to T2DM was associated with carrying fast acting sugar, appearing stupid or drunk, making a mistake or having an accident at school or work and getting bad evaluation at school because of something that happened when sugar was low. These results were not surprising given that T1DM is the one which is more prone to hypoglycemia and has more self-care demands than T2DM. Moreover, the developmental age of individuals with T1DM are the ones below 18 years of age (also the case in this study) which complicates the balance between diabetes self-care and avoiding hypoglycemia.

The strength of the study is that we were able to assess FoH in both T1DM and T2DM. Most studies examine the two types of diabetes separately making it difficult to make comparison. Limitations of the study are that we do not know the refusal rate; therefore, there might have been a selection-bias. The sample size was also small due to recruitment difficulties; as a consequence, probability sampling could not be used. Another limitation is that data on HbA1c, type of medication for T2DM patients (oral or insulin), and number of severe and frequency of hypoglycemia in the past year were lacking. However, despite these limitations, this study still remains important as it shed lights on psychosocial factors associated with FoH in a country with little information on FoH in diabetes patients. Therefore, this study will suggest directions for future studies on the mechanism influencing FoH in Zambia and other SSA countries. Future studies on fear of hypoglycemia should be complimented by interviews to individuals with diabetes and adults living with individuals with diabetes.

In conclusion, FoH was common in individuals with diabetes in Zambia. FoH is positively associated with diabetes specific emotional distress and diabetes self-care. Therefore, physicians need to assess fear of hypoglycemia based on its dimensions since they seem to be associated with demographic characteristics and psychosocial issues differently. Physician need to consider depression, self-care, diabetes-specific distress, younger age, higher SES, and T1DM when intervening on fear of hypoglycemia.

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Authors' contributions GH collected data, analyzed, and drafted the manuscript. AA verified analysis and reviewed the manuscript. FV and FP reviewed and approved the manuscript.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

The study obtained consent from adults participants and assent from children after their guardians consented for their participation.

Ethical approval and consent The study was approved by the ethics committee of the School of Humanities and Social Sciences, University of Zambia on 29th April, 2011 (reference number IRB: 00006464, IORG: 005376). The study only used data from participants that consented to participate in the study. Assent was obtained from participants younger than 18 years old.

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ORIGINAL ARTICLE

Diabetes education and basic insulin related knowledge assessment in nursing staff in a tertiary care hospital in India

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Abstract

Background Nursing staff plays a major role in the delivery of insulin to hospitalized patients. We aimed at studying the adequacy of desired knowledge related to insulin in nursing staff in a tertiary care government-funded teaching hospital in India.
Methods A cross-sectional descriptive design study was conducted to assess the basic insulin-related knowledge in nursing staff by a self-administered questionnaire.
Results A total of 101 nursing staff accepted to participate in the study and returned the completed questionnaire. Forty-one participants were female and 60 were male. Almost 80% of participants denied as trained for diabetes management in hospitalized patients in their nursing curriculum. Eighty-seven participants were comfortable giving insulin. Only two participants could identify basal and bolus insulin as the type of insulin needed to manage hyperglycemia. Twenty-five participants could identify the strengths of insulin vials available in India and only 8 could correctly mention the colour code of the corresponding insulin syringe. Only 15 participants could correctly identify the commonly used routes of inulin administration. Thirty participants could identify all the correct subcutaneous injection sites, however, at least one wrong site was chosen by 78 participants and 8 participants did not answer. A similar deficit in knowledge was seen in hypoglycemia management, glucose monitoring, insulin storage, expiry of inulin and injection techniques.
Conclusion This study shows a significant deficit in basic insulin-related knowledge in nursing staff. There is a need for continuous medical education of nursing staff to stay updated on Diabetes management.

Keywords Insulin · Nursing knowledge · Diabetes education

Introduction

Obesity and Diabetes Mellitus (DM) are one of the biggest non-communicable disease epidemics in human history. World is facing currently the epidemic of DM which if improperly managed in the long run has many complications with significantly increased morbidity and mortality [1]. Human Body has a long-term glycemic memory. A persistently high HbA1c or on higher side which indicates long term uncontrolled hyperglycemia have increased risk of microvascular and macrovascular complications. A long term good glycemic control as monitored by HbA1c within target range has long term benefits on microvascular and macrovascular complications.

India is considered as a high-risk population of diabetes as Type 2 DM has a younger onset even at lower body mass index (BMI). The prevalence of diabetes is estimated to be approximately 7.3% and prediabetes of 10.5% [2]. All patients with type 1 DM and most of the cases with long-standing type 2 DM require insulin therapy for the management of hyperglycemia. Insulin prescription to hospitalized patients is common. Hospitalized patients with DM for infection, major surgery, and other medical conditions are usually started on insulin.

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Nursing staff plays a major role in the delivery of insulin to hospitalized patients. However, Insulin is considered a high-alert drug. High-alert medications pose a health risk to patients if used inappropriately or erroneously [3]. The reported errors and adverse drug events with insulin use remain high worldwide [4]. Even developed countries have reported high insulin administration related errors. A study by National Diabetes Inpatient Audit 2017 suggests that a significant proportion of the patients on insulin experience a medication error related to their insulin (49%) [5]. Lack of adequate knowledge of insulin safe use while administering insulin, for example, insulin dose, route and delivery errors, timings, insulin preparations etc., safe use with other comorbidity, precautions to be taken, while storage and transportation of insulin etc. among healthcare professionals contribute to medications error and patient harm [2, 6–9]. Insulin is one of the most common drugs associated with medication errors and patient harm due to errors.

Pharma industry is evolving to improve the quality of life in patients on insulin treatment by better and new techniques of administration and newer insulin biomolecules with suitable pharmacodynamics. However, this brings a frequent change in product available, increases the complexity of informations and knowledge required associated with new products use in which healthcare professionals may not be updated, and probably contribute to a lack of competence among healthcare professionals [6]. Many studies from developed and developing countries have assessed the knowledge of insulin use in nursing staff. Many of these studies showed a large gap between the actual knowledge of the nursing staff and the desired knowledge.

The role of nursing staff in the administration of prescribed insulin to hospitalized patients with hyperglycemia is vital. The nursing staff of Endocrinology department are usually trained especially for diabetes education, management, and insulin education. However, most of the diabetes mellitus patients are admitted in non-endocrine departments for surgery or other co-morbidity. Hence, Diabetes Education and basic insulin knowledge applicable for nursing care are important for good outcome in these patients.

Our aim of this study was to examine the actual status of the basic insulin-related desired knowledge for adequate management of hyperglycemia in hospitalized patients with diabetes with insulin, their perceived ability to advise diabetes patients and give diabetes education. This study may help in guiding the government in policy decisions to identify the gaps in desired insulin and diabetes knowledge and help in improving the insulin-related desired and expected knowledge in Nursing staff and inclusion in nursing curriculum and training.

Materials and methods

This study was cross-sectional and observational carried out in a recently started tertiary care teaching hospital in the central part of India. This study was conducted from June 2018 to January 2019. The hospital services have been running for the last 5 years and most of the employed nursing staff are young. A questionnaire was prepared by an Endocrinologist in coordination with a nursing faculty. Content analysis of the questionnaire was examined by piloting the questionnaire with a convenience sample of 20 nursing staff in a separate multispeciality teaching hospital. The validity of the questionnaire was assessed by three independent Endocrinologists. The Endocrinologists were requested to assess the questionnaire if it fulfilled the requirement of identifying the desired basic level of insulin education desired from the nursing staff. A few questions were modified and some new questions were added based on the response of the independent reviewers. The modifications were made in the questionnaire based on the response of Endocrinologists and finally based on the pilot study. The questionnaire consisted of 22 items and questions were of multiple-choice type with one or multiple correct answers and few open-ended questions in to achieve the objective of the study. All the nursing staff available on duty were approached and after taking consent the self-administered questionnaire was given to fill in front of research assistants.

Participants were requested to avoid discussion, or referral to any information resources and to provide answers based on their knowledge to answer questions or disclose answers to other participants. There was no set time limit to answer the questions but usually most completed the form in less than 20 min. Information was also taken for the education level age, gender additional training for diabetes management, etc. The study was started only after obtaining ethical clearance from Institute Ethics Body, reference no. IHEC-LOP/2018/STS0138.

Statistics

The descriptive data were analysed and expressed as mean ± SD. The multiple-choice questions and open-ended questions are reported as proportions or percentages. Data were analysed by Microsoft Excel 2017.

Results

A total of 101 questionnaires were completed. All the nursing staff posted in the institute were approached for the study. The departments where participants consented include surgery, dermatology, ophthalmology, cardiology, critical care, psychiatry and surgical oncology. Forty-one participants were female and sixty were male. A total of 55 (45.8%) participants

had no prior work experience or had experience of less than a year. The mean age of participants was 26.7 ± 3.1 years. Almost 66.7% participants (80) denied as trained for diabetes management in hospitals in the nursing curriculum, while 30.8% (37) participants agreed to have received training for diabetes management in hospitals in the nursing curriculum. The baseline training, grade of staff and patients of DM seen in the last 1 week are given in Table 1.

Almost all of the nursing staff reported having adequate knowledge of insulin and were comfortable dispensing insulin to patients. A total of 87 participants reported as comfortable giving insulin, while 10 participants reported as uncomfortable giving insulin (Table 2). The participants were assessed for insulin knowledge and the result is shown in Table 3. Many questions were asked to assess the insulin management knowledge. One question assessed the factors important to consider before injecting pre-lunch insulin in hospitalized patients. A total 67% of participants correctly identified one of the correct options of lunch ready or not, and if patient nil per oral for late afternoon procedure; and 20 participants (19.8%) correctly identified both the given situations. When asked to identify the two types of insulin required for hyperglycemia management in DM patients, only 2 participants correctly identified basal and bolus insulin, however, one of the correct options was identified by 39 participants. A question on somogi effect was asked as leading question as why midnight sugar is monitored.

Table 1 Baseline information of the participants (total participants in the study 101, *n* = absolute numbers)

Baseline Characteristics	n
Trained for diabetes management in hospital in nursing curriculum	
Yes	30
No	70
Not answered	01
Duration of experience as staff	
< 1 year	48
1–3 years	28
3–7 years	18
> 7 years	07
Grade of nursing staff	
Grade 1	25
Grade 2	75
Not answered	1
Additional training (e.g., workshop) for in-hospital diabetes management	
Yes	05
No	96
Number of patients with diabetes seen in last 1 week	
0	43
1–3	41
> 3	17

Table 2 Self-reported comfort level of participants for giving insulin. (*n* = absolute numbers)

Comfort level for insulin injection	n
Very comfortable	49
Comfortable	38
Uncomfortable	01
Very uncomfortable	09
Not answered	04

Route of insulin administration was correctly identified as subcutaneously and intravenous route in 15 (14.8%), and only one route was correctly identified by 79 participants. The strengths of insulin available in India were correctly identified as 40 IU/ml and 100 IU/ml by only 25 participants (24.7%), and at least one was correctly identified by 71 participants (70.3%). Only 8/101 participants correctly mentioned the colour code of insulin syringes used for 40 IU/ml and 100 IU/ml insulin vials, however, 59/101 correctly mentioned at least one insulin vial strength and their colour code of syringe to be used in an open-ended question. In a multiple option question, 30 participants could identify all the correct subcutaneous (s.c.) insulin injection sites, whereas at least one wrong site was chosen by 78/101, two incorrect sites were chosen by 18/101, and three incorrect sites by 12/101 participants. A total of 8 participants did not choose even a single correct site for s.c. insulin injection.

Participants were told to identify premeal Aspart and Regular insulin in multiple choice question answer. A total of 11/101 participants identified both the premeal insulins correctly, while 75/101 participants could identify at least one of the premeal insulins. Long-acting insulins were marked as premeal insulin by 17/101 participants, while 7/101 participants did not answer. In a multiple-choice question, participants were told to identify complication of insulin injection improper technique, and both lipohypertrophy and lipoatrophy were identified by only 7/101 participants while at least one of these complications was identified by 71/101 participants. A total of 23/101 participants did not reply.

Discussion

Studies have reported a prevalence of DM ranging from 38 to 40% in hospitalized patients and 70–80% of critical illnesses and cardiac surgery patients [10]. Almost 20% of hospital discharges have DM as comorbidity. Patients with DM have higher chances of hospitalization as compared to those without DM. Around 30% of patients hospitalized with DM as comorbidity have readmissions twice or more in a given year [11].

Table 3 The Percentage of participants with correct response for questions related to insulin knowledge. (Expected response mentioned in bracket for short answers and for long correct response are below table)

Questions	Correct response (n)	Incorrect response (n)	Not answered
Colour of regular Insulin (colourless)	79	19	22
Colour of NPH Insulin (milky)	37	44	35
Colour of Glargine Insulin(colourless)	58	29	33
Colour of Mixtard insulin (milky)	49	44	27
Food gap required with regular Insulin (20–30 min)	37	72	1
Ml of Syringe used to give insulin (1 ml)	97	3	1
Level of Blood glucose below which is defined as hypoglycaemia by American Diabetes Association (70 mg/dl)	22	76	3
How is insulin mixed in pen or vial before use (gently rolling)	76	19	0
Why midnight glucose is monitored? (midnight hypoglycaemia followed by morning hyperglycaemia)	50	43	8
Temperature for storage of in-use opened vials (12–25°)	68	24	9
Optimum angle for giving insulin injection subcutaneously by insulin syringe (90°)	30	67	3
For how many days opened insulin vials can be used (28 days)	67	23	11
How long to wait for syringe withdrawal after injecting insulin? (count till 10)	23	78	0
At what temperature unopened Insulin vial or pen is kept (2–8°)	25	75	1
How frequently is Human Mixtard 30/70 insulin given to a patient (twice daily)	38	48	15
Food Gap with Aspart Insulin (15 min)	14	68	19
Hypoglycaemia management at blood glucose 65 mg% in non-meal time	43	53	5
How to manage unconscious patient with BG 10 mg %*	74	21	6
How to manage hypoglycaemia at dinner in a patient on NPH and regular at dinner**	28	46	27

Total participants in the study 101. The values expressed are in percentage. The expected responses are as follows * give a candy followed by 3 Marie biscuits and monitor sugar level every 15 min till normalized. ** give 100 ml 25% dextrose, Let the patient take dinner immediately but to take regular insulin after reducing 2 units, and take NPH as prescribed

The present study aimed to assess the prevailing insulin knowledge in a recently started government tertiary care teaching hospital. The study allowed to broadly assess the insulin-related knowledge which is desired for dispensing insulin or providing diabetes education to the patients at the time of discharge. Nursing staff plays a major role in the ongoing care of hospitalized patients with hyperglycemia, like administering of a correct dose, correct technique of insulin to patients, identifying hypoglycemia and its urgent management in patients admitted in almost all clinical departments for various medical and surgical conditions. Good nursing care structure is an important variable determining the patient care quality, safety and overall mortality in hospitalized patients with hyperglycemia [12–14]. Approximately, 5–15% of patients hospitalized experience adverse reactions [15]. Insulin has place in the list of top medicines implicated in adverse drug events and medication errors worldwide [4].

We report a general lack of insulin-related knowledge in nursing staff. Most of the staff were young and had a similar representation of both genders. All the participants were at least a graduate in nursing. A significant proportion of nursing staff denied nursing training in their nursing curriculum. However, a large number of nursing staff reported being comfortable giving insulin and insulin-related knowledge (87%). This study highlights the lack of knowledge of types of insulin, timing of insulin, regimens and their frequency of administration. A study by UK national patient safety agency on review of insulin-related incidents commonly reported an error with insulin administration and reported 24% of the events were causing harm to patients and few were even fatal. In the above study, the commonly reported errors included the error of dosing, insulin strength and frequency (26%), omitted or delayed dose (20%), and wrong insulin product [5]. Our study suggests similar deficits in the knowledge of the nursing staff. Insulin is considered as high-risk medication if not used appropriately, and a sound baseline knowledge is required in the nursing curriculum, and bedside and outpatient training for insulin use in patients with hyperglycemia to avoid errors. Nursing staff plays a major role in the education of patients to patients receiving insulin at home about self-management

of insulin doses, hypoglycemia management, the correct technique of insulin administration, etc. Every patient who is prescribed insulin self-administration at home must receive basic insulin-related education to prevent complications related to improper use of insulin.

Surprisingly, the perceived knowledge had little bearing on the actual desired knowledge related to insulin, and this finding of actual knowledge and perception deficit was consistent with the results of many previous studies [16–18]. As our Institute is an upcoming institute and most of the nursing staff were young, we could not demonstrate the relationship between years of nursing experience and knowledge levels.

The observations of this study highlight significant deficits in pharmacological treatment of diabetes with insulin, e.g., types of insulin and relation to meal, food gap required with insulin, insulin strengths and the used syringes colour coding, regimens of insulin, storage of insulin, hypoglycemia management, need for sugar monitoring in hospitals, site rotation of insulin injection and complications of poor injection techniques etc. A study in Ireland had shown a similar knowledge deficit among nursing staff [14]. The basic insulin-related knowledge is clinically important for safe patient care. Nurses deal with hospitalized patients every day and safe insulin administration is the responsibility of nursing staff. Lack of basic insulin-related knowledge poses a risk to hospitalized patients. This study highlights the importance of insulin-related knowledge in nursing staff to improve the mortality and morbidity outcome of hospitalized patients, prevent potential harm by inappropriate use of insulin by nursing staff, and strengthening the knowledge and confidence of patients discharged on insulin on self-management of diabetes at home.

India is going to be a leader in the number of patients with diabetes in the coming decade posing a major financial health burden on the country by increasing the duration of hospital stay and complications related to a prolonged stay and inadequately managed hyperglycemia. Good management of in-hospital hyperglycemia will reduce the duration of hospital stay and complications arising due to prolonged stay. The study may give an insight into the curriculum developers to stress on the areas on Diabetes Education needed by nursing staff to improve the outcome of hospitalized patients with hyperglycemia and prevent errors with insulin.

The strength of this study is the focus of the questionnaire to assess the knowledge and practices of nursing staff important for in-hospital management of hyperglycemia, prevention of errors with insulin use and knowledge required to educate the patient about self-monitoring and proper use of insulin at home to patients. The key limitation of the study may be that the study was conducted in a newly set up tertiary care teaching hospital where most of the recruited nursing staff has limited practice experience as most staff were recent pass out of nursing graduation. We could enroll the participants only if they gave prior written consent as per the directives of Ethics approval, which might have affected the results. The reason for non-participation in the study merits examination. However, this study highlights the need for continuous medical education for nursing staff for keeping them updated to the new technology and new insulin. As the hospital has attached nursing college, this study may help in modifying the curriculum to help the nursing students.

Conclusion

Knowledge deficit in nursing staff and other health care providers is an important hurdle in safe and effective management of hyperglycemia in hospitalized patients and prevent insulin administration errors and potential harm.

Acknowledgments We like to thank Dr. Pankhudi for contribution in data collection for the study.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval The study was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The data was collected after receiving proper Institutional Human Ethics Clearance.

Informed consent Written informed consent was obtained from each participant before enrolment.

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CASE REPORT

Prolonged diabetic ketoacidosis and glycemic fluctuations associated with dapagliflozin: a case report

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Abstract

Aim Sodium-glucose co-transporter 2 inhibitors (SGLT-2I) are oral anti-diabetic drugs. We aimed to raise awareness by presenting a difficult and long-lasting DKA case that developed after the addition of dapagliflozin to the treatment.

Case presentation We report a case of a 40-year-old woman who developed diabetic ketoacidosis (DKA) after 2 weeks use of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor (SGLT-2I). She had complaints of nausea, vomiting, loss of consciousness, fever, and shortness of breath. DKA was detected in her laboratory results despite the absence of marked hyperglycemia. The patient had metabolic acidosis episodes accompanied by ketonuria on the 5th (mild) and 9th (severe) day despite discontinuation of dapagliflozin. Sudden fluctuations in blood glucose levels of the patient lasted for 10 days and made it difficult to switch to routine basal-bolus insulin treatment.

Conclusion Prolonged DKA may be a result of SGLT-2 inhibition and individualized treatment and follow-up should be performed instead of standard DKA treatment. Also, we suggest that we need to evaluate endogenous insulin reserves of the patients before starting a SGLT-2I treatment. We believe that in order to raise awareness, these cases should be reported.

Keywords Diabetic ketoacidosis · Dapagliflozin · SGLT-2 inhibitors

Introduction

Sodium-glucose co-transporter 2 inhibitors (SGLT-2I) are oral anti-diabetic drugs that have been in use for the past few years in the treatment of diabetes mellitus (DM). They reduce the reabsorption of glucose by 30–50% by inhibiting SGLT-2, which is present in the S1 segment of the renal proximal tubule and is responsible for the reabsorption of 90% of the urine glucose [1]. They can be used at any stage of DM as they provide a decrease in blood glucose levels independent of insulin levels. They provide an average drop of 0.4–1.1% in HbA1c levels and have advantages such as weight loss, drop in blood pressure, and low risk of hypoglycemia [2, 3]. In SGLT-2I users, diabetic ketoacidosis (DKA) cases with slightly increased or normal blood glucose levels are reported with increasing numbers [4–6]. Therefore, it is necessary to follow preventive approaches for DKA development when starting SGLT-2I and their follow-up. In this case report, we aimed to present a difficult and long-lasting DKA case that developed after the addition of dapagliflozin to the treatment of a patient with type 1 DM and to raise awareness on this issue.

Case presentation

A 40-year-old female patient was admitted to the emergency department with complaints of nausea, vomiting, loss of consciousness, fever, and shortness of breath. She had been followed up with type 1 DM for 18 years, was admitted to the internal medicine outpatient clinic of another hospital 2 weeks ago due to non-regulated blood glucose levels. She had been receiving basal-bolus insulin therapy 4 times a day since the diagnosis. It was learned that the patient did not comply with her diet and did not use insulin regularly. Her previous treatment was discontinued, and insulin aspart + degludec insulin 2 times/day, metformin + sitagliptin, and dapagliflozin treatments were started. She had no history of any other disease or drug.

In physical examination, her respiratory rate was 23 breaths/min, heart rate was 110 bpm, blood pressure was

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115/60 mmHg, and body temperature was 36.0 °C. Her mental status was confused with a Glasgow Coma Scale of 10. She was dehydrated with decreased skin turgor-tonus and dry mucosal membranes. Her breath sounds decreased bilaterally, and crackles were present in the left lung. In laboratory investigations, glucose level was 265 mg/dl and there was hypokalemia, metabolic acidosis, and ketonuria confirming the diagnosis of DKA with relatively low levels of blood glucose (Table 1). She had pneumonic infiltration in thorax tomography (Fig. 1).

She was hospitalized to the intensive care unit of our hospital and standardized DKA therapy including fluid replacement, insulin infusion, and antibiotherapy (piperacillin-tazobactam + clarithromycin) was initiated. The patient's previously prescribed medications were discontinued. In the 10-day follow-up, the patient had recurrent metabolic acidosis episodes accompanied by ketonuria on the 5th (mild) and 9th (severe) day, although the infection had improved (Table 1). During the follow-up, hypoglycemia and hyperglycemia attacks were also observed. On the 10th day of hospitalization, her clinical and metabolic status improved.

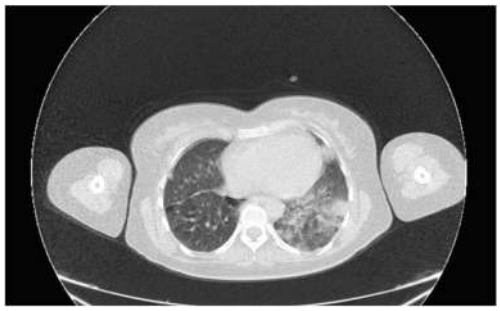

Fig. 1 Thorax computed tomography of the patient on the day of admission. Diffuse pneumonic infiltration is observed especially in the left lung

Discussion

In the SGLT-2I users, DKA is often developed in patients with insulin deficiency (type 1 DM and long-term type 2 DM) and those with increased insulin requirements such as infection, trauma, and inflammation [4, 7]. Therefore, care should be taken in the use of SGLT-2I in these groups of patients. According to the FDA warning in December 2015, 73 DKA cases (44 type 2 DM, 16 type 1 DM) requiring drug-induced hospitalization were reported in patients receiving SGLT-2I between March 2013 and May 2015 [4]. Of these, 48 are caused by canagliflozin, 21 by dapagliflozin, and 4 by empagliflozin [4]. Although DKA improves with standard treatment in most of the cases, treatment-resistant cases are also seen [8, 9]. It has been reported that metabolic recovery lasts up to 6 days, glucosuria up to 11 days, and ketonemia continued for up to 10 days [8, 9]. Similarly, the blood glucose-lowering and ketogenic effects of SGLT-2I seem to persist for a long time despite drug discontinuation [8, 10]. This situation complicates the management of DKA in these patients as in our case. Recovery of our patient lasted 10 days. In this period, since the blood glucose levels measured do not reflect the actual insulin requirement of the patients, switching to oral regimen and basal-bolus insulin therapy becomes difficult and prolonged. This also leads to recurrent episodes of metabolic acidosis, and relapsing ketonuria and ketosis, as seen in our patient.

An increasing number of DKA cases with all SGLT-2I indicate that there are issues to consider when evaluating treatment options and monitoring in diabetic patients. Some studies previously suggested the temporary discontinuation of SGLT-2I during acute illness and acute surgery to prevent

Table 1 Laboratory values of the patient on the day of admission and follow-up

Day	1st	2nd	3rd	4th	5th	6th	7th	9th	10th
Biochemistry									
Glucose (70–110 mg/dl)	265	118	166	139	287	126	88	192	143
Creatinine (0.5–0.9 mg/dl)	1.01	0.9	0.73	0.41	0.55	0.39	0.39	0.5	0.33
Sodium (Na) (135–145 mEq/l)	141	148	145	142	139	138	142	136	144
Potassium (K) (3.5–5.5 mEq/l)	2.95	3.33	4.3	3.39	4.72	3.68	3.48	4.31	4.18
Urine analysis									
Ketone (mg/dl)	≥ 80	40	40	15	≥ 80	40	–	≥ 80	–
Blood gases									
pH (7.35–7.45)	7.13	7.33	7.46	7.45	7.26	7.44	7.44	7.08	7.44
pCO_2 (35–45 mmHg)	16.6	25.9	18.9	31.8	13.7	23.6	32.1	16.1	33.4
pO_2 (80–100 mmHg)	97.6	102.7	143	31.3	147	94.4	91	163	91.3
HCO_3 (22–26 mmol/l)	8.9	15.8	18	22.6	9.8	18.7	22.5	7.6	23.2
O_2 saturation (90–100%)	97.6	98.1	98.9	69.4	98.7	97.6	97.7	98.6	97.3
Lactate (0.4–1.4 mmol/l)	1.48	1.26	1.32	1.17	2	1.36	1	1.15	1.15

progression to DKA [7, 10]. In patients without endogenous insulin reserve, such as type 1 diabetes, the use of SGLT-2I is likely to be inappropriate especially in the presence of conditions such as strict low-carbohydrate diet and increased insulin requirement such as infection. Also, we suggest that in patients with partial insufficiency of endogenous insulin reserve (insufficient beta cell function), likelihood of developing DKA with the use of SGLT-2I is also increased in the presence of strict low-carbohydrate diet and conditions associated with increased insulin need.

In summary, while our patient had an uncontrolled type 1 DM, the treatment of the patient was changed inappropriately. Newly acquired pneumonia in the patient increased the need for insulin. With the changing treatment, the patient's insulin requirement could not be estimated correctly and could not be replaced due to the blood glucose that remained close to normal. As a result, the DKA picture appeared in the patient. Despite the discontinuation of dapagliflozin, the blood glucose-lowering and ketogenic effects persisted for 10 days and complicated the management of DKA.

Conclusion

We suggest that we need to evaluate endogenous insulin reserves of the patients before starting a SGLT-2I treatment. In light of the cases reported in the literature, it should be kept in mind that prolonged DKA may be a result of SGLT-2 inhibition and individualized treatment and follow-up should be performed instead of standard DKA treatment.

Compliance with ethical standards

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

Ethical approval All procedures performed 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 The authors certify that they have obtained all the appropriate consent forms.

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LETTER TO THE EDITOR

SGLT2 inhibitor/GLP-1 receptor agonists as first step monotherapy—evidence and implications

Anil Pareek[1] · Ravi T. Mehta[1] · Shruti Dharmadhikari[1]

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Dear Editor,

The recently published guidance by ESC/EASD on diabetes, pre-diabetes, and cardiovascular diseases (CVD) [1] mentions that metformin should be considered as first-step therapy only in overweight patients with type 2 diabetes (T2D) without CVD and at moderate CV risk. The guideline recommends SGLT2 inhibitors or GLP-1 receptor agonists (RA) as first-step therapy in patients with T2D and CVD, or at very high/high CV risk, to reduce CV events. This recommendation is based on cardiovascular outcome trials (CVOTs) of SGLT-2 inhibitors and GLP-1 RA, in which majority of patients received metformin before and concurrently with SGLT-2 inhibitors and GLP-1 RA. The recommendation on use of SGLT2 inhibitors or GLP-1 RA as monotherapy appears to be based on consensus of the experts and not on CVOTs. Thus, the level of evidence should be changed to C.

Until recently, use of metformin was considered "absolutely" contraindicated in patients with co-morbid heart failure (HF) and chronic kidney disease. Recently, regulatory bodies in the USA and Canada have removed HF contraindication from product labeling for metformin [2]. In the UKPDS trial, patients initially assigned to intensive therapy with metformin had decreased risks of combined diabetes-related end points, diabetes-related deaths, all-cause deaths, and myocardial infarction compared with conventional therapy. In an observational study [3], metformin therapy was associated with survival benefit over 2 years of follow-up in diabetes patients with established HF. In recent retrospective cohort study, among patients with diabetes and reduced kidney function with persistent monotherapy, treatment with metformin was associated with lower risk of MACE compared with sulfonylurea [4]. Thus, despite the absence of dedicated CVOTs on metformin, its benefits on CV outcomes cannot be ignored. The recently published guidance by RSSSDI-ESI recommends that metformin should be considered in combination with lifestyle intervention at the time of diagnosis of T2D. When metformin is contraindicated or not tolerated, other treatment options include sulfonylurea, DPP-4 inhibitors, SGLT2 inhibitors, or alpha-glucosidase inhibitors [5].

The adoption of such recommendations of use of SGLT2 inhibitors by ESC/EASD [1] will have major implications in developing countries including India and will lead to use of higher doses for glycemic control and further leading to increased cost of therapy and possibly higher incidence of adverse effects such as LDL-C elevation [6] and UTI [https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/sodium-glucose-cotransporter-2-sglt2-inhibitors]. This can deter the use of these evidence-based agents, which have good beneficial CV outcomes in combination with metformin. Also, cost-effectiveness analysis needs to be performed before recommending a mass strategy.

Compliance with ethical standards

Conflict of interest Authors are employees of Ipca Laboratories Limited which markets anti-diabetic drugs

Research involving human participants and/or animals This manuscript did not involve participation of human volunteers or animal as this is a letter to the editor (comment on the guideline)

Informed consent This manuscript did not require participation of humans and hence informed consent was not required as this is a letter to the editor (comment on the guideline)

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LETTER TO THE EDITOR

Metformin use in T2D patients with reduced kidney function: reassuring, more evidence needed

Anil Pareek[1] · Shruti Dharmadhikari[1] · Ravi T. Mehta[1] · Kumar Naidu[1]

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Dear Editor,

The recently published trial assessed major adverse cardiovascular events (MACE) among patients with diabetes and reduced kidney function who continued treatment with metformin or a sulfonylurea through well-designed retrospective cohort study [1]. Until recently, the use of metformin was restricted in patients with reduced kidney function due to concerns regarding risk of lactic acidosis. In 2016, the US FDA changed its guidance based on evidence regarding safety of metformin in patients with mild to moderate kidney disease; however, its effect on clinical outcomes in patients with reduced kidney function was unknown. In view of this background, the results of the present study are important and reassuring.

Hypoglycemia and HF are two important events that influence cardiovascular outcomes in diabetic patients, more so in those with kidney disease. Hypoglycemia in T2D patients is a frequent event and is associated with increased risk of cardiovascular events and all-cause mortality. Hypoglycemia is most commonly associated with the use of insulin secretagogues (primarily sulfonylureas) or insulin. Other risk factors include advanced age and cognitive impairment, renal dysfunction, duration of diabetes mellitus, and missed or irregular meals [2]. Both diabetes and kidney disease are major and independent risk factors for the development of HF [3]. In the unweighted cohort, there were substantially more patients with comorbid HF in sulfonylurea group which were adjusted with propensity matching. However, during the observation period, incidence of HF was not assessed, although hospitalization for acute MI, stroke, and TIA was assessed.

Thus, to better understand the influence of hypoglycemia and HF on CV outcomes, there is need to additionally assess the following: (1) overall incidence of hypoglycemia along with its severity, (2) incidence of hypoglycemia in patients who experienced CV events, (3) incidence of CV events in the two groups after excluding patients with hypoglycemia, and (4) hospitalization due to HF.

Finally, as reported, it cannot be determined whether metformin is associated with a reduced risk or sulfonylureas are associated with an increased risk of MACE outcome. Well-designed prospective studies will further reassure the use of metformin in T2D patients with reduced kidney function.

Compliance with ethical standards

Conflicts of interest The authors are employees of Ipca Laboratories Limited which markets anti-diabetic drugs.

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VISION STATEMENT

To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

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2. Empowerment of persons living with diabetes.
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Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

All awardees are encouraged to submit their work to the RSDDI Journal International Journal of Diabetes in Developing Countries.

CALL FOR RESEARCH PROPOSALS FOR GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI.

The proposals may be of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization of funding sought from RSSDI.

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
All applications should be addressed to:
1. The Secretary , RSSDI.
2. Soft copy of the research proposal should be sent to Secretary, RSSDI.

When to apply
Proposals will be accepted Twice a year. Once between 1st Jan - 31st April & then July 1st to 30th Nov.

All research proposals will be reviewed by Research committee over a period of 4-6 weeks & approved proposals will be provided Research Grant after fulfilling all documentation by 30th June & then 31st December of each year.

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof.M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 18 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers fromtime to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

List of RSSDI Accredited Centres

SI. No	Institute Name	Location
1.	Diacon Hospital	Bengaluru, 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	Ahmedabad, Gujrat
6.	Sonal Diabetes Hospital	Surat, Gujrat
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 Cenre	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 Street, Kolkata

COURSE DETAILS

Name of the Course: Advanced Certificate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by screening interview of 50 marks at respective Centres. This will be conducted by the local course Director. The result will be declared in a week's time.

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1-year program)
- Rs. 50000/- (for post MBBS,MDin other branches, 2-year program)

Session: Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

ANNOUNCEMENTS

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile No. after logging in your membership area on our website www.rssdi.in under sub heading Membership Corner, so that we can send you RSSDI Newsletter & Journals.

48th Annual Conference of RSSDI –RSSDI 2020

Virtual Conference by National RSSDI EC Members on 26th - 29th November 2020

THEME: Practical, Clinical, Relevant

Registered with Registrar of News Papers, India vide no. RNI-43019/85

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